Maurice W. Sabelis · Jan Bruin *Editors*

Trends in Acarology

Proceedings of the 12th International Congress

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Editors
Maurice W. Sabelis
University of Amsterdam
Institute for Biodiversity and Ecosystem Dynamics (IBED)
Section Population Biology
P.O. Box 94248
1090 GE Amsterdam
The Netherlands
M.W.Sabelis@uva.nl

Jan Bruin
University of Amsterdam
Institute for Biodiversity and Ecosystem Dynamics (IBED)
Section Population Biology
P.O. Box 94248
1090 GE Amsterdam
The Netherlands
bruinjan@tiscali.nl

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Cover illustration: A female of the predatory mite *Gaeolaelaps* (*Hypoaspis*) *aculeifer*, feeding on a female of the bulb mite *Rhizoglyphus robini*, both cultured by Dr. Izabela Lesna (University of Amsterdam) (Photo: Bert Mans)

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VETERINARY ACAROLOGY

Preface

For the past 50 years, the International Congress of Acarology has been the foremost forum for worldwide communication on the knowledge of mites and ticks. This group of very small arthropods exhibits a bewildering diversity of species that live in many different habitats, in association with the land, freshwater and marine organisms with which they interact. Many mites and ticks have economic consequences as they are pests of agricultural, veterinary and medical importance, and several species have become model organisms for studies in modern biology. While acarologists pur sang focus on evolution and phylogeny of the Acari, they may learn from insights emerging from fundamental and applied biological experiments in which selected species of Acari are the focal object of study. Experimental and applied biologists, on the other hand, may learn from insight in how their pet organism is positioned amidst the diversity of Acari. In this sense the International Congress of Acarology may stimulate biologists to look beyond the borders of their disciplines.

The 12th International Congress of Acarology, held from 21-26 August 2006 in Amsterdam, The Netherlands, succeeded in bringing together scientists that share an innate fondness for mites and ticks, yet differ widely in scientific specialisation. The congress was truly international and well attended, with 386 participants from 59 countries: Australia, Austria, Benin, Belgium, Brazil, Bulgaria, Cameroon, Canada, China, Colombia, Costa Rica, Croatia, Cuba, Czech Republic, Denmark, Egypt, Finland, France, Georgia, Germany, Ghana, Greece, India, Iran, Ireland, Israel, Italy, Japan, Kenya, Latvia, Mexico, Netherlands, New Zealand, Nigeria, Norway, Pakistan, Philippines, Poland, Portugal, Russian Federation, Serbia and Montenegro, Slovak Republic, South Korea, Spain, Sri Lanka, St. Vincent and the Grenadines, South Africa, Sudan, Switzerland, Taiwan, Thailand, Tasmania, Trinidad, Tunesia, Turkey, UK, Ukraine, USA, and Venezuela. Moreover, a wide variety of disciplines were represented, such as molecular biology, biochemistry, physiology, microbiology, pathology, ecology, evolutionary biology, systematic biology, soil biology, plant protection, pest control and epidemiology. As shown in Table 1, there were 14 symposia with invited speakers, 8 regular sessions with submitted papers, and 8 workshops for small groups of specialists. In total, there were 469 presentations/posters with accompanying abstracts, published in an abstract volume edited by Jan Bruin. The keynote address on Molecular Acarology was given by Dr Hans Klompen (Ohio State University, Columbus, OH, USA) and a special invited lecture on Tick Genomics was presented by Dr Catherine Hill (Purdue University, West Lafayette, IN, USA).

Several of the papers presented and some of the sessions held during the congress have been published elsewhere. Most notably are two special issues of the journal Experimental and Applied Acarology, resulting from sessions on the Control of Poultry Mites (volume 48, issues 1-2, 2009; editor: Dr. O.A.E. Sparagano) and on Forensic Acarology (volume 49, issues 1-2, 2009; editor: Dr. M.A. Perotti). In total, 114 papers were submitted for publication in the Congress Proceedings, 18 of which had to be rejected for various reasons. The remaining 96 contributions have all been reviewed and carefully edited. The editors are confident that the Proceedings of the 12th International Congress of Acarology reflect current trends in acarology as a field of science. We sincerely hope that the volume will promote communication throughout the scientific community and may stimulate research for many years to come.

Maurice W. Sabelis & Jan Bruin
December, 2009, Amsterdam, The Netherlands

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Table 1 Symposia with invited speakers, regular sessions with submitted papers, and workshops at the 12th International Congress of Acarology, August 2006, Amsterdam, The Netherlands.

Symposia	Regular Sessions	Workshops
Molecular Phylogeny	Taxonomy	Coconut Mites
Tick Physiology	Morphology	Tomato Mites
Evolutionary Ecology	Ecology	Water Mites
Chemical Ecology	Soil Acarology	Soil Mites
Canopy Acari	Biogeography	Disease Transmission
Mites on Arthropods	Veterinary Acarology	House Dust Mites
Mites on Vertebrates	Agricultural Acarology	Pesticide Resistance
Biological Control	Videographic Acarology	Forensic Acarology
Invasive Acari		
Recognition Systems		
Host Race Formation		
Diseases of Mites and Ticks		
Symbionts of Mites and Ticks		
Non-target Effects of Pesticides		

Plenary Opening Lecture

From sequence to phoresy – molecular biology in acarology

Hans Klompen
Ohio State University Acarology Collection, Museum of Biological Diversity, 1315 Kinnear Road, Columbus, OH 43212, USA.
E-mail: klompen.1@osu.edu

First of all, I would like to thank Maurice Sabelis and the organizing committee of the International Congress for the honor of being invited to present this address. When Maurice invited me to give this address he suggested I stick with the general theme of this congress, ecology and genomics. I had a brief moment of doubt, I do not actually work on either genomics or ecology, and so I decided to broaden the topic to the impact of molecular biology on acarology. More specifically, to use this occasion to emphasize and celebrate some areas where molecular biology has allowed us to make significant advances. Acarology is clearly following in the footsteps of other disciplines in rapidly integrating molecular data and methods in all aspects of research. Anybody doubting this should check the listing of presentations at this congress. In presenting these comments I should stress that in selecting developments to highlight, I have tried to cover a range, but I lay no claim to being comprehensive. These are my choices, reflecting my biases.

I will deal with three main areas, each with a different focus (and different tools). First, population or species level issues. This includes issues of species delimitations, dispersal patterns, and population structure – basic scientific issues which also have considerable relevance in agriculture and medicine. This area relates most directly to the bread and butter research that makes knowledge of mites so important. In terms of techniques this used to be an area for allozymes and RAPD's, techniques partially replaced by sequencing of rapidly evolving markers and analysis of microsatellites. Second, my personal interest: higher-order systematics. This area goes beyond straightforward analyses of relationships. Hypotheses in systematics are often (and correctly) considered indispensable for testing broad evolutionary hypotheses, and in setting the parameters within which any such hypotheses have to operate. Sequencing of slowly evolving markers, such as nuclear rRNA and nuclear protein coding genes, is the main tool, but there are additional options, such as mitochondrial gene order. Finally, in genomics the focus is on the structure of the genome and the function and regulation of the various genes. This will be the area where eventually we might approach the holy grail of complete vertical integration from DNA to phenotype.

POPULATIONS AND SPECIES

Dispersal

How well are mites really getting around? What are average distances for dispersal, and what factors may influence this? Eight years ago Evert Lindquist in a similar keynote speech for the Canberra congress listed these types of questions as a possible priority area for research. A range of molecular techniques is allowing us to get a better handle on these questions. Previously employed molecular methods, such as RAPD's and sequencing of relatively fast evolving loci can be used in this area, but they have clear limits. Analysis of microsatellites, often combined with sequencing or PCR-RFLP, appears to be more powerful. Microsatellites have been used to establish dispersal patterns and gene flow in a wide range of mite taxa, including the mesostigmatic mite *Varroa destructor* Anderson & Trueman (Solignac et al., 2003), the eriophyoid *Colomerus vitis* (Pagenstecher) (Carew et al., 2004), the spider mite *Tetranychus turkestani* Ugarov & Nikolski (Bailly et al., 2004), and the tick *Ixodes uriae* White (McCoy et al., 2003). One would hope that in the near future these techniques would be applied to an even wider range of taxa. For example, they might help provide insights in dispersal patterns of taxa such as Bdelloidea and Raphignathoidea, taxa of potential use in biocontrol, but whose dispersal abilities are quite poorly known. Moving into the soil ecosystem, these techniques may help elucidate dispersal patterns and population structure in oribatid mites.

But there is certainly no need to wait for future developments. Molecular techniques are already proving to be very powerful in current issues such as tracking invasive species. Recent work on *Aceria guerreronis* Keifer, a worldwide pest of coconuts (Navia et al., 2005), demonstrated a New World, probably Neotropical, origin of this mite. The direct importance of that research is that it allows a much more focused search for potential predators. Similarly tracking *V. destructor* invasions of North America and dispersal of *Raoiella indica* Hirst around the world may lead to improved management strategies. As an aside, it is interesting to note the current prominence of research on Tenuipalpidae. Often regarded as a minor player compared to the family Tetranychidae, the group has come into prominence in several areas in the last decade. *Brevipalpus phoenicis* (Geijskes) populations feature some very odd reproductive systems, including all-haploid populations (Weeks et al., 2001), *R. indica* has become yet another major pest of coconuts (http://www.doacs.state.fl.us/pi/enpp/ento/r.indica.html), and tenuipalpids in general have emerged as vectors of major plant diseases (e.g., citrus leprosis) (Childers et al., 2003). We clearly have not exhausted the potential for scientifically and/or economically important discoveries, even in relatively well-known groups of mites.

Development of host races and species limits

The literature is filled with claims and counterclaims of host specificity and host races. Testing such claims is often difficult and very laborious using standard methods. Molecular techniques allow quicker and often more accurate assessments of separation between 'host races' by measuring actual gene flow. Such approaches have shown cryptic species in the genus *Varroa* (Anderson & Trueman, 2000) and significant indicators of host race formation in the

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Sarcoptes scabiei complex (Walton et al., 2004) and in the tick *I. uriae* (McCoy et al., 2005). Meanwhile other molecular-based studies showed a lack of host specificity in *Psoroptes* (Ramey et al., 2000; Zahler et al., 2000; Pegler et al., 2005) and some *Tetranychus* species (Tsagkarakou et al., 1999; Bailly et al., 2004). A mtDNA-based study of genetic variability in *Myialges* spp. on the Galapagos Islands rejected the conventional wisdom that a single species, *Myialges caulotoon* Speiser, was associated with both Galapagos hawks and cormorants (Whiteman et al., 2006, 2007). In fact, the mites associated with the two bird hosts [and with members of two genera of louseflies (Hippobiscidae)] were genetically distinct. A re-examination of morphology showed small, but consistent, morphological differences between members of the two host races, reinforcing the molecular-based conclusions. This is a good example of how morphology and molecular data can be synergistic.

A broader issue is identification of species by molecular methods. In this context it is worthwhile to note the ongoing efforts in DNA barcoding. The idea here is to sequence a small piece of DNA that is species specific, thus providing the equivalent of a 'barcode'. To make this system work, some issues need to be worked out, including assessments of within species variability and the ability of the chosen sequence to distinguish closely related species. Neither issue can be assumed solved for mites until some experimentation has been conducted for a wide range of taxa. Such tests take on extra significance because of the dramatic range of evolutionary rates among mite taxa (Murrell et al., 2005; Klompen et al., 2007). Assuming these problems are solved, this approach can be very useful in specific situations. One example would be border inspections, where quick identifications are needed and experts are not always available. The practical problems in this type of situation should be limited, because such inspections often concentrate on a limited number of target species. Assuming those target species can be tested as needed, it should be possible to make molecular identification feasible soon. Eventually the same could be done for ecological studies of soils, etc., but that would require a much larger set of reference sequences. Of course it is not always necessary to identify specimens to species in ecological studies, but this merely shifts the problem to developing reference sequences for genera, families, or whatever grouping is needed. Some work is already being done in this area, and it is certainly worth exploring. Still I do not expect widespread application of these techniques in this field for some years. I stress that using 'molecular barcode' identifications is not the same as DNA taxonomy, the idea of defining new species based solely on (usually small) bits of DNA sequence. That 'shortcut' to the backlog of species descriptions has great potential for disrupting existing taxonomy, while the benefits are at best unclear.

Mite associates

Molecular techniques, combined with broader interest in the matter, have also expanded knowledge on associates of mites. We generally think of mites as small and being associates of other, larger organisms, but that view is incomplete. Mites themselves are also habitats for even smaller organisms. This includes well-known associations with tapeworms (Denegri, 1993), entomopathogenic nematodes (Samish et al., 2000), and fungi (Hofstetter et al., 2006), but investigations of associations with bacteria and protozoa have largely been limited to medically or veterinary important taxa vectored by ticks or chiggers. Only a few mite associates outside of that setting have been studied in detail. The most prominent example in that category is *Wolbachia*, which in some (but not all) hosts can cause sex ratio distortion and cytoplasmic incompatibility (e.g., Breeuwer & Jacobs, 1996; Vala et al., 2000, 2002, 2003; Gotoh et al., 2003, 2005).

However, it is clear that the number of bacterial or fungal associations of mites is much larger than that, and we are seeing a growing interest in a wider range of microorganisms, especially those that affect the mites themselves (see Samish & Rehácek, 1999; van der Geest et al., 2000). The use of a variety of molecular techniques (e.g., Jeyaprakash & Hoy, 2004), including PCR assays (Hoy & Jeyaprakash, 2005; Reeves et al., 2006), is allowing considerable progress in reliably detecting even very small numbers of microorganisms. This makes it feasible to quickly (and cost-effectively) search for such organisms in a much wider range of mite taxa, and to cover a wider range of microorganisms (not just pathogens). Many of the associations detected in this manner may well be accidental and have little biological significance, but others may provide some real insights. For example, reports of *Anaplasma* nr. *phagocytophilum*, agent of human granulocytic anaplasmosis, in various dermanyssoid Mesostigmata (Reeves et al., 2006) and in syringophilid quill mites (Skoracki et al., 2006) suggest a fairly wide distribution of this pathogen. The impact for epidemiology is not yet clear, but the Dermanyssoidea are potential vectors or reservoir hosts. As for the presence of *A. phagocytophilum* in permanent parasites such as Syringophilidae, the authors found that often the bird hosts did not register as positive, which brings up the possibility that infected mites may provide evidence of past infections (Skoracki et al., 2006). Another area of potential interest is the interaction of various microorganisms in the mite host, for example the potential for facilitation or competition. This general area of microorganisms associated with mites should see lots of exciting developments in coming years, some of which were already shown at a symposium on 'Symbionts of mites and ticks' during this congress.

HIGHER-ORDER SYSTEMATICS

This area has not been exceptionally well developed in the Acari because of the great difficulties in establishing homologies among members of distant lineages. It is simply quite difficult to find characters that can be scored across all Acari, or even across a single order (Acariformes or Parasitiformes). With respect to homology issues, a summary of ongoing efforts to homologize setal designations across all Acariformes is included in the third edition of 'A Manual of Acarology' (Krantz & Walter 2009). There are also some excellent morphology-based studies, such as those by OConnor (1984) and Norton (1998) for Sarcoptiformes, Haumann (1991) for early-derivative oribatid mites, and Lindquist (1986) for Heterostigmata, but equivalent analyses are lacking for most other acarine lineages. The analysis by Lindquist (1984) is still the only phylogenetic analysis of relationships across acarine orders, and even that analysis includes only a relatively small character set. As with the intra-specific issues noted above, molecular biology can make, and is making, a major contribution in this area. It does so mainly by allowing the generation of large new data sets. The ultimate goal will be combined analyses of mor-

phology and molecules, but such studies have so far been limited to relatively small lineages (e.g., Klompen et al., 2000; Dabert et al., 2001).

A critical consideration in deep phylogeny analyses of mites is the choice of marker. Cruickshank (2002) noted that nuclear rRNA's (both 18S and 28S) appear among the best candidates for deep phylogeny in the Acari, whereas nuclear protein coding genes might be most useful at an intermediate level, e.g., from family to subordinal levels. So far, most molecular-based studies looking at higher-order relationships in groups of Acari have indeed been based on nuclear rRNA, mostly 18S small subunit rRNA, but increasingly sections of, or whole, 28S, or on the nuclear protein-coding gene Elongation Factor-1 alpha (EF-1α) (Cruickshank & Thomas, 1999; Klompen, 2000; Lekveishvili & Klompen, 2004; Murrell et al., 2005; Schaefer et al., 2006; Klompen et al., 2007). Again, rate variation among lineages can be substantial, so relatively variable loci, such as the D3 variable region of 28S rRNA, may be relatively informative at higher levels in oribatid mites (Maraun et al., 2004), whereas the very conserved 18S is informative at the genus level in basal Mesostigmata (Lekveishvili & Klompen, 2004). As evidenced in this congress, multiple research groups are working in this area and it is likely that we will see substantial progress on higher-order systematics of Acari in the coming years. Additional molecular markers would be very welcome, and that area is also being addressed (e.g., Xu et al., 2003, 2004; Schaefer et al., 2006). Thus, we can expect not only more data for established markers, but also a considerable increase in diversity of markers over the next few years.

The ultimate goal for much of systematic research is to use acquired insights in relationships among groups to test evolutionary hypotheses on those groups. Such hypotheses cover diverse areas, from parthenogenesis to feeding modes, coloration, and host associations.

Parthenogenesis

Parthenogenesis is common among Acari, but particularly within basal lineages of oribatid mites ('Macropylina') (Norton & Palmer, 1991; Palmer & Norton, 1991). This despite the wide-held notion that all-female parthenogenesis (thelytoky) is an evolutionary dead-end. The long-term existence of thelytoky in bdelloid rotifers (Welch & Meselson, 2000) and darwinulid ostracods (Martens et al., 2003) has attracted a lot of attention, but thelytokous radiation in oribatid mites may be more common, and even more ancient (Heethoff et al., 2002). More astonishingly, we are seeing evidence of repeated reversal to sexuality, both within smaller oribatid lineages (this congress), and perhaps even the entire infraorder Astigmata (Norton, 1994, 1998).

Feeding modes

Most mites are fluid feeding, a feeding mode assumed to be ancestral in the Arachnida. There are exceptions, including Opilioacarida and a few genera of Mesostigmata (e.g., *Asternolaelaps*) among Parasitiformes, and the majority of Sarcoptiform Acariformes. But are they true exceptions, or is ingesting solid food the primitive condition for Acari? To determine this, we need more data on relationships in Chelicerata. Interestingly, molecular data so far have not been able to resolve order-level relationships in Chelicerata with any confidence (Giribet et al., 2001, 2002). Meanwhile, detailed studies of morphology continue to support a close association of the fluid-feeding Ricinulei with Acari, but perhaps as sistergroup to Parasitiformes (that is within Acari), rather than as sistergroup to Acari (Shultz, 2007).

Coloration in water mites

Did the spectacular colors of water mites evolve as UV protectant, aposomatic warning colors, or other? Many Trombidiformes are red in color due to an accumulation of carotenoids in the cuticle, quite possibly as a UV protectant. However it is unclear whether the function of coloration has changed in water mites relative to their terrestrial relatives. Feeding experiments show that fish often reject water mites as prey, suggesting an aposomatic function of color (Proctor & Garga, 2004). Unfortunately observations comparing ponds with fish vs. ponds without generally show more bright-colored water mites in the fish-less ponds. One way to study this is to plot coloration and habitat data on a phylogeny of water mites / Parasitengona. Efforts are underway to generate a molecular phylogeny for Parasitengona, and the first results of these efforts were presented by Heather Proctor et al., during this congress.

Host-parasite associations

Acari are clearly one of the best, if not the best, groups to study host associations. Host associations have evolved numerous times, they include a wide range of association types (from phoresy to permanent parasitism), and involve a stunning diversity of hosts. Although most studies of host association patterns involve slightly smaller lineages than discussed above, molecular-based phylogenies are improving our understanding of mite and host phylogenies, thereby allowing more sophisticated hypotheses of evolution of the associations. Examples of host-parasite systems examined in just this congress include Cheyletoidea (Bochkov), Dermanyssoidea (Dowling, OConnor), and Astigmata (Klimov, OConnor). In most of these cases results have not yet been published, but we can expect whole series of publications quite soon (e.g. Bochkov et al., 2008; Klimov & OConnor, 2008). And it is not only co-evolution scenarios that can be tested, there are additional questions such as the origin (and possible reversal) of parasitism and the notion that phoresy might be a facilitating condition for the evolution of parasitism (Athias-Binche & Morand, 1993; Houck & Cohen, 1995).

GENOMICS

How many coding regions are present in a given organism, what do these genes code for, how are they regulated, what is their distribution? These are just some of the questions asked in genomics research. This is a young field, and it may take some time before we can answer them for mites, but considerable progress is being made in some areas. Worth noting here is research on the sialome of ticks, the set of proteins in tick saliva involved in preventing blood clotting and in interfering with the host immune systems (Valenzuela, 2004). Other areas where similar but smaller-scale studies have been done include dust mite allergens, and Sarcoptiform alarm pheromones.

As for entire genomes, it is worth to briefly look at a relatively simple system, the mitochondrion. The mitochondrial genome of arthropods is relatively small (14-19.000 bp) and considered quite variable in nucleotide sequence, but

conserved in organization, that is in genes present and gene order (Boore, 1999). As with many areas, most early work in the Acari has been done for ticks (Campbell & Barker, 1998; Black & Roehrdanz, 1999). These studies found that the 'hypothetical ancestral pattern' for arthropods is retained in *Limulus*, derived *Ixodes*, and all Argasidae examined so far (Shao et al., 2004). However, although ticks are a morphologically conservative group, mitochondrial rearrangements have been demonstrated for several lineages of Ixodidae, including the addition of a second control region in some Australian *Ixodes* (Shao et al., 2005b), and re-arrangements of a considerable part of the genome in all Metastriata (Black & Roehrdanz, 1999). The only other Parasitiform for which the entire mitochondrial genome is known, *V. destructor*, again shows considerable changes (Evans & Lopez, 2002; Navajas et al., 2002), specifically a different position of several tRNA's and of the small subunit rRNA (12S rRNA). Recently the first Acariform mitochondrial genome was published, suggesting that observed deviations from the hypothetical ancestral pattern in Parasitiformes may be relatively minor. The genome of the chigger *Leptotrombidium pallidum* Nagayo et al. features several rearrangements of tRNA's, four control regions, plus an apparent doubling of a major section that includes a complete copy of the large subunit rRNA (16S), and a partial one for 12S rRNA (Shao et al., 2005a). Of course it is currently unclear whether this result is just an aberration, or whether it is representative of mitochondrial genome structure in Acariformes as a whole. Clearly we will need complete mitochondrial genomes for more Acariformes to evaluate that question.

The main area of excitement in mite genomics is certainly represented by projects focusing on the nuclear genome. Overall this type of research in Chelicerata is well behind similar efforts in insects, but acarology is doing quite well. We are already seeing the first results of the *Ixodes scapularis* Say genome project (http://www.entm.purdue.edu/igp/), and we can now add another, the *Tetranychus urticae* Koch project (approval announced during this congress). This means that complete genomes will soon be available for representatives of both Parasitiformes and Acariformes. Again, it will take some time before results of these projects will filter down to a wider range of projects, but it will happen, and it will be a major boost for our field, even if I do not dare speculate where the main impact will be.

CONCLUSION

In conclusion, molecular biology has a lot to offer for acarology, whether by itself or integrated with existing morphology- or ecology-based research. As a group, acarologists may complain about the decline in our numbers, the lack of funding, the scarceness of positions in the field, etc., all of which legitimate issues, but I am optimistic. The field attracted us because there are so many truly remarkable things left to be discovered, whether it is in systematics, physiology, ecology, or genetics. Molecular biology will be no different. What little we know so far has included a host of exiting and unanticipated results, and there is no reason to expect that we will not find many more. The ongoing genome projects will just add more possibilities to discover something amazing. So go out and dig.

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Phylogeny and Taxonomy of Acari

Systematic relationships of Lohmanniidae (Acari: Oribatida)

Roy A. Norton
S.U.N.Y. College of Environmental Science and Forestry, Syracuse, NY 13210, USA. E-mail: ranorton@esf.edu

Lohmanniidae is a moderately diverse family of macropyline oribatid mites that is usually grouped with taxa having opisthonotal glands, even though they themselves lack these glands. Morphological traits are examined for evidence that lohmanniids are instead members of Enarthronota, particularly the superfamily Hypochthonioidea. Six traits are consistent with membership in Enarthronota, eight support a relationship with Hypochthonioidea and its close outgroup Arborichthoniidae, seven support membership in Hypochthoniidae, and five others support a sister-group relationship with its subfamily Nothrolohmanniinae. Two alternative classifications are suggested to accommodate this relationship.

Key words: Oribatid mites, Enarthronota, Hypochthoniidae, Hypochthonioidea, *Malacoangelia, Nothrolohmannia*

Lohmanniidae is a moderate-size family comprising 21 nominal genera and 179 species (Subias, 2004), most with tropical or subtropical distributions (Hammer & Wallwork, 1979; Balogh & Balogh, 1992). Its present composition was attained gradually, as several fundamentally different mites (now in Epilohmanniidae, Eulohmanniidae, Perlohmanniidae, and the enarthronote *Malacoangelia*) were removed from Berlese's (1916) original tribe Lohmanniini.

Adults have a characteristic facies (Fig. 1A-C): elliptical to ovate shape in dorsoventral view, convex dorsum, flat venter, and pedofossae that receive retracted legs when an individual is disturbed. A large anterior notogastral tectum, unique among dichoid mites, covers the sejugal articulation dorsally. Their biology also seems rather uniform: they consume decomposing leaves and often woody substrates, within which they feed as endophages (Shereef, 1976; Haq, 1984; Ramani & Haq, 1991). Males are unknown (Grandjean, 1950) and parthenogenesis has been proven in the laboratory (Shereef, 1976). They are one of several families of early to middle derivative oribatid mites that show modest evolutionary radiation in the absence of sexual reproduction (Norton & Palmer, 1991; Maraun et al., 2004).

In a foundational paper, Grandjean (1950) considered Lohmanniidae an isolated family that exhibits interesting contrasts. It is specialized, yet rich in primitive characters and despite its homogeneous facies some traits show high variation. The latter include a wide range of dorsal setations (holotrichy to extreme neotrichy) and many combinations of shapes, subdivisions and fusions in plates of the anogenital region that, as Grandjean predicted, underlie the current multitude of recognized genera. He first expressed this isolation by listing Lohmanniidae as one of 11 distinct groups of oribatid mites (Grandjean, 1954a), then merged it with several others to form Mixonomata, one of six major groups in a later classification (Grandjean, 1969) that continues to be used (e.g., Subias, 2004). Mixonomata is probably a paraphyletic group (Norton, 1998) that ancestrally has opisthonotal glands (see below). Haumann (1991) had a similar view: his cladogram included Lohmanniidae between Eulohmanniidae and Perlohmanniidae (both mixonomatans) in a pectinate part of Novoribatida – the sister-group of Enarthronota in his study.

Lee (1984, 1985) first closely linked Lohmanniidae with taxa usually included in Enarthronota, although his unique terminology partly masked this insight (see Norton, 2001). He thought ancestral transverse scissures were lost from Lohmanniidae (essentially his cohort Affisurina), but thought its sister-group included all enarthronotes but Protoplophoroidea. Instead, Norton (2001) and Alberti et al. (2001) suggested that Lohmanniidae represents a derived family of Hypochthonioidea. This enarthronote superfamily exhibits much evolutionary plasticity, with both ptychoid (Mesoplophoridae) and holonotic (*Nothrolohmannia*) clades. Woas (2002) subsequently included Lohmanniidae within Hypochthonioidea, a classification followed by Weigmann (2006). However, the original suggestion was based on few traits, and no overview of morphological support exists. A preliminary DNA analysis is consistent with this hypothesis (Maraun et al., 2004), but lacks taxa important for testing it.

My objective is to examine how traits of Lohmanniidae fit the phylogeny proposed earlier for Hypochthonioidea (Norton, 1984, 2001). Lohmanniidae is included as the sister group of Nothrolohmanniinae (Fig. 2), where the weight of evidence seems to place it, and various branches are numbered for discussion. This is a preliminary study, because few new characters are considered and some alternative relationships are not yet examined. Still, traits of many taxa have been examined, particularly those of mixonomatan families, and no more plausible relationship was uncovered.

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MATERIALS AND METHODS

The overall approach is to climb the tree from bottom to top, testing Lohmanniidae against characters that support relevant clades, as linked by numbers to Figure 2. Except for the first section (below) the apomorphic state is given, followed by the plesiomorphic state (pl) in parentheses. Principal sources are Grandjean (1950) for Lohmanniidae, Grandjean (1935) for *Malacoangelia*, Norton (2003) for *Nothrolohmannia*, and Fernandez (1984) for *Eohypochthonius*; the latter also summarizes traits of *Hypochthonius* and *Eniochthonius*. Traits of Endeostigmata and Palaeosomata are from Grandjean (1939, 1954b, respectively). Other sources are indicated where relevant. *Arborichthonius styosetosus*, in the monotypic Arborichthoniidae (Norton, 1982), was chosen as the outgroup of Hypochthonioidea, for reasons noted below. New developmental data for *Malacoangelia*

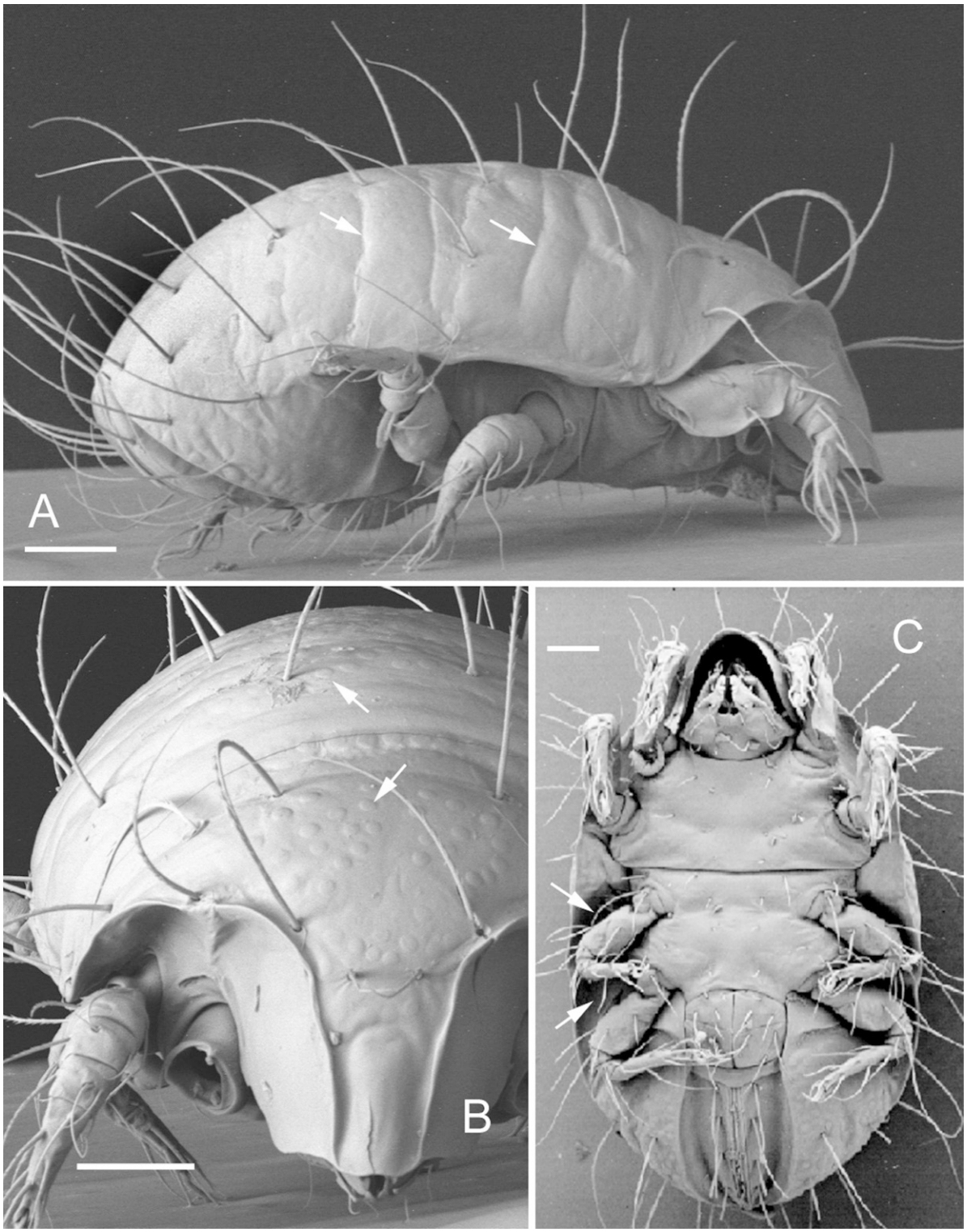

Figure 1 *Meristacarus* sp. A. Lateral aspect (arrows on mineralized bands). B. Anterior aspect (arrows on porose areas). C. Ventral aspect (arrows on pedofossae). Scales: 100 µm. (Australian specimen, images by Sue Lindsay).

remigera came from specimens and exuviae collected in Quintana Roo, Mexico, by the author, and protonymphal data for *Nothrolohmannia calcarata* came from a topotypic specimen donated by the late János Balogh.

Are Lohmanniidae members of Enarthronota?

As Lohmanniidae are holonotic (have a one-piece notogaster), the most diagnostic trait of Enarthronota – one or more transverse notogastral scissures (Grandjean, 1947) – is absent, but most other traits are consistent with this placement. Except scissures, and the first two characters below, there are no recognized synapomorphies of Enarthronota. Other listed traits are plesiomorphies that help exclude Lohmanniidae from derived oribatid mite groups with apomorphic states.

Subcapitular anarthry. The subcapitulum of known Enarthronota is anarthric, i.e., it lacks a labiogenal articulation (Grandjean, 1957). By contrast, species in more derived macropyline groups (Parhyposomata, Mixonomata, Desmonomata) are stenarthric, i.e., they have an oblique articulation between mentum and genae. Lohmanniidae are all anarthric. Some have a pair of oblique lines on the subcapitular venter, but these are ridges or changes in cuticular structure, not articulations. Weigmann (1996) considered anarthry a synapomorphy of Enarthronota.

Immature instars with moderately sclerotized hysterosomal cuticle. This is a common trait of Enarthronota (Grandjean, 1969) that is most easily seen in the rather rigid exuviae (see below). Except for some Brachypylina, glandulate taxa have immatures with unsclerotized, weak hysterosomal cuticle that easily crumples during molting.

Absence of opisthonotal gland. Of the six major groups recognized by Grandjean (1969), members of Palaeosomata and Enarthronota lack opisthonotal glands, like Lohmanniidae. Nearly all Parhyposomata, Mixonomata, Desmonomata, and Brachypylina (Circumdehiscentiae) have them, and are referred to below as 'glandulate' taxa (Norton, 1998).

Plesiomorphic rutellum. The rutellum of Lohmanniidae clearly shows its setal origin (Grandjean, 1950, 1957). Although it is broader distally than those of Enarthronota, its narrow base does not overlap the lateral lips and is not incorporated with the gena in the manner of glandulate taxa.

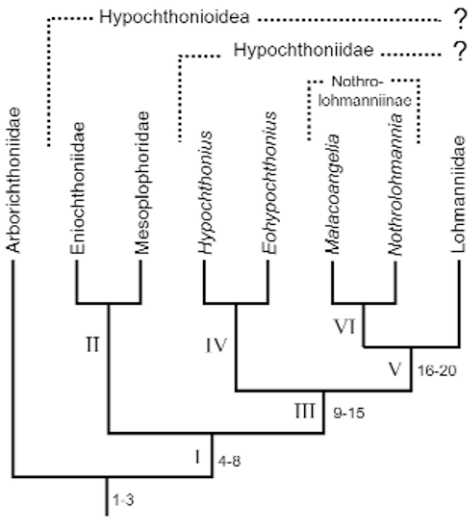

Figure 2 Hypothesis of cladistic relationships in Hypochthonioidea and Lohmanniidae, with Arborichthoniidae as outgroup. Roman numerals indicate clades referred to in text; arabic numerals indicate apomorphic traits for the clade as discussed in text. See Norton (2001) for support of clades II, IV, and VI. See text for discussion of indicated classification.

Absence of lyrifissures iad and ian. Grandjean (1950) considered their absence in Lohmanniidae a regression. However, all Enarthronota and Palaeosomata lack these lyrifissures, as do Endeostigmata. They are present in glandulate taxa, except most Brachypylina lack *ian*.

Ten pairs of genital setae. The plesiomorphic number of genital setae in oribatid mites seems to be 10 pairs. This is the common number in Palaeosomata and Enarthronota, and lesser setations seem attributable to losses (Grandjean, 1949, 1961b). Lohmanniidae have 10 pairs, but no glandulate group has more than nine.

Arborichthoniidae as an outgroup of Hypochthonioidea

Arborichthoniidae shares three apomorphies with Hypochthonioidea and Lohmanniidae that are not known in other Enarthronota.

1. *Adoral seta or$_2$ medially with deep notch and tooth* (pl = without notch). In Lohmanniidae, this apomorphy is present only in some genera (e.g., *Meristacarus, Torpacarus*). Hypochthoniioidea have additional cilia distal to the tooth that are not known in Lohmanniidae.

2. *Subcapitular genae with paired dorsal rasps* (pl = rasp absent). A patch of rasp-like teeth arranged in transverse rows lies on the dorsal face of each gena, posterolateral to the rutellum and close to the mouth opening (Fig. 3A). It is not known from other oribatid mite taxa.

3. *Lyrifissure im on notaspis* (pl = *im* on pleuraspis or soft lateral cuticle). In Hypochthonioidea, *im* is anterior to setal row *e*, but is behind it in Arborichthoniidae. In Lohmanniidae *im* is above the suprapleural scissure, essentially aligned with row *e*.

Are Lohmanniidae members of Hypochthonioidea (clade I)?

Several synapomorphies proposed earlier (Norton, 1984, 2001) to distinguish Hypochthonioidea from other Enarthronota were based on the assumption that ancestors had the plesiomorphic enarthronote body architecture in which setal rows *e* and *f* are enlarged, erectile, and inserted on paired intercalary sclerites in two respective transverse notogastral scissures (type-S scissures of Grandjean, 1947). Arborichthoniidae has an autapomorphic arrangement in which setae *f* are erectile, but insert in a pair of soft cuticular patches rather than a single scissure.

4. Loss of erectile function in setal rows e and f; i.e., full or partial incorporation of intercalary sclerites bearing setal row e and f into notogaster and size regression of setae (pl = e and f setae enlarged, erectile, on independent sclerites). Lohmanniidae have the apomorphic state but, unlike Nothrolohmannia, they have no vestige of ancestral scissures, so the 'loss' is equivocal.

5. *Sternal apodeme present* (pl = sternal apodeme absent). A distinct linear apodeme runs along the midline of fused epimera III and IV; it may be long, short, or in two parts. (lost in the ptychoid Mesoplophoridae). In Lohmanniidae it is relatively short and approximately centered in the plate.

6. *Aggenital plates fused with epimere IV* (pl = aggenital plates articulated with epimere IV). In Hypochthonioidea aggenital plates form what appear to be triangular extensions of the coxisternum that frame the genital plates anteriorly. Some Lohmanniidae have this state (e.g., *Torpacarus gramineus* and *Heptacarus graminosus*), but in most species the plates articulate with epimere IV.

7. *Trochanter II glabrous* (pl = trochanter II with one seta). Trochanters I and II have one seta each in Arborichthoniidae and in most glandulate taxa. But trochanter I is glabrous in the majority of Enarthronota, perhaps in all Palaeosomata, and in many Endeostigmata. In contrast, trochanter II has one seta in most Endeostigmata, all Palaeosomata, and most Enarthronota. Hypochthonioidea is unusual, with trochanter II also glabrous: the typical setal formula is 0-0-2-2 (I to IV). Trochanter II is also glabrous in the distant enarthronote lineage Brachychthoniidae, and in a paedomorphic clade of Protoplophoroidea (Norton et al., 1983), but only Lohmanniidae share the 0-0-2-2 formula.

8. *Proximal part of chelicera inserted into body* (pl = chelicera not inserted). In Endeostigmata, Palaeosomata, Parhyposomata, Mixonomata, and most Enarthronota the entire chelicera projects from the body wall like other appendages. In Desmonomata and Brachypylina (Norton, 1998), and independently in Hypochthonioidea, the wall attachment encroaches such that part of the chelicera projects internally. The internal part comprises only about 5-6% in *Eniochthonius*, but 20-30% in other hypochthonioid genera, and in Lohmanniidae (Fig. 3B).

Are Lohmanniidae members of Hypochthoniidae (clade III)?

Apomorphies 9-15 characterize Hypochthoniidae, which currently includes *Hypochthonius, Eohypochthonius, Malacoangelia*, and *Nothrolohmannia.* In 2001 the loss of lyrifissure *ip* was listed, but this character appears to be incorrect and is deleted here. The lyrifissure exists along with the other four typical lyrifissures at least in *Hypochthonius* and *Eniochthonius* (Fujikawa, 2003 and RA Norton, unpubl. observations) and dense spicules make finding lyrifissures in Nothrolohmanniinae difficult. Members of Lohmanniidae retain all five lyrifissures

9. Epicuticular chambers form as indentations over pore canals and contain nonbirefringent calcium-potassium mineral, probably apatite (pl = chambers form as caverns within epicuticle and contain birefringent calcium oxalate).

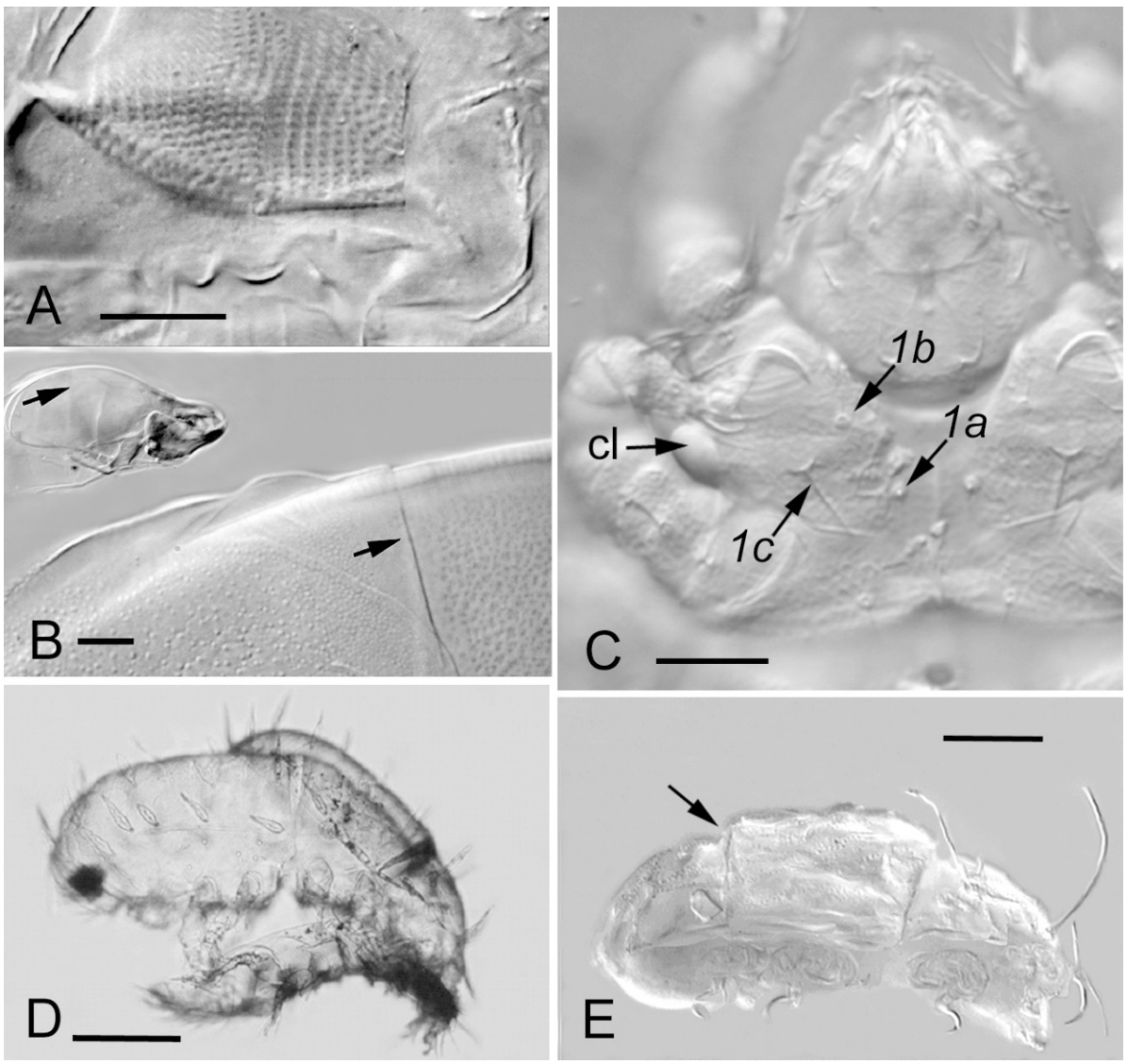

Figure 3 A. *Lohmannia carolinensis*, rasp on gena of subcapitulum. B. *L. carolinensis*, chelicera (insert) and enlargement of body wall attachment (arrows). C. *Malacoangelia remigera* larva, ventral proterosoma. D. *Lohmannia banksi*, molting deutonymph. E. *Nothrolohmannia calcarata,* protonymph (layered image: arrow on transverse scissure). Abbreviations: cl, Claparède's organ (stalk out of focus); 1a, 1b, 1c, epimere I setae. Scales: 10 µm (A-C), and 50 µm (D, E).

Table 1 Setation of adult legs (I to IV) of Hypochthonioidea (Mesoplophoridae excluded), Arborichthoniidae and representative Lohmanniidae.[a]

	Trochanter	Femur[b]	Genu	Tibia	Tarsus[c]
Arborichthoniidae					
Arborichthonius styosetosus Norton[d]	1-1-2-2	5-6-3-3	5-5-4-4	5-4-4-4	18-16-13-13
Eniochthoniidae					
Eniochthonius minutissimus (Berl.)[e]	0-0-2-2	3-5-3-3	5-3-3-3	5-4-3-3	18-16-13-13
Hypochthoniidae					
Hypochthonius rufulus C. Koch	0-0-2-2	5-5-3-3	3-3-2-2	5-5-3-3	19-15-13-13
Eohypochthonius spp.	0-0-2-2	5-5-3-3	3-3-2-2	5-5-3-3	19-15-13-13
Malacoangelia remigera Berlese	0-0-2-2	5-5-3-3	3-3-2-2	5-5-3-3	19-13-11-12
Nothrolohmannia baloghi Norton	0-0-2-2	5-5-3-3	3-3-2-2	5-5-3-3	19-13-11-12
Lohmanniidae					
Lohmannia lanceolata Grandjean	0-0-2-2	5-**6**-3-3	3-3-2-2	5-5-3-2	17-13-12-12
Torpacarus omittens Grandjean	0-0-2-2	4-5-**4**-3	3-3-2-2	4-4-2-2	15-13-11-10
Annectacarus mucronatus Grandjean	0-0-2-2	5-**6**-**4**-3	3-3-2-2	5-5-2-2	17-13-10-10
Cryptacarus promecus Grandjean	0-0-2-2	5-**6**-3-3	3-3-2-2	5-5-1-1	17-11-9-9

[a]Data from Grandjean (1950), Fernandez (1984), Norton (1982, 2003), and new observations. [b]Counts in boldface exceed those of Hypochthonioidea. [c]Famulus included in count. [d]Ancestral seta *m"* (monotrope) is absent (regressed) from tarsus I of *A. styosetosus* but retained in other listed taxa; *bv"* was inadvertently omitted from Figure 7 of Norton (1982). [e]Seta *it"* is absent (regressed) from tarsus I, present in Arborichthoniidae and Hypochthoniidae.

Lohmanniidae have chambers of the derived type (Alberti et al., 2001) that form the transverse bands previously thought to indicate primitive segmentation (Grandjean, 1950; Wallwork, 1963). The suggested plesiomorphic state is found in clade II, but not in Arborichthoniidae. This is problematic, since similar chambers probably also occur in some Protoplophoroidea: *Phyllozetes* (Cosmochthoniidae) has large chambers with birefringent contents, but the mineral is unidentified; *Prototritia* (Protoplophoridae) has calcium oxalate (Norton & Behan-Pelletier, 1991), but its epicuticle has not been studied. Mineralized epicuticular chambers are not known from other superfamilies.

10. *Aggenital setae absent* (pl = at least one pair present). Clade II ancestrally has aggenital setae, as does Arborichthoniidae, but all Hypochthoniidae lack them. Lohmanniidae also lack aggenital setae, but so do various families or genera throughout oribatid mites (Grandjean, 1949), so the character is homoplasious.

11. *Ontogeny of genital setae accelerated; deutonymph with five pairs of setae* (pl = deutonymph with four pairs). All adult Hypochthoniidae have the ancestral complement of 10 pairs, but the specific ontogeny of this setation is shared only by Lohmanniidae: 1-5-8-10 (protonymph to adult). All oribatid mites have a single protonymphal seta, and 10 adult pairs is common, but the unusual deutonymphal (5) and tritonymphal (8) setations presented problems for Grandjean's (1949, 1961b) interpretation of evolution in genital setation. Since deutonymphs of other enarthronotes and Palaeosomata have a maximum of four pairs, the fifth pair probably results from accelerated development. Having eight tritonymphal setae may be correlated with the deutonymphal acceleration, but not necessarily. *Palaeacarus* and the enarthronote *Gozmanyina* (Marshall & Reeves, 1970) have eight pairs in the tritonymph, but no other known oribatid mite has more than seven.

12. *Tarsus I famulus simple* (pl = famulus with lateral bract-like branch). Like Hypochthoniidae, Lohmanniidae has a simple famulus, but it has become short and peg-like. Simplification seems highly homoplasious (Haumann, 1991) and of little value.

13. *Iteral setae lost from tarsi II-IV* (pl = iteral setae retained on at least some of tarsi II-IV). Grandjean (1961a, 1964a) reported many patterns for iteral setae on leg tarsi, and among the rarest is to have a pair on adult tarsus I, but none on tarsi II-IV. All four hypochthoniid genera have this pattern. The single pair first forms in the protonymph; under Grandjean's model, they have strongly 'resisted' regression, whereas those of other tarsi were gradually delayed to the point of loss. Studied Lohmanniidae either lack iterals altogether or only *it'* forms on tarsus I. Total loss of iterals is convergent in many lineages; however, when *it'* is present in Lohmanniidae it forms unusually early, in the deutonymph, which suggests that past resistance to regression was greater on tarsus I than on II-IV. In this sense, the iteral ontogeny is considered a derivative of the hypochthoniid type.

14. *Ventral setae absent from all leg genua* (pl = at least some ventral setae present). In contrast to the ancestral state in clade II, Hypochthoniidae have only fundamental genual setae (larval on I-III, deutonymphal on IV; Grandjean, 1942). The resulting formula (I-IV) is 3-3-2-2 in all known species (*d, l'* and *l'* on genua I and II; *d* and *l'* on III and IV). Ventral setae have been lost in parallel in Mesoplophoridae and several other groups of Enarthronota, but with formulas other than 3-3-2-2. Lohmanniidae share the rare genual setation of Hypochthoniidae (Table 1), which otherwise is known only for some Brachychthoniidae.

15. *Palp tarsus with distal setiform organ trifid* (pl = distal setiform organ bifid). All hypochthonioid mites have the ultimal pair of palp setae fused basally. This is common in Enarthronota, and found in some species of Palaeosomata, Parhyposomata, and Brachypylina. Clade II retains this form, but in Hypochthoniidae the unpaired subultimal seta joins the ultimals to form a trifid structure, and this is true of Lohmanniidae. Since the trifid state is also found in both close (Arborichthoniidae) and distant (*Gozmanyina, Nipponiella*) enarthronote outgroups, the character is homoplasious.

Are Lohmanniidae and Nothrolohmanniinae sister-groups (clade V)?

Of the many apomorphies proposed earlier for Nothrolohmanniinae (Norton, 2001, 2003), the following five (16-20) are shared by Lohmanniidae. *Papillacarus* possibly has another one, spicules developed from epicuticular chambers, but the ultrastructure of its spicules is unknown and they are distributed in areas *other* than the mineral-containing transverse bands.

16. *With pedofossae for accommodation of folded legs* (pl = pedofossae absent). Many Brachypylina have defensive reactions in which legs are folded into concave niches in the body wall. In macropyline taxa this behavior and the associated niches, or pedofossae, are known only from clade VI and Lohmanniidae.

17. *Seta p" absent from tarsus IV* (pl = *p"* present). Proral setae are rarely lost from tarsus IV in oribatid mites, but only in clade VI and Lohmanniidae is one lost unilaterally; *p"* is absent from all studied species. This loss is most obvious on the highly regressed protonymphal leg IV, where the normal count of seven tarsal setae is reduced to six. All studied Lohmanniidae have the rare protonymphal leg IV setation of 0-0-0-0-6 (Grandjean, 1946a, 1950), and the same is true of *Malacoangelia* and *Nothrolohmannia*.

18. *Coxisternal seta 1c setiform in larva, independent of Claparède's organ* (pl = seta *1c* scaliform, covers retracted Claparède's organ). Ancestrally in acariform mites coxisternal seta *1c* is modified to form a protective cap over Claparède's organ when the latter is retracted (Grandjean, 1933, 1939, 1954b). Concomitant with the disappearance of that organ in the protonymph, *1c* transforms to a normal seta. Rarely *1c* is setiform in the larva of oribatid mites: previously known examples were Epilohmanniidae and Lohmanniidae (Grandjean, 1946b, 1950), but *Malacoangelia* shares the trait (Fig. 3C; the larva of *Nothrolohmannia* is unknown). Grandjean (1955) considered the setiform larval state primitive and strongly believed that reversion from scaliform to setiform was impossible, but since *1c* is consistently scaliform in basal acariform groups (Endeostigmata and Palaeosomata) it must be the plesiomorphic larval form within Enarthronota. The genetic-epigenetic mechanism producing the scale form is probably disabled in these rare cases, and I interpret setiform *1c* as an acceleration of the protonymphal transformation, rather than a reversion. The acceleration in *Epilohmannia* is convergent.

19. *Anal plate regressed, strap-like* (pl = anal plate well formed, independent of adanal plate). In clade V adanal plates comprise most of the adult paraproctal valves, with the anal plate reduced to a narrow band at their medial edge. *Malacoangelia* has complete paraproctal atrichosy (At3 of Grandjean, 1954a), and the anal plate is not delineated from the adanal until the adult. Lohmanniidae have no such setal delays, but anal setae can lack altogether. A convergent internal lineage of *Eohypochthonius* also has the apomorphy (Fernandez, 1984).

20. *Porose organs present on notogaster* (pl = porose organs absent). Discrete porose organs are rare on the notogaster of macropyline mites, but they occur in clade VI and in some Lohmanniidae. Their ultrastructure and distribution differ between the two groups (Alberti et al., 1997, 2001), so this apomorphy is equivocal.

Characters states incongruent with Figure 2

If Figure 2 is correct, four traits listed above (1, 6, 13, and 20) are variable within Lohmanniidae and represent autapomorphies or homoplasies. Eight other problematic trait distributions (a-h) are not known to be variable: (a), (b), and (g) seem to be autapomorphic, the others are homoplasious.

a. *Molting*. In studied Hypochthonioidea molting is prodehiscent (Norton & Kethley, 1994): exuviae of immatures split anteriorly above the appendages in a U-shape and the animal emerges forward (new observations show *M. remigera* is also prodehiscent). The cuticle of immature Lohmanniidae splits posteriorly in a U-shape, and the animal emerges backward (Fig. 3D), as in Brachypylina. A transition from prodehiscence is difficult to explain.

b. *Rutellum expanded distally into hyaline, thin, apparently flexible lobe* (pl = rutellum without distal expansion). The distal projection of Lohmanniidae is thumb-like, not a hyaline lobe.

c. *Notogastral fusion.* If Figure 2 is correct, the holonotic state of *Nothrolohmannia* and Lohmanniidae would be convergent, or the scissure in *Malacoangelia* would represent a reversal. Based on a single known protonymph, immatures of *Nothrolohmannia* have a functional transverse scissure (Fig. 3E) that bears small setae of row *e*, so the fusion occurs only in the adult instar. Immatures of Lohmanniidae show no evidence of a scissure.

d. *Proterosomal structure*. In *Malacoangelia*, *Nothrolohmannia*, and *Eohypochthonius* the prodorsal aspis is isolated from fused epimera I-II by articulating soft cuticle (a plesiomorphy). *Hypochthonius* and Lohmanniidae share the derived fusion of aspis and epimera I-II into a single proterosomal unit.

e. *Setation of femora.* The femoral chaetome of Lohmanniidae (Table 1) seems too rich to be consistent with the hypothesis in Fig. 2. Hypochthoniidae all have a setation (I to IV) of 5-5-3-3. But some lohmanniids have six setae on femur II, greater than any member of Hypochthonioidea, and some have four setae on femur III, greater than any known member of Enarthronota. If the hypothesis is correct, and if the rich femoral setations of Palaeosomata are ancestral in oribatid mites, then setae previously lost by regression have reappeared in Lohmanniidae. In contrast, the tibial and tarsal setations of Lohmanniidae are never richer that those of Hypochthoniidae, and all could have been derived by simple regressive losses.

f. *Solenidial complements* (Grandjean, 1964b). Lohmanniidae have solenidial formulae for tibiae and tarsi that are similar to those of Hypochthoniidae: 1-1-1-0 and 2-[1,2]-0-0, respectively [brackets indicate interspecific variation]. Lohmanniidae have a second genu I solenidion (2-1-1-1) that is absent from Hypochthoniidae (1-1-1-1), but not from Eniochthoniidae or Arborichthoniidae (each with 2-1-1-1). Figure 2 requires independent losses of the second solenidion in clades IV and VI, or its reappearance in Lohmanniidae.

g. *Coupling of solenidia with seta d on genua*. Overall, the distribution of seta/solenidion coupling within Enarthronota is complex, but it is rather uniform within Hypochthonioidea. Hypochthoniidae, Eniochthoniidae, and early derivative Mesoplophoridae (*Archoplophora*) have seta *d* independent of solenidia on all genua. Arborichthoniidae shares the pattern on genua I-III, but coupling occurs on genu IV. In Lohmanniidae *d* couples to a solenidion on all genua.

h. *Pretarsal claw structure*. *Malacoangelia*, *Nothrolohmannia*, and *Eohypochthonius* have a tooth along the dorsal marginal of the claw, but none on the ventral edge. *Hypochthonius* has a proximoventral claw, but none on the dorsal margin. Claws in Lohmanniidae are often smooth, but if a tooth is present it is proximoventral.

Conclusions

The weight of evidence suggests that Figure 2 is correct, and that Lohmanniidae should be included in Hypochthonioidea. Particularly convincing are progressive apomorphies 2, 3, 5, 9, 11, 16, and 18, and rare regressive apomorphies 14 and 17. Incongruencies exist, yet none is exceptional, and similar levels and types of homoplasy are found in other mite taxa.

Feeding biology is perhaps the most striking incongruence with Figure 2. Enarthronotes are primarily fungivores or scavenger/necrophages (Schneider et al., 2005). Loh-

manniidae are unique among them in being saprophages of higher plant structural material (see above). Their strong, broad rutella and robust chelicerae are quite similar to those of more derived groups with the same feeding biology (e.g., Mixonomata, Desmonomata), but the primitive rutellar base and anarthric subcapitulum reveal an enarthronote origin.

If Figure 2 is correct, a classification problem arises of a type discussed previously (Norton, 2001). Cladistically, Lohmanniidae form a clade within Hypochthoniidae and would fit well at the subfamily rank (Lohmanniinae) in a sequenced classification. But its divergent morphology and species diversity argues for retaining family rank, restricting Hypochthoniidae to the genera *Hypochthonius* and *Eohypochthonius*, and once again recognizing Nothrolohmanniidae for the sister-genera *Malacoangelia* and *Nothrolohmannia*. No classification change is recommended at this time, pending the results of an ongoing molecular study (with K. Domes, M. Maraun and S. Scheu)

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Anomalies of notogastral structures in poronotic oribatid mites (Oribatida: Poronota) interpreted as cryptic ancestral characters modulated by regulatory genes

Gerd Weigmann
Institute of Biology, Zoology, Freie Universität Berlin, Koenigin-Luise-Str. 1-3, 14195 Berlin, Germany.
E-mail: weigmann@zedat.fu-berlin.de

Occasionally, specimens of oribatid mites have abnormal asymmetric characters, e.g., regarding notogastral setation or the porose areas of the octotaxic system, similar to those in other more or less related taxa, or similar to those in ancestors. Exemplarily, anomalies in specimens of Scheloribatidae and Phenopelopidae are presented. A model is proposed involving chains of regulatory genes that explains evolutionary changes within branches of poronotic Oribatida as well as the notogastral anomalies discussed. This model may contribute towards a revised strategy for taxonomy and phylogenetical systematics.

Key words: Phylogenetic systematics, taxonomy, regulatory genes, Scheloribatidae, Phenopelopidae

Occasionally, specimens of oribatid mites have asymmetric characters, e.g. regarding notogastral setation or the porose areas of the octotaxic system. Often, the character expression of the aberrant side is strongly suggestive of the normal character expression in other more or less related taxa. Are such anomalies negligible mutations or developmental defects? Can we interprete them as an atavistic reminiscence of ancestors or should we maintain the view that they are reversal mutations? Grandjean (1948a,b, 1952) discussed anomalies ('écarts') within clones of *Platynothrus peltifer*, mostly concerning losses of setae, whether asymmetric or not, as well as their frequency of occurrence in populations. He concluded that these types of anomalies are not small mutations (Grandjean, 1948b, p.882: '…les écarts ne sont pas de petite mutations'), but phenotypical expressions in the context of evolution in the number of organs.

Some simple notogastral characters, as loss of centrodorsal setae (*da*, *dm*, *dp*), loss of setae c_1 or c_3, or realization of the octotaxic system either as areae porosae or as sacculi, seem to be widely distributed within the families of poronotic oribatid mites. The mosaic-like distribution of these characters in the systematic branches makes a cladistic analysis nearly impossible without assuming several convergent disappearances; patterns of above mentioned, presumably 'homologous' character expressions contradict other characters which are assumed to be of systematic relevance.

In the following, I discuss some examples of notogastral anomalies in the light of phylogeny, ontogeny and I introduce a model of regulatory gene complexes influencing morphogenesis and gene expression in the instars of oribatid mites to explain these anomalies. The results may help to critically revise taxonomy and systematics of oribatid mites, based on knowledge of modern molecular genetic processes underlying phenotypical character expression. A similar approach regarding enzymatic patterns in *Platynothrus peltifer* has been proposed earlier as a 'new concept of evolution' by Ziegler & Wauthy (1987).

Abnormal patterns of notogastral setation and areae porosae in Phenopelopidae

Eupelops acromios

Within the genus *Eupelops* there are two well-known groups of species with different notogastral setation pattern: (A) the setae h_3 are very close to the areae porosae *A1* and the associated setae *lp*; (B) the setae h_3 are in normal lateral position and distant from setae *lp*, which are associated with areae porosae *A1*.

Eupelops acromios (Hermann) belongs to the species group B; its diagnostic characters are: short club-like sensillus, notogastral setae of median length and distally broadened, setae h_3 in normal lateral position. It is the most common arboreal bark-dwelling *Eupelops* species in Europe. Surprisingly, I recognized three single specimens with asymmetric setation pattern, two of them within populations of normal specimens and one specimen in a microscopical slide in the Willmann-Collection. The abnormality in all three specimens is the setation type A on the right side (with juxtaposed setae h_3 and *lp*; Fig. 1a) and the 'normal' setation type B on the left side. Using a determination key (Willmann, 1931; Weigmann, 2006) we must decide to follow either the right or the left side pattern. The left side morphology leads without any doubt to *E. acromios*.

Willmann (1931) ignored the asymmetry of his specimen, he regarded the right side as relevant and described his single specimen as '*Pelops geminus* Berlese'. Examining the mounted specimen (collected by C. Willmann on 25 July 1919 from Stoteler Forest, near Bremen, Germany), I

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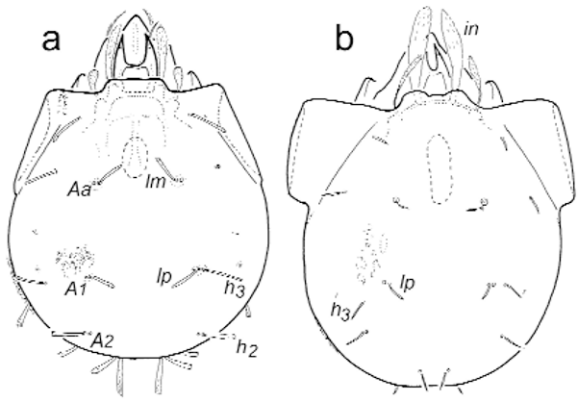

Figure 1 Abnormal *Eupelops* specimens. (a) *E. acromios*: left side with normal notogastral setation pattern, right side abnormal. (b) *E. occultus*: left side abnormal. Abbreviations: *in*, interlamellar seta; *Aa*, *A1*, *A2*, Areae porosae; *lm*, *lp*, h_2, h_3,notogastral setae.

assumed the specimen as probably belonging to *Eupelops claviger* (Berlese) (see Weigmann, 2006, p. 344). But now, compared with the other two abnormal *E. acromios*, the Willmann-specimen is most probably conspecific.

The second specimen with this asymmetry (drawing in Fig. 1a) has been collected by Stephanie Sobek in 2001 from a canopy branch of an oak tree, near Basel in Switzerland (Weigmann et al., 2004), together with several normal and symmetric specimens of *E. acromios*. The third specimen has been found in a dune area on the Isle of Sylt, Northern Germany (in 2005; G Weigmann, unpubl.), together with some normal *E. acromios*. These three independent findings from three distinct populations give rise to the assumption of a common cause for the misdevelopment, possibly a unilateral defect in the morphogenesis of the adult. The first idea was that the right side with the juxtaposed setae h_3 and *lp* should be a special apomorphy. In that case, the disjunct position of h_3 (as represented in nearly all other families of poronotic Oribatida) should be plesiomorphous: this seems to be a misinterpretation as will be discussed below.

Eupelops occultus

Eupelops occultus (CL Koch) is quite common in meadows. It belongs to the species group A as characterized in the previous section; the setae h_3 and *lp* are juxtaposed and form a complex together with *A1*. In 2005, I got some specimens collected by Andreas Toschki (Aachen, Germany) to verify the species identity. There was one specimen with abnormal notogastral setation (together with a normal specimen) which shows imperfect juxtaposition on the right side and far distant position of h_3 on the left (Fig. 1b). This observation seems to support the idea of a plesiomorphous character expression, representing an abnormal atavistic regression towards disjunct setae, yet with asymmetric configuration.

The described anomalies in *E. acromios* on the one hand and in *E. occultus* on the other seem to be incompatible and antagonistic: in *E. acromios* the setae h_3 and *lp* are juxtaposed abnormally, whereas in *E. occultus* the setae h_3 and *lp* are separated abnormally! Is a common explanatory model conceivable? This phenomenon begs for detailed phylogenetical discussion.

Abnormal patterns of notogastral setation in Scheloribatidae

In *Scheloribates* species and in most other genera of the family the notogastral setation is 'multideficient', following the terminology of Grandjean (1954), i.e., there are 10 pairs of notogastral setae in the adults. Some taxa, as *Topobates*, which is related to *Scheloribates*, have 12-14 pairs of notogastral setae in the adults, representing an intermediate status between 'unideficient' (15 pairs) and 'multideficient' (10 pairs). Some species with intermediate setation have been described as further genera, which seems to be punctilious splitting, based only on a character of minor taxonomic value (discussed in Weigmann & Miko, 1998). In this context, it is of highest importance that the third nymphs of all Scheloribatidae and related families (as far as I know) represent the unideficient status, i.e., with all 15 pairs of notogastral setae (the 16th seta f_1 in basic Oribatida is lost in all poronotic Oribatida).

Looking through the literature I discovered a lot of individual abnormalities in the notogastral setation of *Scheloribates* and *Topobates* species. In the following only some examples will be presented, constricted to both taxa, but the same phenomenon can be observed in some other genera and families as well; for instance, cf. Seniczak et al. (1990) on the ceratozetid *Fuscozetes fuscipes*. The first described *Topobates* was *T. granifer* Grandjean. It has 14 pairs of notogastral setae in the adult; compared with *Scheloribates* we find the mediodorsal setae c_1, *da*, *dm*, and *dp* in addition. Yet, even the first author marked a unilateral vestigial seta c_3, which represents the 15th seta of the unideficient pattern (Grandjean, 1958). Adult *Topobates holsaticus* Weigmann have 13 pairs of notogastral setae (without c_1 and c_3), but in one individual out of ca. 200, the unilateral seta c_3 was present and stronger than all other setae (as normally observed in some *Oribatula* species). In a Spanish *T. holsaticus* population, Subias & Arillo (2000) found a unilateral seta c_1 in a single specimen. Csiszar & Jeleva (1962) published a new species, *Scheloribates labyrinthicus* Jeleva, which differs from the widespread *S. laevigatus* (CL Koch) only by bilateral expression of hypertrophied c_3-setae: *S. labyrinthicus* is assumed a junior synonym of *S. laevigatus* (Weigmann & Miko, 1998). Ingrid Wunderle has found a specimen of the arboreal *Scheloribates ascendens* Weigmann et Wunderle, with additional alveoles of notogastral setae c_3 and the centrodorsal *da*, *dm*, *dp*, partly unilateral, though all other studied adults have 10 pairs as usual (Weigmann & Wunderle, 1990). *Topobates carpathicus* Weigmann et Miko has 12 pairs of notogastral setae in the adults (*dm* and *dp* present), but some individuals show an additional vestigial pair of *da*-setae.

All these examples of abnormal additional notogastral setae in adult Scheloribatidae beg for a unifying explanation. The existence of 15 pairs of notogastral setae in the juveniles indicate that genes for these setae are present. It seems most probable that the additional setae of the unideficient pattern of the nymphs are repressed morphogenetically in the adult phenotype, yet there is a latent potential to develop these setae in the adult instar! One consequence of this hypothesis is that additional notogastral setae of the adults are atavistic reminicences of ancestral characters, in abnormal (often asymmetric) cases as well as in *Topobates* and other genera of the *Scheloribates* complex. There are no convincing apomorphies to define distinct genera in a suffi-

cient phylogenetical manner. Thus, a second consequence is that *Topobates* should merely be a subgenus of *Scheloribates* (Weigmann & Miko, 1998).

Abnormal appearance of a notogastral sacculus in Peloptulus

Peloptulus phaenotus (CL Koch) is most common in fresh and wet meadows in Europe. As normal in all genera of Phenopelopidae, the octotaxic system is characterized by four pairs of small areae porosae which are associated with a notogastral seta each (see Fig. 1 for *Eupelops*, Fig. 2a for *Peloptulus*). Within some populations from the North Sea coast, one single specimen was found (Dagebüll, Schleswig-Holstein, Germany; leg. Weigmann, 1967) with a sacculus at the right side instead of an area porosa *Aa*, which is expressed typically at the left! Figure 2b-d shows the drawing, figure 3 a light-microscopical photo.

This abnormal expression of a single sacculus within the normally four pairs of areae porosae of *P. phaenotus* provokes the question how the transformation of an area porosa into a sacculus may occur. This phenomenon must be a misdevelopment during the formation of the adult instar, because it is unilateral and unique within a larger population. It is conceivable that this misdevelopment is an atavistic phenomenon. The consequence should be that ancestors of *Peloptulus* must have had sacculi instead of the notogastral areae porosae. The phylogenetic implications of this hypothesis will be considered below. Recently, *Peloptulus sacculiferus* Weigmann was described as the first species of the family Phenopelopidae, which shows notogastral sacculi in all positions of the octotaxic system (Weigmann, 2008).

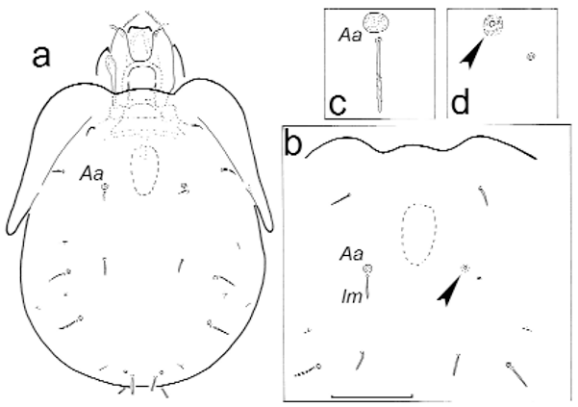

Figure 2 *Peloptulus phaenotus*. (a) Normal symmetric specimen. (b) Anterior part of notogaster from an abnormal specimen: left side with normal Area *Aa*, right side with sacculus (scale bar: 100 µm). (c) Left *Aa* with seta *lm*, enlarged. (d) Right sacculus with insertion of *lm*, enlarged. The arrow head points to the sacculus.

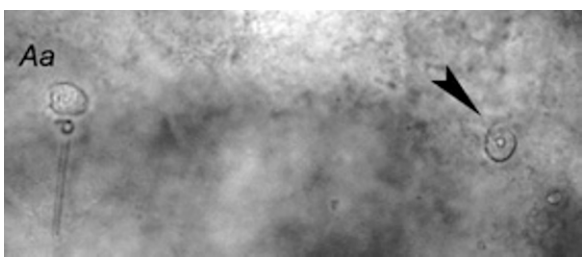

Figure 3 *Peloptulus phaenotus*. Light-microscopical photo from the specimen in figure 2: Left area *Aa* with seta *lm,* the arrow head points to the sacculus on the right side.

DISCUSSION

The concept of regulatory genes as a tool for systematics

In modern evolutionary biology it is commonly accepted to study and discuss ontogenetic differentiations, mutations as well as phylogenetic transformations, in the light of molecular-genetic processes (Kutschera & Niklas, 2004; Futuyma, 2007). In the following, I try to take up models from molecular genetics and adapt them to phylogenetic aspects within poronotic Oribatida with the goal of explaining the morphological anomalies, using the simple case of the asymmetric appearance of a sacculus in *Peloptulus* to begin with.

Ontogenetic and morphogenetic differentiation is processed by regulatory gene complexes or 'gene clusters'. Regulatory genes control the activity of other genes which may be determinants of specific characters in the phenotype. In *Drosophila* genetics or the morphogenesis of vertebrates, the so-called HOX-genes are known as gene clusters that regulate patterns of morphological structures. There is no doubt that similar gene complexes regulate morphological patterns of notogastral structures in oribatid mites, too. Furthermore, occasional developmental anomalies, for instance in *Drosophila*, have been proven to be defects in the regulation by regulatory genes, who work as molecular switches to turn on or off a specific gene ('operon model'), or curtailing the rate of synthesis of the products of other distant genes. MicroRNA molecules are known to regulate gene expression, partly by blocking translation of mRNA (Futuyma, 2007). The latter process may explain punctual or quantitative modifications of phenotypical characters or enzyme activities.

Several models in molecular biology (widespread in bacterial genetics, often regarding enzyme activities) construct hierarchical complexes of regulatory genes which regulate protein synthesis; additive gene complexes may modify the process or may block it causing alternative pathways or even mutations. Against this background, Ziegler & Wauthy (1987) explained different enzyme activities in adult and juvenile *P. peltifer* as an effect of regulatory genes. In addition, they discussed the theoretical application of those regulation models to numerical regressions of morphological characters. Unfortunately, these ideas did not influence recent research in systematics and taxonomy of oribatid mites. The anomalies in notogastral setation of Scheloribatidae concern 'regressive characters', being numerically reduced or lost in the course of phylogeny. The setation patterns in *Eupelops* and the areae-sacculi-modifications in *Peloptulus* are transformations. Both phenomena can be explained by the following model.

Table 1 Model of morphogenetic regulatory gene chains: the example of an abnormal *Peloptulus phaenotus*, evolutive level C represents the abnormal expression of a sacculus (Fig. 2b).

genetic regulation	gene complex a	gene b *modifying*	gene c *blocking gene b*
	↓	↓	↓
evolutive level	**A**: basic plesiomorphous	**B**: derived apomorphous	**C**: reverse plesiomorphous
phenotype of octotaxic system in **Peloptulus**	sacculus	area porosa	sacculus

Sacculi or areae porosae in the octotaxic system of Poronota: A simple genetical switch?

At least since the fundamental analysis of porose integumental organs by Alberti & Norton (1997), there is no doubt that the octotaxic areae porosae are homologous to the notogastral sacculi. There are some genera of Poronota with sacculonotic *and* poronotic species, for example *Anachipteria*, *Punctoribates*, and *Tegoribates*. Some closely related genera differ namely by having areae resp. sacculi – examples are *Lepidozetes–Scutozetes* and *Parachipteria–Achipteria*. The occurrence of both area resp. sacculi can be found in several families, and there is no convincing correlation with important characters that have been used to establish families within Poronota.

Furthermore, a rare case of sexual dimorphism of the octotaxic porose organs in *Glanderemaeus hammerae* Balogh et Csiszar (Cymbaeremaeoidea) has been reported. The male has large sacculi, whereas the female has small areae porosae A_3 (Norton & Alberti, 1997). Both phenotypical expressions can switch apparently from one to the other type, possibly supporting different functions in male or female. The reported anomaly of an asymmetric sacculus in *Aa*-position of a single specimen of *P. phaenotus* (Figs. 2, 3) provides strong support for the conception that a simple genetic switch changes the phenotypical expression between sacculus and area porosa (Table 1). In this case the realization of a sacculus is an atavistic phenomenon: a reversion to the plesiomorphous status as commonly present in ancestors. Consequently, differential diagnosis of related genera is poor and doubtful, if based only on the presence of sacculi resp. areae porosae.

A model of hierarchical control of gene expression: the example *Scheloribates*

The basic notogastral setation of holonotic Oribatida shows 16 pairs ('holotrich'). In early derivative Brachypylina (ancestors of Poronota) we regularly find 15 pairs of notogastral setae ('unideficient'), setae f_1 are lost (Table 2: evolutive level B). This numerical regression is interpreted as genetical repression of f_1. All Brachypylina lack setae f_1 in juvenile and adult stages. In Poronota, the octotaxic system is evolved possibly as sacculi (Table 2: evolutive level C), as has been discussed above. The octotaxic system is modified from sacculi to areae porosae in a branch of Poronota (Table 2: evolutive level D), which can be interpreted as effect of a modifying gene d.

In another branch of Poronota (Table 2: evolutive level E) the setation pattern changes from 15 to 10 pairs of notogastral setae, a very frequently observed numerical regression in the 'multideficient' types. This process is interpreted as caused by a regressive gene, which is effective and phenotypically visible only in the adult stage. Within several families we regularly find the character combination of '10 pairs of notogastral setae' plus 'areae porosae in the octotaxic system' (e.g., Ceratozetidae part., Scheloribatidae part., Phenopelopidae). A special apomorphic character of *Scheloribates* is the expression of the octotaxic system as multiporal sacculi (cf. Weigmann, 1969; Norton et al., 1997), which is interpreted as regression from areae-porosae-status back to sacculi, but these modified from one to several orifices (Table 2: evolutive level F).

A next phylogenetic step to the evolutive level G in Table 1 reactivates some of the repressed pairs of notogastral setae. This process is interpreted as effect of blocking genes, which make the setal regression from level D to E partly inactive. This character is typical for *Topobates*, but similar 'reappearances' of centrodorsal setae *da*, *dm*, and *dp* as setae c_1 or c_3 occur in several families and genera, e.g., *Peloribates* (Haplozetidae), *Diapterobates*, *Fuscozetes*, and *Melanozetes* (Ceratozetidae), with completely or partly recompensated setal loss. Even in Oppiidae (non-poronotic oribatid mites) with mostly 9-10 notogastral setae in adults we find the unusual setal number of 12 pairs (*Multioppia*).

This list of examples could be continued and it is hard to imagine that all these apparent parallelisms within diverse families or systematically related groups of genera, partly being gradually or completely multideficient, partly being unideficient, are independent convergencies. Also, it makes no difference whether uni- or multideficiency is declared to be apomorphic. The general evolutionary trend in oribatid mites is a numerical regression of setal numbers, but in those taxa with different notogastral setation the complete number is not obviously plesiomorphous, but often the exception.

These anomalies from the *Scheloribates* complex help to solve our problem in explaining exemplarily the obviously mosaic-like occurrence of uni- and multideficient species or genera: We know that all setae of the unideficient set are

Table 2 Evolution model of notogaster structures in Scheloribatidae.

genetic regulation	evolutive level	taxon	phenotypical character
regressive gene b →	A	Holonota	Holotrich notogaster: 16 ng Ad: no octotaxic system (OS)
additive gene c →	B	Early derivative Brachypylina	unideficient notogaster: 15 ng Ad: no OS
modifying gene d →	C	Poronota I	unideficient notogaster: 15 ng Ad: OS with sacculi 
regressive gene e →	D	Poronota II	unideficient notogaster: 15 ng Ad: OS with areae porosae 
	E	Poronota III e.g. basic Scheloribatidae	Ad: multideficient notogaster: 10 ng Ad: OS with areae porosae
regressive + modifying gene f →	F	*Scheloribates* s. lat.	Ad: multideficient notogaster: 10 ng Ad: OS with multiporal sacculi 
block gene g partly blocking e →	G	*Scheloribates* s.gen. *Topobates*	Ad: 12-14 ng Ad: OS with multiporal sacculi

manifested in the genome of all individuals, because all nymphs 3 possess all these setae. Non-expressed setae in the adult stage must be suppressed phenotypically, if they nevertheless occur, then their position is mostly identical with the homologous setae in more complete designs in related taxa. Asymmetry or incomplete development of setae form a clear picture of morphogenetic defects. The use of the model of regulatory genes explains these individual anomalies within normally developed populations as well as the problem of multiple 'parallelisms' with regard to notogastral setation patterns in distinct taxa.

A model of hierarchical control of gene expression: the example Phenopelopidae

With regard to the notogastral setation and the octotaxic system, the ancestors of the Phenopelopidae (with the only genera *Propelops*, *Eupelops*, and *Peloptulus*) might have represented an evolutive level E of Poronota, as characterized in Table 2: a poronotic oribatid mite with 10 pairs of notogastral setae and with notogastral areae porosae. This type serves as a basis in Table 3 to discuss the evolution of the genera based on a model of genetic regulation. Following Norton & Behan-Pelletier (1986), the family is strongly characterized by association of each notogastral area porosa with a notogastral seta; the typical pincette-like 'peloptoid' chelicera in *Eupelops* and *Peloptulus* is *not* developed basically because *Propelops* has a normal chelicera.

It can be assumed that the normal octotaxic pattern of free areae porosae is modified in the hypothesized basic species/genus of Phenopelopidae towards the association pairs (seta/area): *lm*/*Aa*, *lp*/*A1*, h_2/*A2*, h_1/*A3* (Table 3: evolutive level H). This pattern is modified in the evolutive level I to a juxtaposed group of the setae *lp* and h_3 with area *A1*: this character is a synapomorphy of both subfamilies Propelopinae and Phenopelopinae (cf. Norton & Behan-Peletier, 1986), where the latter evolved the special pincette-like chelicera from the so-called peloptoid type (Table 3: evolutive level K). The genus *Eupelops* is – among other criteria – characterized by hypertrophic leaf-like interlamellar setae (Table 3: evolutive level L), not being large in *Propelops* and *Peloptulus*. Within *Eupelops*, two species groups are distinguished: group A with *E. occultus* keeps the juxtaposed form of *lp*, h_3+*A1*; in group B with *E. acromios* the setae h_3 become secondarily separated from *A1*+*lp* (Table 3: evolutive level M). This phenotypical character represents the hypothesized basic species/genus of characters for the Phenopelopidae (as in level H), thus being a regression that can be explained by genetic blocking of gene i, as indicated in Table 3. This character is therefore not apomorphous but a regression to a plesiomorphous character expression!

In *Peloptulus*, the association group is modified in another way than in *Eupelops* group B: here the area *A1* remains juxtaposed with h_3, but *lp* becomes disjunct (Table 3: evolutive level N).

This phylogenetic interpretation is supported by the anomalies in *E. acromios*, as argued above. If the juxtaposed group *lp*, h_3+*A1* is a family character, as is thought plausible by Norton & Behan-Peletier (1986), this status is plesiomorphous within *Eupelops* and consequently group B is derived. The right side of *E. acromios* in Figure 1a is regressive and abnormal. In Figure 1b the left side of *E. occultus*, normally showing the association group with *lp* and h_3, is abnormal and is a regression below the family-typical evolutive level.

Conclusions

The rare anomalies in adult specimens of poronotic Oribatida discussed in this article are not accidental individual misdevelopments, but represent phenotypical characters that are expressed in the same manner in the ancestors. The morphogenetic model makes plausible that these anomalies of adults are atavisms, i.e., characters being cryptically present in the genome (occasionally expressed phenotypically in juvenile stages). This view leads to the plausible hypothesis that numerical regressions of the notogastral setation or transformations of sacculi to areae porosae in the notogastral octotaxic system of the Poronota can be switched on or off by regulatory genes. Following this explanation, convergent appearances of these phenotypic characters in more or less related taxa must not be independent parallelisms, but emerge from the same regulatory gene switches as postulated for the anomalies discussed: the phenotypic characters are homologies in a plesiomorphous, apomorphous, or secondary-plesiomorphous (regressive) development.

In consequence, while evaluating characters for cladistics and phylogenetics we should not only look for synapomorphies to establish sister-group-relationships, but we have to be aware of cryptic persistence of plesiomorphous characters in the genome, which may become apparent by

genetic regulation	evolutive level	taxon	phenotypical character
modifying gene h →	E	Poronota III	Ad: multideficient notogaster: 10 ng Ad: OS with areae porosae (A.p.)
modifying gene i →	H	hypothetical basic **Phenopelopidae**	A.p. associated with *lm, lp, h2, h1*
modifying gene k →	I	Propelopinae *Propelops*	*lp, h3+A1* juxtaposed
modifying gene l →	K	hypothetical basic Phenopelopinae	*lp, h3+A1* juxtaposed Chelicers pincette-like ("peloptoid")
	L	*Eupelops* group **A**	*lp, h3+A1* juxtaposed interlamellar setae large, leaf-like
block gene m blocking i →	M	*Eupelops* group **B**	*h3* disjunct from *A1+lp*
modifying gene n →	N	*Peloptulus*	*h3+A1* disjunct from *lp*

Table 3 Evolution model of notogaster structures in Phenopelopidae.

genetical regression in a derived systematic clade. Such phenomena of erroneously assumed parallelisms, especially in cases of numerical regressions, may be reasons of 'mosaic-like' character distribution in the system of Oribatida. Also in taxonomy it is recommended to take the phylogenetical and morphogenetical quality of the diagnostic characters into account when intending to create a new genus or family.

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Phylogeny and host-parasite associations of feather mites of the *Pteroherpus* generic group (Astigmata: Pteronyssidae)

Sergey Mironov[1] & Georges Wauthy[2]

[1]Zoological Institute, Russian Academy of Sciences, 199034, Saint Petersburg, Russia. E-mail: astigmata@zin.ru; and
[2]Institute royal des Sciences naturelles de Belgique, rue Vautuer 29, B-1000, Brussels, Belgium

Cladistic reconstruction of phylogenetic relationships within the *Pteroherpus* generic group, consisting of the genera *Dicrurobius*, *Micropteroherpus*, *Pteroherpus*, and *Vanginyssus*, has been carried out, here, for the first time. This analysis was based on 46 morphological characters and 29 operational taxonomic units, including 21 species of the *Pteroherpus* group (ingroup), eight species representing three pteronyssid genera known from passeriforms and piciforms (close outgroups), and two species of the family Avenzoariidae (distant outgroups). Maximum parsimony analysis confirmed monophyly of the *Pteroherpus* group and four of its genera. The analysis also revealed four distinct lineages within the genus *Pteroherpus* that we treat as species groups: *hoplophorus*, *diploplax*, *nicator*, and *josephi*. The *Pteroherpus* group displays two contrasting tendencies in morphological modifications: (1) reduction of shield areas in the posterior part of the female opisthosoma by splitting the hysteronotal shield into fragments and decreasing their size; this trend has been realized to various extents in all genera of the group, and (2) development of additional sclerotization in the sejugal area in both sexes of the most derived species of the *holoplax* and *diploplax* groups of *Pteroherpus*. Based on phylogenetic relationships within the *Pteroherpus* generic group and among their avian hosts, and on parasite-host associations, we conclude that co-speciation with their hosts was the main mode of diversification – although in a few cases host shift has taken place.

Key words: Feather mites, Pteronyssidae, *Pteroherpus* group, phylogeny, host associations, host shift, Passeriformes

The feather mite family Pteronyssidae Oudemans (Astigmata: Analgoidea) currently includes approximately 170 species in 23 genera (Gaud, 1952; Gaud & Mouchet, 1959; Faccini & Atyeo, 1981; Gaud & Atyeo, 1996; Mironov, 1989, 1992, 2001, 2003; Mironov & Kopij, 2000; Mironov & Wauthy, 2005a). Mites of this family are mainly distributed on hosts from the orders Passeriformes and Piciformes, with only a few species recorded from the Coraciiformes. Within the plumage of their hosts, pteronyssids inhabit the ventral surface of feathers with large vanes, the flight feathers, and the large upper coverts of wings. Mites are commonly located in narrow corridors on the ventral surface of vanes formed by barbules (Mironov, 1987).

Among nine pteronyssid genera restricted in their host associations to passerines, four genera – *Dicrurobius* Mironov, *Micropteroherpus* Mironov, *Pteroherpus* Gaud, and *Vanginyssus* Mironov – constitute the *Pteroherpus* generic group (Mironov & Wauthy, 2005b, 2006a,b, 2008). This generic group is clearly characterized by the bifurcate dorsal palpal seta *dp2* and the long ventral membrane of tarsus I, which is subequal in length to this segment. As in many other pteronyssids, the clearest difference between the genera of the group is expressed in the general pattern of the hysteronotal shield in females, which is quite stable within genera, whereas males of different genera are rather similar in general appearance.

Mites of the *Pteroherpus* group are medium sized for pteronyssids (about 300-550 µm). They are known from hosts of eight avian families belonging to 'higher' passerines (Oscines) from the Old World; the genera *Dicrurobius*, *Micropteroherpus*, and *Vanginyssus* are restricted to drongos (Dicruridae), cisticoles (Cisticolidae), and vangas (Vangidae), respectively; the genus *Pteroherpus* is mainly distributed on the Old World warblers and allies (superfamily Sylvioidea).

In the course of our studies (Mironov & Wauthy 2005a,b, 2006a,b,c, 2008) dealing with biodiversity and systematics of feather mites associated with passerines, and, particularly, mites of the family Pteronyssidae, we examined specimens of all species of the *Pteroherpus* group, including type material of most species. The main target of the present paper is cladistic analysis of the *Pteroherpus* group based on morphological characters. A brief analysis of host-parasite associations of the generic group and co-evolutionary patterns is also provided.

MATERIAL AND METHODS

Specimens

We examined material from four museums (Table 1): Musée royal de l'Afrique central (Tervuren, Belgium), Institut royal des Sciences naturelles de Belgique (Brussels, Belgium), Muséum national d'Histoire naturelle (Paris, France), and Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia). General morphological terms and nomenclature of chaetotaxy used in the analysis are those of Gaud & Atyeo (1996); terms regarding structure of dorsal shields follow Mironov (1992) and Mironov & Wauthy (2005a). Scientific names of avian hosts follow 'The Howard and Moore Complete Checklist' (Dickinson, 2003), and passerine phylogeny follows recent concepts based on molecular studies (Ericson et al., 2002; Ericson & Johanson, 2003; Barker et al., 2004; Beresford et al., 2005).

Phylogenetic analysis

Qualitative morphological characters implying the presence/absence or the form of a certain structure were used in the parsimony-based cladistic analysis (Table 2). Two species of the family Avenzoariidae, *Avenzoaria calidridis* (Oudem-

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Table 1 Feather mite species used in phylogenetic analysis, their host associations, location, and the source of material.

Mite species	Host species	Host family	Location	Source[1]
Avenzoaria calidridis Oudemans, 1904	*Calidris alpinus*	Scolopacidae	Russia	ZISP
Zachvatkinia sternae (Canestrini et Fanzago, 1876)	*Sterna hirundo*	Sternidae	Russia	ZISP
Neopteronyssus pici (Scopoli, 1763)	*Dendrocopos major*	Picidae	Russia	ZISP
N. picinus (Koch, 1841)	*Dryocopus martius*	Picidae	Russia	ZISP
Pteronyssoides motacillae Mironov, 1987	*Ploceus griseus*	Motacillidae	Russia	MRAC
P. holoplax (Gaud et Mouchet, 1959)	*Pycnonotus barbatus*	Pycnonotidae	Cameroon	MRAC
Sturnotrogus truncates (Trouessart, 1885)	*Sturnus vulgaris*	Sturnidae	Russia	ZISP
S. subtruncatus (Trouessart, 1885)	*Gracula religiosa*	Sturnidae	Netherlands	IRSNB
Dicrurobius monacrotrichus (Gaud, 1952)	*Ducrurus forficatus*	Dicruridae	Madagascar	MRAC
D. cameroonensis (Mironov et Wauthy, 2005)	*D. adsimilis*	Dicruridae	Cameroon	MRAC
Micropteroherpus benoiti (Faccini et Atyeo, 1981)	*Cisticola brachyptera*	Cisticolodae	Mozambique	ZISP
M. orthotomi (Mironov, 1992)	*Orthotomus sutorius*	Cisticolidae	Vietnam	ZISP
Pteroherpus hoplophorus (Gaud, 1952)	*Hypsipetes madagascariensis*	Pycnonotidae	Madagascar	MRAC
Pt. hypposathes (Trouessart, 1887)	*Astrapia nigra*	Paradisaeidae	New Guinea	NMHN
Pt. doleoplax (Gaud et Mouchet, 1959)	*Thescelocichla leucopleura*	Pycnonotidae	Cameroon	MRAC
Pt. josephi (Gaud et Mouchet, 1959)	*Muscicapa comitata*	Muscicapidae	Cameroon	MRAC
Pt. megathyrus (Gaud et Mouchet, 1959)	*Bleda exima notata*	Pycnonotidae	Cameroon	MRAC
Pt. pycnonoti Mironov, 1992	*Pycnonotus jocosus*	Pycnonotidae	Vietnam	MRAC
Pt. africanus (Mironov et Kopij, 2000)	*Pycnonotus barbatus*	Pycnonotidae	South Africa	ZISP
Pt. nicator Mironov et Wauthy, 2005	*Nicator gularis*	Pycnonotidae	South Africa	MRAC
Pt. pyrrhuri Mironov et Wauthy, 2005	*Pyrrhurus scandens*	Pycnonotidae	Cameroon	MRAC
Pt. trinoton Mironov et Wauthy, 2005	*Phyllastrephus terrestris*	Pycnonotidae	South Africa	MRAC
Pt. diploplax (Gaud et Mouchet, 1959)	*Turdoides plebejus*	Timaliidae	Cameroon	MRAC
Pt. dentilobus Mironov, 1992	*Timalia pileata*	Timaliidae	Vietnam	ZISP
Pt. krivolutskii Mironov, 1992	*Stachyris nigriceps*	Tymaliidae	Vietnam	ZISP
Pt. zosteropis Mironov, 1992	*Zosterops japonica*	Zosteropidae	Vietnam	ZISP
Pt. pallens (Berlese, 1886)	*Acrocephalis arundinaceus*	Sylviidae	Russia	ZISP
Vanginyssus schizurus (Gaud, 1952)	*Leptopterus chabert*	Vangidae	Madagascar	ZISP
V. euryceros Mironov et Wauthy, 2006	*Euryceros prevostii*	Vangidae	Madagascar	ZISP

[1]MRAC, Musée royal de l'Afrique central (Tervuren, Belgium); IRSNB, Institut royal des Sciences naturelles de Belgique (Brussels, Belgium); MNHN, Muséum national d'Histoire naturelle (Paris, France); ZISP, Zoological Institute of the Russian Academy of Sciences (St. Petersburg, Russia)

ans) (Avenzoariinae) from *Calidris alpina* (L.) and *Zachvatkinia sternae* (Canestrini et Fanzago) (Bonnetellinae), belonging to an analgoid family that retains many plesiomorphic features (Dabert & Mironov, 1999), were used as distant outgroups. Representatives of three pteronyssid genera, *Neopteronyssus* Mironov, *Pteronyssoides* Hull, and *Sturnotrogus* Mironov, were used as potential close outgroups; each of these genera was represented by a pair of species. *Pteroherpus*, the most species-rich genus of the group in question, was represented by 15 of 17 known species. Two species were excluded, because we consider *Pteroherpus oxyplax* (Gaud et Mouchet) as a synonym of *P. pallens* (Berlese), and *P. aciaepiginius* is known only by the male and its true host association is unknown (Faccini & Atyeo, 1981). Three other genera of the group each consist of a few species differing by autopomorphic or continuous characters – *Dicrurobius* (three species), *Micropteroherpus* (three), and *Vanginyssus* (five) –, they are restricted to one host family and are clearly monophyletic. To simplify the analysis, they were represented by one pair of species.

In total, 29 mite taxa and 46 characters were included in the analysis (Tables 1-3). Constructing and editing of the data matrix was done using NEXUS Data Editor 0.5.0 (Page, 2001). All characters were treated as unordered; characters having multiple states were not modified into binary characters. Reconstruction of phylogenetic relationships was performed with PAUP 4.0 beta version for Windows 95/NT (Swofford, 1998). The branch and bound algorithm was used for the maximum-parsimony analysis and the reconstruction of phylogeny. For a posteriori optimization of character states, we used the DELTRAN option (delayed transformation), which favours parallelism over reversal when the choice is equally parsimonious. Bremer indices used for estimating support for branches were calculated with the program Autodecay (Eriksson, 1998). Trees were drawn using Winclada, version 1.0 (Nixon, 1999).

RESULTS AND DISCUSSION

Phylogeny

The branch-and-bound search produced a single shortest tree with a length 98 steps and the following standard indices (excluding uninformative characters): CI = 0.7447, RI = 0.8636, RC = 0.6521 (Fig. 1). The *Pteroherpus* generic group appeared monophyletic and is a sister group of the *Pteronyssoides* generic group. Monophyly of the *Pteroherpus* group is supported by five synapomorphies: long ventral membrane of tarsus I (character 5.1), and bifurcate palpal setae *pd2* (6.1) in both sexes; splitting of pygidial shield from the main body of hysteronotal shield (32.1), and loss of sclerotization around hysteronotal gland openings *gl* in females (40.1); developing of V- or Y-shaped transventral sclerite in males (18.1). The monophyly of all the four genera currently recognized within the *Pteroherpus* group is also well supported.

The genus *Dicrurobius* splits first from the base of the *Pteroherpus* group branch and is characterized by the following apomorphies: in males, the posterior part of prodorsal shield is large, encompasses setae *c1*, and has rectangular posterior angles (8.1), an opisthosomal membrane along the posterior margin of lobes has a pair of spine-shaped protrusions (15.6); in females, the prodorsal shield has greatly elongated posterior angles extending to the bases of setae

Table 2 Characters used in the phylogenetic analysis.

No	Characters and coding
	Both sexes
1	Verical setae *vi*: absent (0), one unpaired seta (1), pair of setae (2).
2	Idiosomal se setae *h1*: present (0), absent (1).
3	Lateral sclerites of ambulacral discs: without lacunae (0), with lacunae (1).
4	Ventral membrane on tarsus I: absent (0), present (1).
5	Length of ventral membrane on tarsus I: about 1/2 of tarsus (0), about 3/4 of or equal to tarsus (1), greatly reduced (2).
6	Form of palpal setae *dp2*: simple setiform (0), bifurcate (1).
7	Proportion of prodorsal shield (length from anterior end to level of scapular setae / distance between setae se): ratio 0.8-1.2 (0), shield noticeably elongated, ratio1.3-1.6 (1).
Males	
8	Posterior part of prodorsal shield: slightly extending beyond scapular setae *se*, posterior margin convex or sinuous, posterior angles not expressed (0), slightly extended beyond scapular setae, posterior angles acute (1), extended by 1/3 of shield length, encompassing setae c1, posterior angles rectangular (2), extended by 1/3 of shield length, posterior angles acute (3), extended by 1/3 of shield length, posterior angles extended and rounded, surface striated (4), not shield beyond scapular setae (5).
9	Anterior additional fragment of hysteronotal shield: absent (0), present, large unpaired plate encompassing setae *c1* (1), pair of little rudimentary sclerites in sejugal area (1).
10	Epimerites I: fused as a Y (0), free (1).
11	Length and form of terminal cleft: large, long, and wide, about 1/4-1/3 of hysterosoma length (0), as a small and narrow V or U (1), as a relatively wide U, but shorter than 1/4 of hysterosma (2), not expressed (3).
12	Opisthosomal lobe length: long, about 1/4-1/3 of hysterosoma length (0), shortened (1).
13	Form of small opisthosomal lobes: widened, with rounded posterior margin (0), narrow, close to each other (1), narrow, distant from each other (2).
14	Supranal concavity: short or not expressed (0), long, anterior end extended to level of setae *e1* (1)
15	Opisthosomal membranes: absent or scarcely developed (0), narrow membrane along posterior margin of lobes (1), membrane on lobar apex with several large teeth (2), small rectangular membrane on lobar apex (3), narrow membrane with numerous small teeth along posterior margin of lobes (4), triangular membrane on lobar apex (5), narrow membrane along posterior margin of lobes with large spine (6).
16	Narrow membrane on inner margin of opisthosomal lobes: absent (0), present (1).
17	Transventral sclerite: absent (0), present (1).
18	Form of transventral sclerite: transverse sclerite (0), as a V or Y with short fused part (1), as a Y with very long handle (2)
19	Epiandrium: absent (0), present, fused with transventral sclerite (1).
20	Tips of epiandrium: well developed (0), strongly reduced, not extended to genital arch apex (1).
21	Cupule *ih*: indistinct (0), well-developed (1).
22	Coxal fields III: open (0), closed (1).
23	Leg III: not modified, subequal in size to legs IV (0), hypertrophied (1).
24	Tarsus III: claw-like apical process (0), bidentate apical process (1).
25	Position of solenidion ϕ on tibia III: dorsal (0), ventral or submarginal (1).
25	Dorsobasal spines on tarsus IV: absent (0), one spine (1), two spines (2), 3-4 small spines on tubercle (3).
Females	
27	Idiosoma (ratio of length to width): moderately elongated, ratio 1.8-2.6 (0), greatly elongated, ratio 2.8-3 (1).
28	Posterior part of prodorsal shield: slightly extending beyond scapular setae *se*, posterior margin convex or sinuous, posterior angles not expressed (0), slightly extended beyond *se*, posterior angles acute (1), posterior angles long and rounded, extending to bases of setae *c2*, posterior margin deeply concave (2), extended by 1/3 of shield length, posterior angles acute (3), extended by 1/3 of shield length, posterior angles extended and rounded, surface striated (4), shield not extending beyond level of scapular setae (5), completely fused with anterior hysteronotal sclerite (6).
29	Epimerites I: fused as a Y or V (0), free (1).
30	Anterior hysteronotal sclerite: absent (0), present (1)
31	Form of anterior hysteronotal sclerite: paired, not encompassing setae (0), paired, encompassing *c2* (1), paired, encompassing *c1*, *c2* (2), unpaired, encompassing *c1*, *c2* (3).
32	Pygidial sclerite: not separated from hysteronotal shield (0), separated (1).
33	Form of pygidial sclerite: unpaired (0), paired (1).
34	Opisthosomal sclerite: not separated from hysteronotal shield (0), separated completely or at least partly, paired (1), completely separated, unpaired (2).
35	Connection between opisthosomal and central sclerites: completely separated (0), entire opisthosomal sclerites connected to central sclerite by striated band or relatively wide bridge (1), inner fragments of opisthosomal sclerite fused with central sclerite (2).
36	Surface of opisthosomal sclerite: monotonously punctured (0), with longitudinal striae or narrow lacunae (1), with longitudinal heavily sclerotized ridges (2).
37	Structure of opisthosomal sclerites: entire plate (0), split into inner and lateral fragments (1), lateral fragments only split from main body of hysteronotal shield (2)
38	Size of central sclerite: large plate (0), smaller than prodorsal shield (1)
39	Surface of central sclerite: monotonously punctured (0), with cell-like pattern (1).
40	Position of hysteronotal gland openings *gl*: situated on hysteronotal shield or its fragment, situated on striated integument (1).
41	Position of openings *gl* regarding to midline: closer to lateral margins of hysterosoma (0), closer to midline (1).
42	Position of setae *c2*: posterior or at level of sejugal furrow (0), distinctly anterior to level of sejugal furrow (1).
43	Length of setae *c2*: filiform, shorter than half-width of idiosoma (0), large setae, 1.5-2× longer than half-width of idiosoma (1).
44	Position of setae *e1* regarding to openings *gl*: posterior to level of *gl* (0), anterior to *gl* (1).
45	Setae *e1*: present (0), absent (1).
46	Epigynum: semicircular or horseshoe-shaped (0), roughly trapezoidal, with straight or slightly divergent lateral parts (1).

Table 3 Data matrix of character states for *Pteroherpus* generic group and outgroup taxa. Character states are scored as 0 to 6, inapplicable states as '-'.

	0000000001	1111111112	2222222223	3333333334	444444
	1234567890	1234567890	1234567890	1234567890	123456
Avenzoaria calidridis	0000-00001	00-0200-0-	0000000010	-0-0-0-000	000000
Zachvatkinia sternae	2000-00000	00-0300-0-	1110030000	-100-0-000	000100
Neopteronyssus pici	1111000500	1100000-00	0010120500	-110-1-000	000100
N. picinus	1111000400	1100000-00	0010120400	-0-0-1-000	000100
Pteronyssoides motacillae	1111000001	1100001010	1011110010	-0-1-00101	000000
P. holoplax	1111200101	1100001010	1011110110	-0-0-00001	000000
Sturnotrogus truncatus	1111000001	3100001010	0010100010	-0-2-02000	000000
S. subtruncatus	1111000001	3100001010	0010100010	-0-0-02000	000000
Dicrurobius monacrotrichus	1111110200	1100601110	0010100200	-100-00011	000000
D. cameroonensis	1111110200	1100601110	0010100200	-100-00001	000000
Vanginyssus schizurus	1111110000	1101101111	1110100000	-111-00001	100000
V. euryceros	1111110000	1101101110	1110100000	-111-00001	100000
Micropteroherpus benoiti	1111110000	2120011210	0111101000	-101000001	001101
M. orthotomi	1111110000	2120011210	0111101000	-101000001	001111
Pteroherpus hoplophorus	1111110000	1100101110	0111100001	3111010000	010101
Pt. africanus	1111110000	1100101110	0111100001	0111010000	010101
Pt. hypposathes	1111110000	1100501010	0111100001	2111110000	010101
Pt. doleoplax	1111110010	1100101110	0111100001	3111010000	010001
Pt. megathyrus	1111110000	1100101110	0111100601	3111110000	010101
Pt. pycnonoti	1111110020	1100101110	0111100001	3111110000	010101
Pt. trinoton	1111110010	1100101111	0111100001	3111011000	010001
Pt. pyrrhuri	1111110010	1100101111	0111100001	3111211000	010001
Pt. nicator	1111110000	1100101110	0111100000	-111010100	010101
Pt. dentilobus	1111111000	1110401110	0111100000	-111011000	010101
Pt. diploplax	1111111000	1110101110	0111100000	-111011000	010101
Pt. krivolutskii	1111111000	1100101110	0111100001	1111011000	010101
Pt. zosteropis	1111111000	1100101110	0111100001	0111011000	010101
Pt. pallens	1111110000	1100101110	0111100000	-111011010	010101
Pt. josephi	1111110300	1100101110	0111100300	-111000000	000101

c2 (28.2). The structure of the hysteronotal shield in females of this genus is apparently the most primitive within the *Pteroherpus* group, because the hysteronotal shield is large, covers the mesal part of the hysterosoma, and only the unpaired pygidial sclerite is split off the main body of the hysteronotal shield. *Dicrurobius* is also the only genus of the *Pteroherpus* group retaining opened coxal fields III in males as in the representatives of the *Pteronyssoides* group and in *Neopteronyssus*.

The remaining part of the *Pteroherpus* group branch is characterized by closed coxal fields III in males (22.1) and by, at least partial, separation of the opisthosomal sclerites from the hysteronotal shield in females (34.1). The genus *Vanginyssus* demonstrates a number of derived features: in males, the supranal concavity is long, extending to setae *e1* (14.1), the opisthosomal membrane is well developed along the posterior margin of the opisthosomal lobes (15.1), the cupules *ih* are well developed (21.1); in females, the pygidial shield is paired (33.1) and openings *gl* are moved closer to midline (41.1). A very long supranal concavity in males and moving of openings *gl* in mesal direction are unique characteristics of this genus within Pteronyssidae.

The upper part of the *Pteroherpus* group branch, bearing the derived genera *Micropteroherpus* and *Pteroherpus*, is characterized in males by the development of a bidentate apical process of tarsus III (24.1), and in females by the moving of setae *e1* anterior to the level of openings *gl* (44.1) and the trapezoidal shape of the epigynum (46.1). Development of the bidentate apical process on tarsus III is a rather common derived feature among various groups of feather mites, males of which have hypertrophied legs; thus, in Pteronyssidae, it is independently developed in the genus *Pteronyssoides* (*Pteronyssoides* group).

The genus *Micropteroherpus*, a lineage of miniaturized forms of the *Pteroherpus* group, is supported by six unambiguous synapomorphies. In males, the terminal cleft is small and semi-ovate (11.2), opisthosomal lobes are small, elongated, and distant from each other (13.2), the inner margin of opisthosomal lobes is provided with a narrow interlobar membrane, whereas the terminal margin of the lobes lacks any membrane (16.1), and the transventral sclerite is Y-shaped with the median part much longer than the free branches (18.2). In females of this genus, the idiosoma is noticeably elongated, about 3× longer than wide (27.1), and the idiosomal setae *c2* are relatively long, their length being over half the width of the idiosoma (43.1).

The cluster of *Pteroherpus* species, forming the core of the generic group, is characterized by three synapomorphies: in males a narrow opisthosomal membrane along the posterior margin of the opisthosomal lobes (15.1), and in females a paired pygidial shield (33.1) and opening *gl* placed on the opisthosomal shields (40.0). In relation to other genera of the group, the latter feature appears in *Pteroherpus* as a reversion of a plesiomorphic state. The alternative hypothesis that *Pteroherpus* retains the plesiomorphic state is theoretically possible, but it requires an additional assumption that *Dicrurobius*, *Micropteroherpus*, and *Vanginyssus* have independently lost sclerotisation around the openings *gl* and this assumption is not confirmed by our cladistic analysis.

Four clades are recognized within the genus *Pteroherpus*, which we taxonomically treat as species groups: *hoplophorus*, *diploplax*, *nicator*, and *josephi*. The clade bearing the first three groups is characterized by the following apomorphies in females: pattern of striae or narrow lacunae on opisthosomal shields (36.1) and position of dorsal setae *c2* anterior to the sejugal furrow (42.1). The lat-

ter character state is a unique feature within the family Pteronyssidae. The *josephi* lineage, with a single species *P. josephi*, apparently represents the most plesiomorphic form within the genus. The most species-rich group *hoplophorus* is characterized by the development of the anterior hysteronotal sclerite in females (30.1). In the early-diverging species, *P. africanus*, this sclerite is represented by a pair of small plates in the sejugal region. In derived species of the *hoplophorus* group, this is a large transverse plate covering the posterior part of the prodorsum and encompassing the bases of the dorsal setae *c1* and *c2* (31.3); in *P. megathyrus*, this plate is completely fused with the prodorsal shield (28.6). The *diploplax* group is characterized by longitudinal splitting of the opisthosomal shield into inner and lateral fragments (37.1). The lineage represented by *P. nicator* shows the maximal degree of reduction of the hysteronotal shield in females: dorsal surface of hysterosoma bears only a pair of relatively small opisthosomal sclerites and the small central sclerite is ovate (38.1).

In tracing the main evolutionary changes in the *Pteroherpus* group it is clear that the main trend is reduction of the hysteronotal shield in the female, displayed in splitting of the entire hysteronotal shield into several fragments and decrease of their size. Thus, in *Dicrurobius*, only the unpaired pygidial fragment is split from the large main body of the hysteronotal shield (Fig. 2A). In *Micropteroherpus*, *Vanginyssus*, and *Pteroherpus* (*josephi*, *hoplophorus*, and *nicator* groups), the hysteronotal shield is split into the unpaired central sclerite, paired opisthosomal sclerite, and paired or unpaired pygidial sclerite (Figs. 2B-F). Finally, in the *diploplax* group of *Pteroherpus*, the opisthosomal sclerites are split longitudinally into inner and outer fragments (Figs. 2G,H).

It is worthwhile to add that the genus *Pteroherpus* also demonstrates a contrasting trend to the main tendency in the generic group. The progressive sclerotisation in the sejugal region is observed in females; this trend was in various degrees independently realized in lineages of the *hoplopho-*

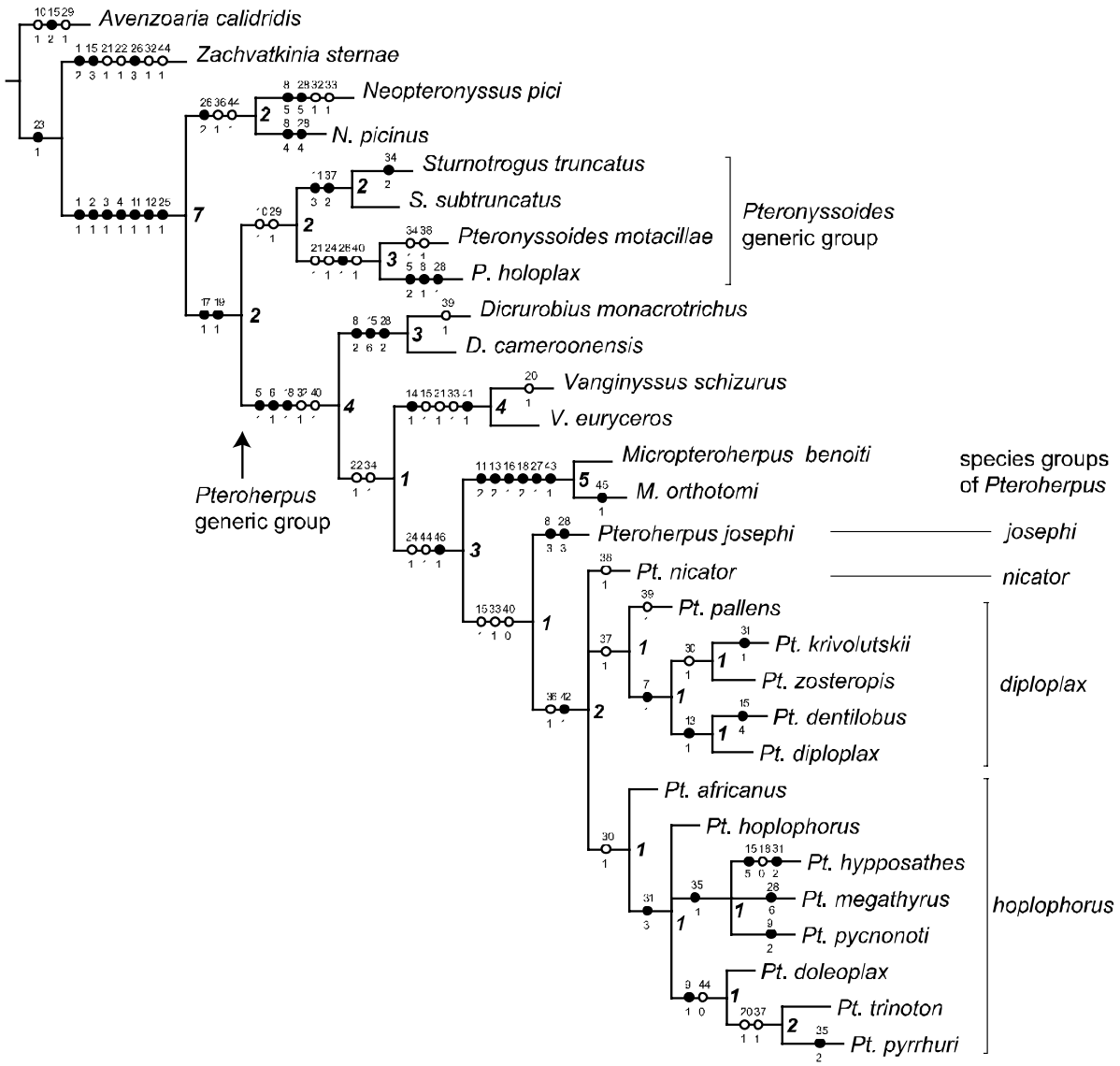

Figure 1 Phylogeny of the *Pteroherpus* genus group. Single most parsimonious tree. DELTRAN character optimization. Numbers above dots (black, unique apomorphy; white, homoplasy) refer to characters, numbers under dots refer to a character state achieved in the respective node. Numbers in bold italics next to the nodes are Bremer indices.

rus and *diploplax* groups. In *P. africanus* (*hoplophorus* group), and *P. krivolutskii* and *P. dentilobus* (both *diploplax* group), the anterior hysteronotal sclerite is represented by a pair of small plates (Fig. 2D); in most other species of the *hoplophorus* group, this sclerite is a large transverse plate, covering the sejugal area and the posterior part or the prodorsum (Fig. 2E); and in *P. megathyrus*, the anterior hysteronotal shield is completely fused with the prodorsal shield forming a complex shield covering almost the entire prodorsum (Fig. 2F).

Host associations

Analysis of host associations of the *Pteroherpus* group, based on the materials examined and also on reference data (Faccini & Atyeo, 1981; Mironov, 2001; Mironov & Wauthy, 2005b, 2006a,b, 2008) shows that these mites are distributed on several familial groupings of the infraorders Passerida and Corvida (Fig 2, Table 1). Regarding the host range at species level, all species of *Micropteroherpus* and *Vanginyssus*, and most species of *Dicrurobius* and *Pteroherpus* are known so far as monoxenous parasites, some species of the two latter genera occur on several host species of one genus. Three genera are restricted to a single bird family: *Dicrurobius* inhabits drongos (Dicruridae), *Vanginyssus* inhabits vangas, Vangidae (Corvida), and representatives of *Micropteroherpus* occur on cistocolas, Cisticolidae (Passerida). Only the genus *Pteroherpus* is distributed on hosts of different families and superfamilies, although most of its species occur on birds of the superfamily Sylvioidea (Passerida). The *hoplophorus* and *nicator* groups are associated with bulbuls, Pycnonotidae; species in the *diploplax* group occur on warblers, Sylviidae, babblers, Timaliidae, and white eyes, Zosteropidae (Sylvioidea). *Pteroherpus josephi* inhabits an African flycatcher *Muscicapa comitata* (Cassin) (Muscicapoidea, Passerida). The most enigmatic host association known for the genus *Pteroherpus* is the occurrence of *P. hipposathes* (*hoplophorus* group) on birds-of-paradise of the genus *Astrapia* Vieillot (Paradisaeidae) which belong to the infraorder Corvida.

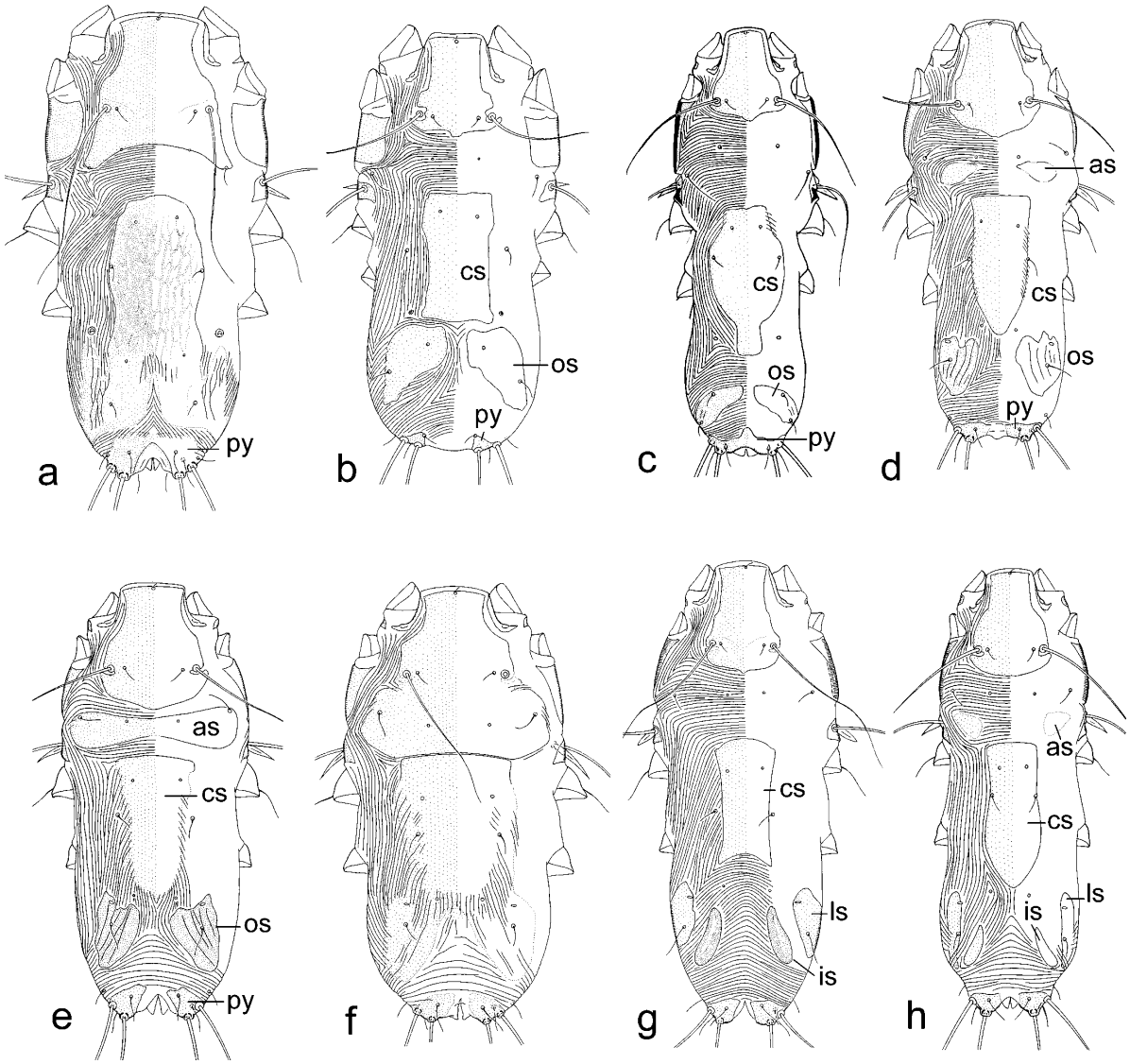

Figure 2 Schemes of dorsal idiosomal shields in females of *Pteroherpus* group. A, *Dicrurobius monacrotrichus*; B, *Vanginyssus schizurus*; C, *Micropteroherpus orthotomi*; D, *Pteroherpus aficanus*; E, *P. hoplophorus*; F, *P. megathyrus*; G, *P. diploplax*; H, *P. zosteropis*. Fragments of hysteronotal shield: as, anterior hysteronotal sclerites; cs, central sclerite; is, inner fragment of opisthosomals sclerite; ls, lateral fragment of opisthosomal sclerite; os, opisthosomal sclerite; py, pygidial sclerite.

Restriction to certain families and high host specificity of species of the *Pteroherpus* group suggest that their diversification could be the result of cospeciation with hosts. Realising that the species diversity of the *Pteroherpus* group is known incompletely, we nevertheless propose a provisional hypothesis explaining the origin of this distribution, based on the currently known host associations and a comparison of the phylogeny of the *Pteroherpus* group (Fig. 3) with current phylogenetic hypotheses for suprageneric taxa of passerines. Elaboration of this hypothesis is complicated by uncertainty of relationships between a number of passerine families, although significant progress in the phylogeny of passeriforms has been achieved, and relationships among most superfamilies have become relatively clear, owing to numerous recent molecular studies (Barker et al., 2001, 2004, Ericson et al., 2001, Ericson & Johanson, 2003; Beresford et al., 2005).

According to our hypothesis, the *Pteroherpus* generic group originated on an ancestral passerine prior to the separation of the infraorder Passerida and the so-called 'core Corvoidea' (Corvida), branching out from the common stalk of pteronyssids associated with passeriforms. The core Corvoidea is the most species-rich monophyletic branch of the paraphyletic infraorder Corvida (Barker et al., 2004).

Two genera, *Dicrurobius* and *Vanginyssus*, separated early (Fig. 1) and clearly diversified within the limits of the corvoidean families Dicruridae and Vangidae, respectively. Our analysis did not show that *Dicrurobius* + *Vanginyssus* make a monophyletic lineage. Therefore, it is impossible to state firmly that the ancestors of these genera appeared on drongos and vangas as the result of co-speciation with corvoids, rather than due to a host shift. However, restriction of *Dicrurobius* to Dicruridae, a well outlined and widely dispersed corvoid family of tropical origin, and restriction of *Vanginyssus* to Vangidae, the endemic family of Madagascar, as well as the high specificity of their species, provide evidence that these genera had a long period of cospeciation from the origin of these avian families. Besides, both corvoid families are older in origin (Barker et al., 2004) than most passeridan families that are hosts of the *Pteroherpus* group.

With the infraorder Passerida, the lineage of the derived genera *Pteroherpus* and *Micropteroherpus* successfully diversified only on several families of the Old World warblers and allies (superfamily Sylvioidea), and apparently were not retained (or failed to disperse) onto other major branches of the infraorder, such as Passeroidea and Muscicapoidea (Fig. 3). The genus *Micropteroherpus* was formed on cisticolas, Cisticolidae, a diverse family of small warbler-like birds, formerly included into Sylviidae. The representatives of *Pteroherpus* have diversified on two branches of this superfamily: the *hoplophorus* group was formed on bulbuls,

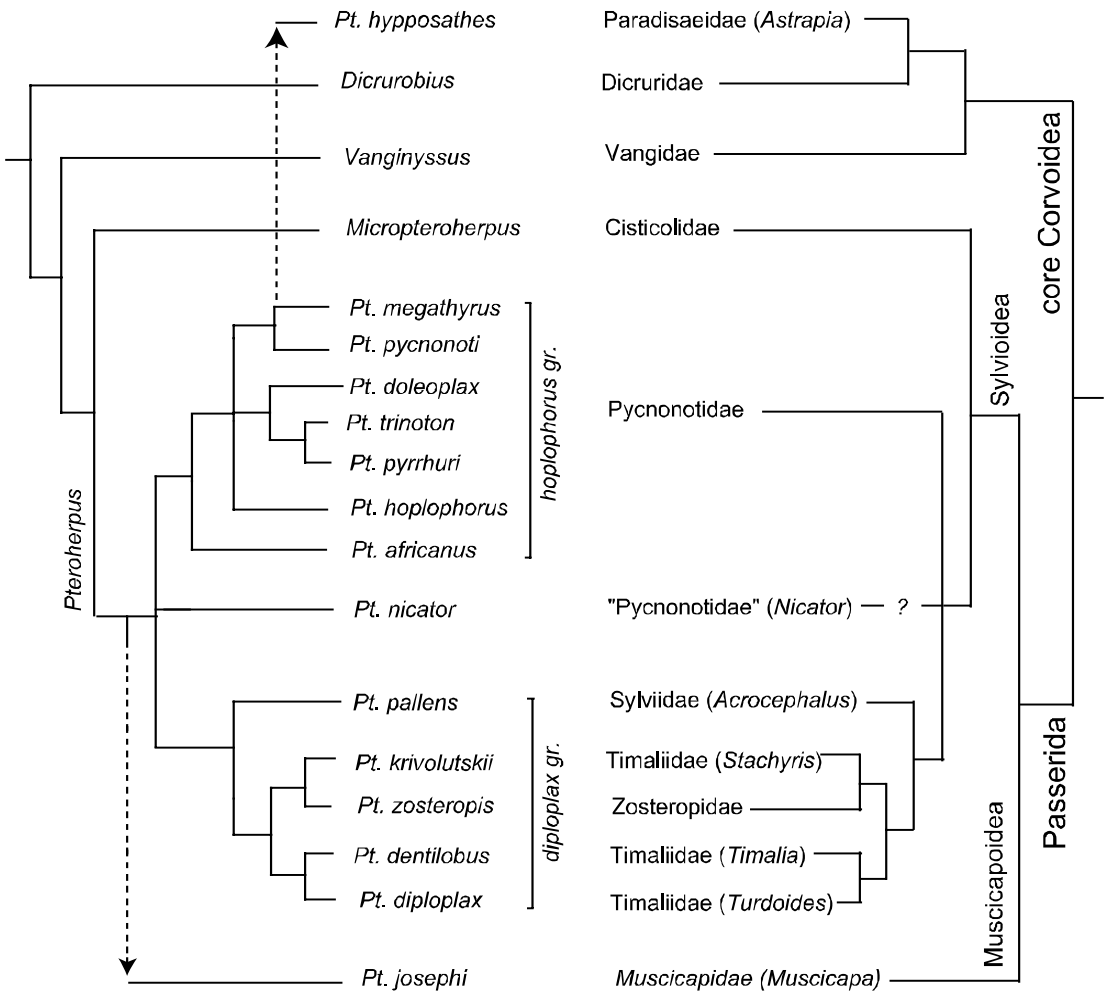

Figure 3 Scheme of host associations of the *Pteroherpus* generic group with passerine families. Phylogenetic relationships between avian families are modified and simplified from Barker et al. (2004) and Beresford et al. (2005); only branching of host families of the *Pteroherpus* group are depicted in the scheme. A bird genus is provided in those cases when mites are known from a single genus of a host family. ?, questionable relationships of avian taxon. Dashed lines indicate probable host shifts.

Pycnonotidae, and the *diploplax* group occupied hosts of three closely related families, Sylviidae, Timaliidae, and Zosteropidae. *Pteroherpus hipposathes* is the only species of the *hoplophorus* group occupying birds-of-paradise of the genus *Astrapia* (Paradisaeidae, Corvoidea), whereas other species of the group are associated with bulbuls. Association of this species with birds-of-paradise, very distant hosts belonging to another infraorder than bulbuls, is an obvious case of host-shifting. Within the *diploplax* group, it is worthwhile to note that *P. zosteropis* living on white-eyes is placed in the cladogram (Fig. 1) inside the cluster of *Pteroherpus* species (*P. diploplax*, *P. krivolutskii*, and *P. dentilobus*) associated with babblers (Fig. 3). This fits recent concepts that Zosteropidae originated within the Timaliidae (Beresford et al., 2005), i.e., babblers in the traditional sense are a paraphyletic group. Occurrence of *P. josephi* on one flycatcher species (Muscicapidae) belonging to the superfamily Muscicapoidea is also likely the result of an early host shift. An uncertain position of the unique species *P. nicator* may be explained by its association with nicators, *Nicator* Hartlaub et Finsch. Relationships of this avian genus, which was referred to shrikes (Laniidae, Corvoidea) till the 1990s, are still enigmatic: at present it is treated either as Pycnonotidae 'incerta sedis' (Dickinson, 2003) or as a separate lineage among families of Sylvioidea (Cibois et al., 2001, Beresford et al., 2005).

We expect that extensive studies of the mite fauna of passeriforms, especially those from the centres of passerine diversification in the Old World, will reveal many more pteronyssid species, which will help us better understand the phylogenetic relationships within Pteronyssidae and their evolution on avian hosts.

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Ontogeny of the famulus in selected members of Damaeidae (Acari: Oribatida) and its suitability as a phylogenetic marker

Jan Mourek[1,2] & Ladislav Miko[3]

[1]Department of Zoology, Charles University Prague, Faculty of Science, Viničná 7, CZ 128 44 Prague 2, Czech Republic. E-mail: jan_mourek@yahoo.co.uk; [2]Department of Biology and Environmental Education, Charles University, Faculty of Education, MD Rettigové 4, CZ-116 39 Prague 1, Czech Republic; and [3]Directorate ENV/B: Protecting natural resources, Avenue de Beaulieu 9, BE-1160 Brussels, Belgium

The famulus is a specialised seta on the dorsal side of the tarsus of the first pair of legs in acariform mites. It has various shapes, but a stable location within the whole group. In the oribatid family Damaeidae, two states of famulus are known: emergent and sunken. The emergent famulus is a simple short seta with fully emergent insertion. It is present in the adult of all known Damaeidae and in immature stases of many damaeid genera. The sunken famulus is reduced in size and submerged in a fovea with an elevated rim, so that only its tip is visible. In this study, the ontogenetic development of the famulus in selected Central European damaeid species, namely *Damaeus* (*Adamaeus*) *onustus*, *D.* (*Paradamaeus*) *clavipes*, *Epidamaeus tatricus*, *Spatiodamaeus verticillipes*, *Kunstidamaeus lengersdorfi*, and *Belba compta*, was studied with light and scanning electron microscopy and compared with that of *Gymnodamaeus bicostatus* (Gymnodamaeidae). Literature data on the ontogeny of the famulus in Damaeidae are summarized and the significance of the famulus as a phylogenetic marker is discussed. In agreement with previous studies, adults of all studied members of Damaeidae had an emergent famulus. The immatures of all studied members of *Damaeus* sensu lato, except for *E. tatricus*, had a sunken famulus, whereas the immatures of *B. compta* had an emergent famulus. Immatures as well as adults of *G. bicostatus* had a sunken famulus. In contrast to Norton's phylogenetic hypotheses, all immature stases of *E. tatricus* possessed an emergent famulus, similar to *B. compta*. Therefore, either the monophyly of *Epidamaeus* is questionable, or, more likely, reversal to a plesiomorphic state occurred in *E. tatricus*.

Key words: Famulus, ontogeny, morphology, Oribatida, Damaeidae, *Epidamaeus tatricus*, SEM

The current generic classification of the oribatid family Damaeidae is largely artificial (Bulanova-Zachvatkina, 1967; Balogh & Balogh, 1992; Perez Iñigo, 1997). Except for the preliminary phylogenetic study and partial revisions published by Norton (1977, 1978, 1979a,b) it has not been subjected to a cladistic analysis. Diagnoses of most damaeid genera are based mainly on leg chaetotaxy. According to Norton (1977a), identical setal formulas of a given leg segment may, at least in some cases, be the result of convergence due to reduction of particular setae or due to neotrichy, and thus are of limited value in phylogenetic classification. Therefore, careful homologisation of particular setae (including tracing their fate during ontogeny) and inclusion of qualitative characters are necessary.

Norton (1979a) attributed high value in phylogenetic classification of Damaeidae to the state of the famulus (sensu Grandjean, 1935) in immature stases. This minute setiform organ is present in proximal parts of the dorsal or dorsolateral side of tarsi I in Acariformes, usually close to the tarsal solenidia (Grandjean 1940, 1941, 1954a,b). Due to its stable location, the famulus can be homologised within the Acariformes. In several families of Prostigmata and rarely in species of Palaeosomata, a famulus is present also on tarsus II (Grandjean, 1957; Norton, 1977a). The famulus has various shapes, but in most Brachypylina it has the form of a simple short pointed seta (Grandjean, 1935, 1940, 1941, 1946, 1951). Unlike the isotropic solenidia, the famulus is birefringent under polarised light (Grandjean, 1940). Alberti & Coons (1999) classified the famulus as a seta-like sensillum with a cytoplasmic core. The biological function of the famulus has not been explained completely. Based on its ultrastructure, it is probably a thermo-hygroreceptor (Leonovich, 1994; Alberti & Coons, 1999).

Among damaeid genera, two character states of the famulus are known (Grandjean, 1954b; Norton, 1979a): emergent and sunken (we consistently use the neutral descriptive terms 'emergent' and 'sunken' famulus, instead of 'normal' and 'reduced', to avoid phylogenetic assumptions about the character polarity).

Emergent famulus (Figs. 1a-d, 3a) – a simple short seta with fully emergent insertion. This is the form in adults of all known damaeid genera and also in immature stases of the majority of damaeid genera, e.g., *Belba*, *Caenobelba*, *Metabelba*, *Quatrobelba*, and *Porobelba*. According to Norton (1979a) the emergent famulus (referred as 'normal') in immatures represents the plesiomorphic state within the family.

Sunken famulus (Figs. 2a-c) – the seta is reduced in size and submerged in a fovea with an elevated rim ('sclerotised cup'), so that only its tip is visible. It is known from all immature stases of *Damaeus* (including all subgenera), *Epidamaeus*, *Spatiodamaeus*, *Dyobelba*, and *Lanibelba* (Grandjean, 1954b; Norton, 1979a). Norton (1979a) proposed that the sunken famulus (referred to as 'reduced' or 'vestigial') in immatures is apomorphic within the Damaeidae, and suggested that the group of genera sharing this character is monophyletic.

The ontogeny of the famulus is known only for a limited number of damaeid species (Table 1) and detailed studies of more species might change the distribution pattern within the family. For example, Norton & Ryabinin (1994) described *Dyobelba reevesi* Norton et Ryabinin as having an emergent famulus in the immatures. In out-group comparisons, the polarity of the character is not so clear, because the sunken famulus is also known in the immatures, as well as in the adults, of several other families of the group Eupheredermes sensu Grandjean (1953), such as Zetorchestidae and Gymnodamaeidae (Grandjean, 1951, 1954a).

In the present study, the ontogeny of the famulus in five European members of *Damaeus* sensu lato, representing dif-

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ferent genera/subgenera, was studied with light and scanning electron microscopy (SEM) and compared with members of *Belba* (Damaeidae) and *Gymnodamaeus* (Gymnodamaeidae).

Notes on the nomenclature

The studied species were identified according to Miko (2006) and we follow the same generic concepts. *Epidamaeus* Bulanova-Zachvatkina, *Spatiodamaeus* Bulanova-Zachvatkina, and *Kunstidamaeus* Miko are considered separate genera, whereas *Damaeus* (*Paradamaeus*) Bulanova-Zachvatkina and *Damaeus* (*Adamaeus*) Norton are classified as subgenera within *Damaeus* CL Koch. We use the term '*Damaeus* sensu lato' for members of all these taxa including *Epidamaeus*. We accept the single family concept of Damaeidae as proposed by Norton (1979a) and reject the splitting into three separate families (Damaeidae, Belbidae, Belbodamaeidae), as proposed by Bulanova-Zachvatkina (1967). On the other hand, we accept the transfer of *Hungarobelba* Balogh from Damaeidae to Hungarobelbidae Miko et Travé.

MATERIAL AND METHODS

Species studied and collection of specimens

Adult specimens of the species studied were obtained in the following 11 localities of the Czech Republic (CR) and Slovak Republic (SR).

Belba compta (Kulczynski): CR, north-eastern Bohemia, Krkonoše Mountains, Údolí Bílého Labe, 50°44'46"N, 15°37'47"E, about 1,000 m above sea level (asl), dead spruce forest (*Picea abies*) with young spruce trees in the undergrowth, wet mosses (*Polytrichum commune*, *Sphagnum* sp.) on granite blocks, 11-06-2003, J Mourek & J Materna leg.

Damaeus (*Paradamaeus*) *clavipes* (Hermann): CR, central Bohemia, west from Karlštejn village, western slope of the Prostřední vrch hill, 49°56'13"N, 14°10'1"E, about 280 m asl, decidous forest of hornbeam (*Carpinus betulus*) and oak (*Quercus* sp.), mixed litter and upper soil layers, 26-05-2003, J Mourek leg.

Damaeus (*Adamaeus*) *onustus* CL Koch: CR, central Bohemia, east from Davle town, valley of the last left tributary of the Záhoranský brook near the confluence, 49°53'50"N, 14°25'25"E, about 260 m asl, deciduous forest of hornbeam, oak, and lime, mixed litter and upper soil layers, 28-05-2003, J Mourek leg.; CR, eastern Bohemia, Nekoř village, 50°3'57"N, 16°32'41"E, about 480 m asl, shrubs of hazel (*Corylus avellana*) and European elder (*Sambucus nigra*), mixed litter and upper soil layers, 18-06-2006, J Mourek leg.

Epidamaeus tatricus (Kulczynski): CR, north-eastern Bohemia, Krkonoše Mountains, beech forest (*Fagus sylvatica*) Buková stráň, eastern slope, 50°44'31"N, 15°32'14"E, about 1100 m asl, south from Malá Kotelní jáma, beech litter and upper soil layers, 11-06-2003, J Mourek & J Materna leg.; SR, Malá Fatra Mountains, mixed growth of dwarf pine (*Pinus mugho*), rowan tree (*Sorbus* sp.), willow (*Salix* sp.), 49°13'5"N, 19°5'40"E, about 1,350 m asl, mixed litter and upper soil layers, 09-08-2004, J Mourek leg.

Spatiodamaeus verticillipes (Nicolet): CR, southern Bohemia, Třeboňsko Biosphere Reservation, peat bog 'Červené Blato' and adjacent scots pine forest (*Pinus sylvestris*), 48°51'20"N, 14°47'46"E, about 470 m asl, mixed litter of scotch pine and birch (*Betula* sp.) and upper soil layers, 17-05-2003, J Mourek leg.; CR, southern Bohemia, Trebonsko Biosphere Reservation, avenue of old oaks on dam between ponds Nový Vdovec and Ženich, 49°1'24"N, 14°50'30"E, 430 m asl, oak litter and upper soil layers, 17-05-2003, F Štáhlavský leg.; CR, central Bohemia, east from Družec village, 50°5'49"N, 14°3'2"E, about 390 m asl, mixed forest of scots pine, beech, lime, and hornbeam, mixed litter and upper soil layers, 25-05-2003, J Mourek leg.

Kunstidamaeus lengersdorfi (Willmann): SR, Slovak karst, 'Čertova diera pri Domici' cave, 49°28'0"N, 20°27'30"E, about 350 m asl, rotten wood, 20-05-2005, P Ľuptáčik leg.

Gymnodamaeus bicostatus (CL Koch): CR, southeastern Moravia, northeast from Pouzdřany village, Pouzdřanská step national reserve, eastern slope, shrubs of bladdernut (*Staphylea pinnata*), plum tree (*Prunus domestica*), and field maple (*Acer campestre*), 48°56'35"N, 16°38'37"E, about 390 m asl, mixed litter, 16-09-2006, J Mourek leg.

The mites were extracted in modified Berlese-Tullgren extractors. Part of the samples was extracted into 80% ethanol and adults were sorted and used for light microscopic and SEM observation. The rest of the samples were extracted into plastic vials with a layer of moistened plaster of Paris on the bottom; live adult specimens were sorted and used as stock for laboratory cultures. The specimens of *K. lengersdorfi* were hand-collected directly in the field, using a fine brush.

Laboratory cultures

Immature stases were obtained from laboratory cultures kept in polyethylene test-tubes (3 cm in diameter, 3.5 cm high). The bottom of the tube was removed and replaced with a 5-8 mm layer of plaster of Paris mixed with charcoal (20:1). A central hole (3 mm in diameter) in the polyethylene lid covered with fine mesh (0.1 × 0.1 mm) ensured aeration. The bottoms of the rearing tubes were embedded in regularly moistened sand in plastic containers, to avoid dessication. Five to 20 adults were introduced in a tube. The mites were fed green bark algae (*Desmococcus* spp.) and kept at 16 °C. Culturing of *K. lengersdorfi* failed, therefore field-collected immatures were studied.

Sample preparation and SEM-observation

Adults and immatures were fixed in 80% ethanol, then dehydrated in an ascending series of ethanol (80, 96, and 100%), next in a series of mixtures of 100% ethanol and acetone (2:1, 1:1, and 1:2) ending with 100% acetone, and finally dried with the critical point method. Specimens were mounted on aluminium stubs with double-sided carbon adhesive tape and coated with gold. Adults of *E. tatricus* were macerated in hot pure lactic acid and the thick layer of filamentous cerotegument was removed with fine needles prior to preparation for SEM. This procedure allowed the observation of details of the setae. Mites were observed and photographed with a Jeol 6100 SEM in the Lab. of Electron Microscopy (Inst Parasitology, Acad Sciences, Ceské Budejovice, CR) and with a Jeol 6380 LV SEM in the Lab. of Electron Microscopy (Dept Parasitology, Fac Science, Charles Univ, Prague, CR). Three-five specimens per particular stase of each species were examined.

RESULTS

The famulus did not vary noticeably within any particular developmental stase of any species studied. The famulus in

all stases of all species, except for the immatures of *E. tatricus*, was inserted on a line connecting the insertions of solenidia ω1 and ω2 (or lateral to solenidion ω1 in the larva, since ω2 appears first in the protonymph). By contrast, the famulus in immatures of *E. tatricus* was inserted slightly distally from the line connecting both solenidia. Adults of all studied damaeids had a normally developed emergent famulus (Figs. 1d, 2d), in the form of a simple short pointed seta, either straight or slightly curved.

In all immature stases of *D.* (*A.*) *onustus*, *D.* (*P.*) *clavipes*, *K. Lengersdorfi*, and *S. verticillipes*, the famulus was sunken (Figs. 2a-c). Its base was submerged in a narrow fovea beneath the general tarsal surface and cuticle surrounding the fovea formed a slightly elevated rim. The foveal opening was either oval or guttiform. At high magnification (≥4,000×) the minute pointed famulus, with slightly emergent tip, was recognizable inside the fovea.

In contrast to other species of *Damaeus* sensu lato, all immature stases of *E. tatricus* had a fully emergent famulus (Figs. 1a-c) of the same appearance as that of adults. It did not differ noticeably among particular stases, except that it was relatively shorter in the larva than in later stases. In some specimens, the famulus was sparsely covered with a fine granular cerotegument. In immatures of *B. compta* (Fig. 3a) the famulus was emergent as in *E. tatricus*. In *G. bicostatus*, the famulus was sunken in all developmental stases, including the adult (Fig. 3b). The tip of the seta was not visible even at high magnification (≥4,000×). The famulus was inserted slightly distally from the line connecting the tarsal solenidia.

DISCUSSION

All published data on the ontogeny of the famulus in Damaeidae are summarized in Table 1. Morphology of immature Damaeidae is known in a few species and most of the genera are insufficiently represented. Moreover, several authors did not include small morphological details such as the ontogeny of the famulus (Sitnikova, 1959; Moraza et al., 1990; Enami, 1992).

Our observations of the emergent famulus in all stases of *B. compta* are consistent with findings of Grandjean (1954b), Norton (1977a, 1978), and Norton & Pallacios-Vargas (1982) on several other species in this genus. Also our finding of a sunken famulus in immatures of *D.* (*A.*) *onustus*, *D.* (*P.*) *clavipes*, and *S. verticillipes* agrees with observations of Grandjean (1935, 1954b) and Norton (1977a) for the same species. The same character state was previously documented in seven species of the nominal subgenus *Damaeus* (*D.*), which was not included in our study (Grandjean, 1954a, 1960; Norton, 1977b). The hypothesis of Norton (1979a) on the synapomorphy of the sunken famulus in immatures of

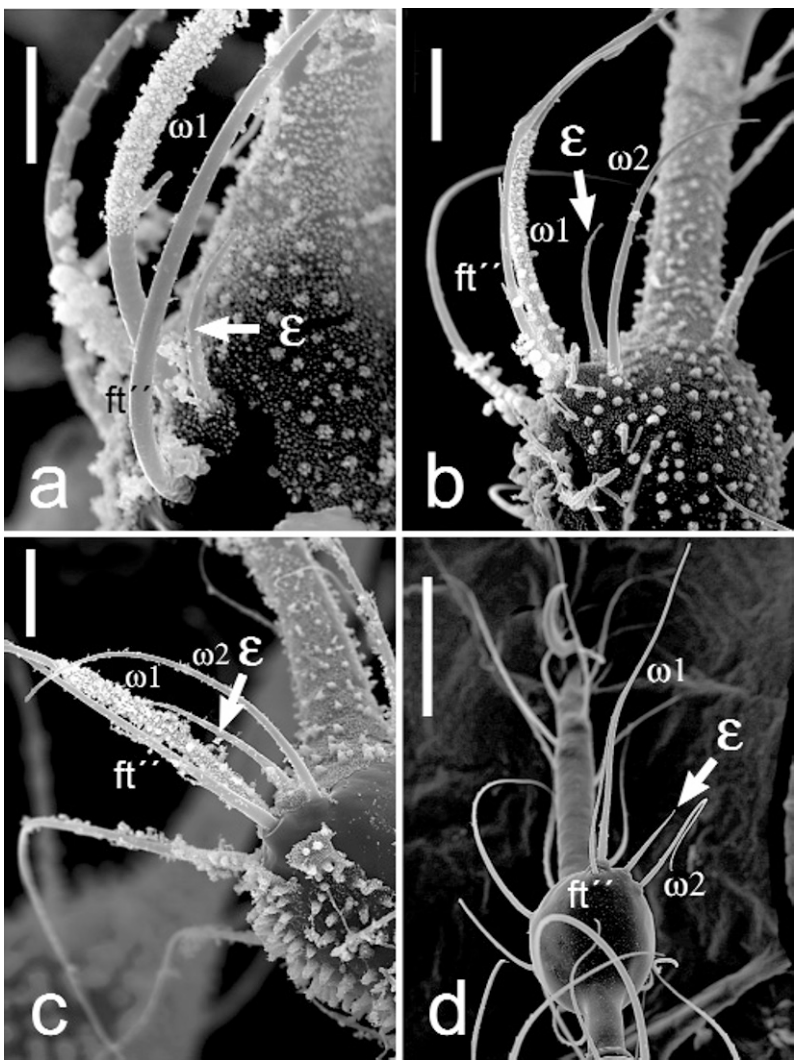

Figure 1 SEM micrographs showing ontogeny of the famulus (arrows) and solenidia on tarsus I (on the right leg) in *Epidamaeus tatricus*. The famulus is emergent in all stases of this species. (a) larva, dorsolateral aspect; (b) protonymph, dorsolateral aspect; (c) deutonymph, dorsolateral aspect; (d) adult, dorsal aspect (specimen macerated in hot lactic acid, cerotegument removed). Scales: (a) 5 μm; (b,c) 10 μm; (d) 50 μm. Abbreviations used: ft", antiaxial fastigial seta; ε, famulus; ω1,ω2, tarsal solenidia.

Damaeus sensu lato, *Lanibelba*, and *Dyobelba* is further supported by our findings on *K. lengersdorfi*.

The unexpected presence of an emergent famulus in immatures of *E. tatricus* is the second known case within the clade including *Damaeus* sensu lato, *Lanibelba*, and *Dyobelba*. Norton & Ryabinin (1994) found emergent famuli in all nymphal stages of *Dy. reevesi* (the larva was not available), although they were uncertain about the generic placement of this species. These two exceptions do not necessarily disprove synapomorphy of the sunken famulus in this clade, but they show that it should be given less 'weight' or at least be interpreted with caution. Pending a more detailed

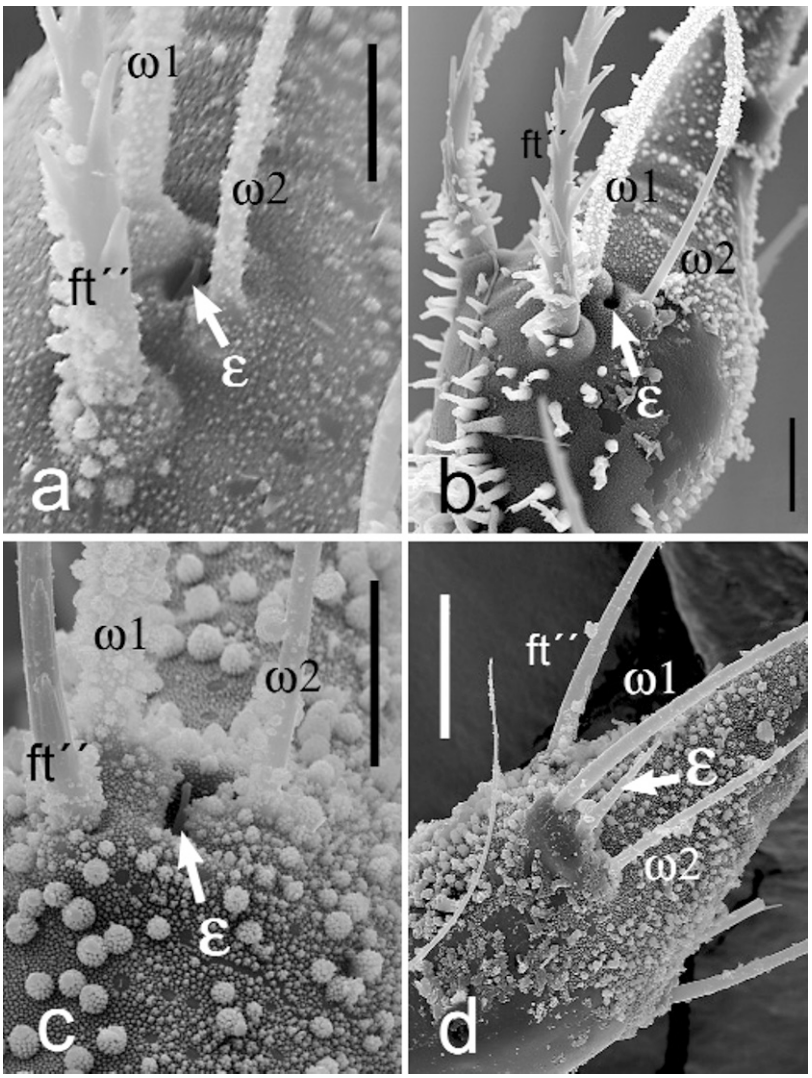

Figure 2 SEM micrographs showing ontogeny of the famulus (arrows) and solenidia on tarsus I (on the right leg) in *Spatiodamaeus verticillipes* and *Kunstidamaeus lengersdorfi*. The famulus is sunken in immature stases of these species and emergent in adults. (a) *S. verticillipes*, deutonymph; (b) *S. verticillipes*, tritonymph; (c) *K. lengersdorfi*, tritonymph; (d) *K. lengersdorfi*, adult. All figures are in dorsolateral aspect. Scales: (a) 5 μm; (b,d) 20 μm; (c) 10 μm. Abbreviations used: ft", posterior fastigial seta; ε, famulus; ω1,ω2, tarsal solenidia.

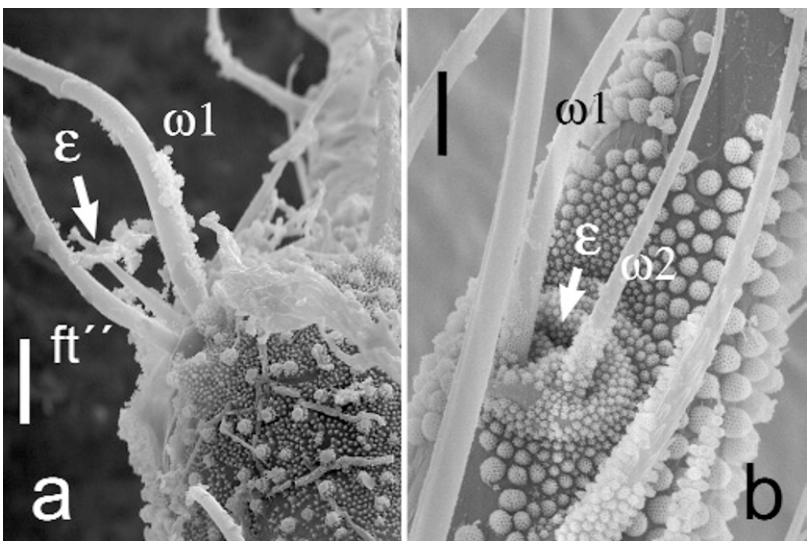

Figure 3 SEM micrographs showing ontogeny of the famulus (arrows) and solenidia on tarsus I in *Belba compta* and *Gymnodamaeus bicostatus*. The famulus is emergent in all stases of *B. compta* and sunken (externally invisible) in all stases of *G. bicostatus*. (a) *B. compta*, protonymph, dorsomedian aspect; (b) *G. bicostatus*, adult, dorsolateral aspect. Scales: (a,b) 5 μm. Abbreviations used: ft", antiaxial fastigial seta; ε, famulus; ω1,ω2, tarsal solenidia.

Table 1 Current knowledge of famulus ontogeny in members of the family Damaeidae. All species with described morphology of immatures are included. Famulus in immatures is emergent (E), sunken (S), or the description does not include information on the state of the famulus (?).

Species	Famulus	Source
Belba (B.) compta (Kulczynski)	E	present study
Belba (B.) corynopus (Hermann)	E	Grandjean, 1954b
Belba (*B.*) *clavasensilla* Norton et Pallacios-Vargas	E	Norton & Pallacios-Vargas, 1982
Belba sp. A[1]	E	Norton, 1977a
Belba sp. B[1]	E	Norton, 1977a
Belba (Protobelba) californica (Banks)	E	Norton, 1978
Caenobelba alleganiensis Norton	E	Norton, 1979b
Metabelba pulverosa Strenzke	E	Norton, 1977b
Metabelba papillipes (Nicolet)	E	Grandjean, 1954a
Porobelba spinosa (Sellnick)	E	Grandjean, 1954a
Quatrobelba montana Norton	E	Norton, 1979b
Damaeus (*Adamaeus*) *onustus* CL Koch	S	Grandjean, 1935, 1954b; Norton, 1977a; present study
Damaeus (*D.*) *angustipes* (Banks)	S	Norton, 1977a,b
Damaeus (D.) arvernensis Grandjean	S	Grandjean, 1960
Damaeus (*D.*) *grossmanni* (Wilson)	S	Norton, 1977b
Damaeus (*D.*) *opilioides* Norton	S	Norton, 1977b
Damaeus (*D.*) *atlanticus* Norton	S	Norton, 1977b
Damaeus (*D.*) *riparius* (Nicolet)	S	Grandjean, 1954b
Damaeus (D.) crispatus (Kulczynski)	S	Grandjean, 1954b
Damaeus (*Paradamaeus*) *clavipes* (Hermann)	S	Grandjean, 1954b; Norton, 1977a; present study
Epidamaeus (Akrodamaeus) *longiseta* (Banks)	S	Norton, 1978
Epidamaeus (E.) tatricus (Kulczynski)	E	present study
Epidamaeus (E.) verrucatus Enami et Fujikawa	?	Enami & Fujikawa, 1989; Enami, 1992
Epidamaeus sp. A[1]	S	Norton, 1977b
Epidamaeus sp. B[1]	S	Norton, 1977a
Epidamaeus sp. C[1]	S	Norton, 1977a
Kunstidamaeus lengersdorfi (Willmann)	S	present study
Spatiodamaeus boreus Bulanova - Zachvatkina	?	Sitnikova, 1959
Spatiodamaeus verticillipes (Nicolet)	S	Grandjean, 1954b; present study
Dyobelba carolinensis (Banks)	S	Norton, 1978
Dyobelba reevesi Norton et Ryabinin	E[2]	Norton & Ryabinin, 1994
Dyobelba dindali Bayartogtokh et Norton	S	Bayartogtokh & Norton, 2007
Lanibelba pini Norton	S	Norton, 1979b

[1] subgenus is unknown; [2] larva is unknown.

cladistic analysis that includes more species and other independent characters, it is unclear whether these two cases represent ancestral or derived states within the clade, or whether they are misplaced in *Epidamaeus* and *Dyobelba*.

In *Epidamaeus*, ontogeny of the famulus is known in few of the >70 described species (Bayartogtokh, 2000c). Norton (1977a, 1979a) did not explicitly list the species of *Epidamaeus* he studied and some of them are still undescribed (Norton, pers. comm.). *Epidamaeus longiseta* (Banks), a member of the subgenus *Epidamaeus* (*Akrodamaeus*), is the only species of the genus for which the sunken state of famulus in immatures was explicitly documented (Norton, 1978). *Epidamaeus tatricus* belongs to the nominate subgenus *Epidamaeus* (*E.*). Furthermore, even the nominate subgenus *Epidamaeus* in the current sense represents a morphologically heterogeneous group and might be polyphyletic or paraphyletic (e.g., Behan-Pelletier & Norton, 1983, 1985; Luxton, 1989; Enami & Fujikawa, 1989; Enami, 1992; Enami et al, 1994; Bayartoktokh, 2000a,b,c, 2001). The genus diagnosis of *Epidamaeus* is weakly defined and should be further revised.

In our view, *E. tatricus* is not a typical member of *Epidamaeus* (*E.*). This species, together with, e.g., *E. setiger*, differs from most other known species of the genus by the presence of four setae (instead of three) on genua III and IV. They might represent a sister taxon to the rest of the genus. However, complete reduction of prodorsal tubercles in *E. tatricus* (seen also in several other members of *Epidamaeus*) does not indicate a basal position within the group. There is no doubt that *E. tatricus* is a member of *Damaeus* sensu lato and we believe that independent reversal of the famulus in its immatures from the sunken to the emergent state is the most likely explanation, and perhaps the same is true of *D. reevesi*, if indeed the later is congeneric with other species of *Dyobelba* (see also Bayartogtokh & Norton, 2007). Because the emergent famulus is retained in adults, its reappearance in immatures would probably require only a minor change in the genetic control of development. Even such a conserved character as the famulus is subject to variation; Grandjean (1960) described a case of accidental loss of the famulus in some adult specimens of *Damaeus arvernensis* (Grandjean).

The polarity of famulus character states in immatures of Damaeidae proposed by Norton (1979a) also needs careful revision (see Norton & Ryabinin, 1994). It is uncertain whether the sunken famulus in all stases (including adults) of Gymnodamaeioidea (Grandjean, 1954a; confirmed in our study) and Zetorchestidae (Grandjean, 1951) developed independently from that in immatures of Damaeidae. The phylogenetic relations of Damaeidae and other families within *Eupheredermes* are still unresolved (Maraun et al., 2004).

Functional aspects of the sunken famulus have not yet been studied. We believe that this state represents a specialized and functional sensory organ, rather than a vestigial or nonfunctional state. It is possible that the fovea provides

some protection of the setal part of the famulus or that it somehow increases the efficiency of the reception.

Acknowledgements

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Food competition and feeding behavior and its implications for the phylogeny of the Histiostomatidae (Astigmata)

Stefan Wirth
Museum für Naturkunde Leibniz, Institut für Evolutions- und Biodiversitätsforschung an der Humboldt-Universität zu Berlin, Abteilung Spinnentiere, Königin-Luise-Str. 1-3, 14195 Berlin, Germany. E-mail: wirthstef@web.de

The feeding behavior of *Bonomoia opuntiae* Wirth was studied in comparative studies on other species of Histiostomatidae, a large monophyletic subgroup of the Astigmata. *Bonomoia opuntiae* was collected from rotting pieces of *Opuntia* sp. in the Mediterranean area and is morphologically derived, especially with respect to the mouthparts, which are distinctly modified relative to the typical histiostomatid gnathosoma. Distal pedipalps and distal chelicerae are adapted so as to pick up pieces of the natural microorganism cover from the ground. To enable feeding on this substrate, the distal chelicerae are spoon-shaped and projected inwards and they can be moved against each other like a clasp organ. This mite *B. opuntia* was frequently observed to move into muddy substrates and to swim in more watery habitats. The leg movements supporting locomotion in these habitats are quite unusual: Each pair of legs I and II move from anterior to posterior position, but the two pairs do so asynchronously. When legs II move to the anterior position over legs I, legs I just start to move backwards. This mode of locomotion is termed 'leg-crossing'. In the field this mite was found to live syntopically with *Histiostoma* sp. Probably due to the unusual adaptations to use other food resources, *B. opuntiae* survives under laboratory conditions even in the presence of *Histiostoma* sp. This is striking, because most observed histiostomatid species show very similar food preferences and feeding mechanisms in the laboratory, so that they are strong food competitors and cannot be cultured in mixtures.

Key words: Astigmata, *Bonomoia opuntiae*, feeding behaviour, food competition, *Histiostoma* sp., Histiostomatidae, SEM

The Histiostomatidae are characterized by a high biological and ecological diversity (e.g., Scheucher, 1957; Hughes & Jackson, 1959). Various arthropods, mainly insects, enabled the mites to colonize new habitats, where sometimes distinctly modified mouthparts evolved (Wirth, 2004b, 2006).

In all species, the digitus mobilis of the chelicera is reduced to an immovable remnant structure, whereas the distal pedipalp article is projected laterally and connected to a complex membranous structure shaped by the coxal endites. In the stem species of the Histiostomatidae both structures form an organ to push off bacteria in an emulsion and to gather them in small heaps in front of the gnathosoma (Wirth, 2004a).

Due to the taxonomic focus in earlier research on the Histiostomatidae (Scheucher, 1957; Hughes & Jackson, 1959) biological information on these mites is scarce (Wurst & Kovac, 2003). Competitive and other interactions between histiostomatid species have not been studied. *Bonomoia opuntiae* Wirth and an unidentified *Histiostoma* sp. associated with *Opuntia* sp. were studied with regard to their behaviour and competitive interactions in the same habitat. Because the mouthparts of *B. opuntiae* are modified in a way distinct from *Histiostoma* sp. (Wirth, 2006), the difference in feeding behaviours between these species, was studied. Details on how food particles are crushed were observed, using scanning electron microscopy (SEM) to fix mites during their feeding activities.

MATERIALS AND METHODS

Mite species were reared in small culture vials (5 cm in diameter) with 1.5% water agar at room temperature (26-28 °C) and with rotting pieces of potato to stimulate microorganism growth. Movements and feeding behaviour of *B. opuntiae*, *Histiostoma* sp., *H. feroniarum*, and *H. palustre* were studied in liquid drops that were picked out of the culture dishes using a Zeiss Axiophot light microscope and a digital video camera. At least 10 specimens of each species were studied in all behavioural studies.

SEM objects for feeding behaviour studies were fixed in vivo with hexamethyl-disilazane (Nation, 1983) that was dropped over mites while feeding on the surface of a rotting piece of *Opuntia*. Thereafter, these objects were left to dry for at least 24 h in an incubator at 37 °C. Next, the objects were sputtered with gold.

RESULTS

Habitats

The Histiostomatidae live in habitats that exist for a short while only. To disperse to fresh habitats, phoretic transport is required. Different kinds of habitats are colonized, such as animal dung, wooden environments, slime flows of trees, watery habitats, and fluids of pitcher plants (*Sarracenia*, *Nepenthes*). Many morphological and behavioural adaptations to these habitats have been discovered, but when reared in the same units under laboratory conditions most histiostomatid species are competitors, one quickly replacing the other (Wirth, 2004a).

Contrary to these laboratory experiences, *B. opuntiae* and *Histiostoma* sp. were found syntopically inside fast-rotting pieces of *Opuntia* sp. from southern Italy (Napoli, Sardinia). Both species survived in the same culture dishes for months or even longer which is unusual for histiostomatid species (Wirth, 2004a). Therefore, the habitat structure was observed more closely to describe the preferred substrates of these species (Fig. 1A).

Both species live in partially rotting pieces of the cactus *Opuntia* sp., found usually dislodged from the plant, but

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nearby on the ground. Mites, nematodes, and other arthropods colonize this microhabitat. *Bonomoia opuntiae* and *Histiostoma* sp. were found in cactus pieces that had green (= live) areas and dark-brown rotting areas, sometimes of a watery consistence. Subhabitats are not clearly distinguishable and instead form smooth transitions. Around green intact areas, microorganism growth produces a lightly brown-yellow film that separates larger intact plant pieces from each other (Fig. 1A). Further beyond remnants of intact plant material are more decomposed and, here, the microorganism film is much thicker, light-brown and viscous (Fig. 1A). Fully decomposed areas appear watery, muddy and dark-brown (Fig. 1A). Numerous fissures usually run along the plant surface. *Bonomoia opuntiae* (Fig. 1A) was observed to prefer submerging in the muddy subhabitat, whereas it is more rarely seen 'swimming' in watery areas. The *Histiostoma* sp. (Fig. 1A), however, moves superficially on the thin film of microorganisms in the area of lightly decomposed plant material. Because the subhabitats in cactus pieces form a continuum, there are always areas in which both species live next to each other. Eggs of both species are laid into the wetter areas within the cactus pieces.

Mouthparts and feeding behavior

From a morphological point of view, the gnathosoma of all Histiostomatidae is derived (Wirth, 2004a). The adaptations probably evolved to enable feeding on a larger quantity of microorganisms in emulsion. These modifications usually concern structures at the distal gnathosoma, such as the laterally projected distal pedipalp segments and some complex membranous structures. The gnathosoma of *B. opuntiae* is distinct from that of other Histiostomatidae. The distal digitus fixus consists of a proximal single lappet pointing downwards and a spoon-shaped apex, which is ventrally divided into two rounded parts (Fig. 1B, right and left). When both chelicerae are extended maximally, one spoon encloses the neighbouring anteriorly. Usually the right chelicera (observed at the dorsal side) was observed in the most anterior position.

Contrary to *B. opuntiae*, the terminal parts of the chelicerae in *Histiostoma* sp. run parallel to each other, as is usual for histiostomatids. The distal digitus fixus is similar to that of *H. feroniarum* and has a constant number of ventral cuticular teeth of different sizes (Figs. 1E, 2D): starting from the proximal side, there are six large and four small teeth, of which three are stuck together. The distal end is seta-shaped and points to the anterior end.

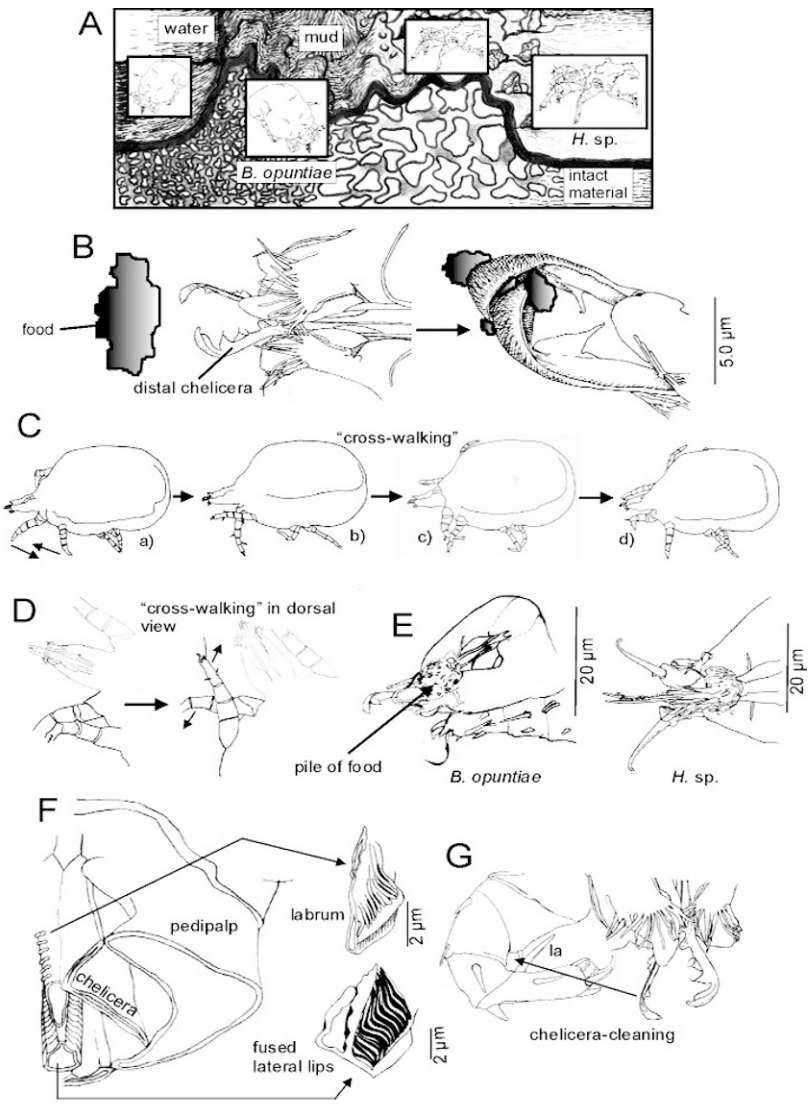

Figure 1 (A) Schematic section through the *Opuntia* habitat, showing habitat changes from intact plant material to muddy and watery areas. The preferred subhabitats of *Bonomoia opuntiae* and *Histiostoma* sp. are shown. (B) Use of the chelicerae as a clasp organ in *B. opuntiae*. On the left chelicerae are protruded and therefore at some distance from each other; on the right the chelicerae hold the food substrate and touch each other following complete protrusion. (C) 'Leg-crossing' mode of locomotory behaviour, (a)-(d) reconstruction of subsequent steps during walking based on video-sequences, taken from a lateral viewpoint. (D) Two sequences from a dorsal viewpoint, leg II before moving over leg I (left) and afterwards (right). (E) Aspect of feeding in both histiostomatid species: partitioning of food particles in the distal area of the gnathosoma after collecting the food first as dorsal piles. (F) SEM-based reconstruction from a razor blade section of the distal mouthparts of *B. opuntiae*, with labrum and fused lateral lips (on the right) in separate drawings. (G) Drawing to illustrate the cleaning behaviour of the distal chelicerae: distal digitus fixus is pulled through seta la and the proximal leg area.

Cheliceral movements differ in the two species, and different food types are preferred. The chelicera of *B. opuntiae* is used as a clasp organ (Fig. 1B, right and left drawing). Both chelicerae are moved simultaneously while clasping the food. They alternate to macerate the food into pieces and to transport the pieces to the preoral cavity. When moved outwards, the terminal ends are still without contact to each other and the 'clasp organ' is opened (Fig. 1B, left). Because the whole chelicerae are lightly vaulted and close to each other, their distal endings are pressed together due to the opposition of the internal walls of the distal pedipalps when the chelicerae are completely extended (Fig. 1B, right). Contrary to other histiostomatids, *B. opuntiae* feeds on flakes of microorganisms that are stuck to each other. They are picked up from watery areas on the ground or from the muddy subhabitat by the aid of the cheliceral clasp organ. After clasping the food, the chelicerae usually are retracted simultaneously. In this way, they divide the flakes into smaller pieces thereby enabling transport to the distal gnathosoma. Additional alternating movements with the chelicerae enable the splitting of food into particles. Now and then, the chelicerae are subject to cleaning, a behaviour observed in *B. opuntia* only. Using the right or left leg, the distal digitus fixus gets pulled through tarsal setae la and the femora of legs I (Fig. 1G).

As usual in histiostomatids (Wirth, 2004a), *Histiostoma* sp. feeds on a thin film of microorganisms in an emulsion. Two feeding modes were differentiated in this and a related species, i.e. *H. feroniarum,* which has a similar saw-like digitus fixus: (1) During locomotion (Fig. 2F), the microorganisms in emulsion are pushed into small heaps in front of the gnathosoma by the aid of laterally directed distal pedipalp articles (Fig. 2E). (2) The distal chelicerae are driven deep into the substrate (Fig. 2C). The distal laterally vaulted pedipalp articles together with ventral palparmembrane lobes perform a counterpressure to facilitate extraction of the chelicerae out of the substrate (Figs. 2A, B). Meanwhile the chelicerae are alternately moved, and food particles adhere to the teeth, ventral of the digitus fixus, via which they are transported to the distal preoral cavity (Fig. 2D).

In all observed histiostomatid species, food particles accumulate around the distal labrum and fused lateral lips, and finally surmount the dorsal surface of the gnathosoma (Fig. 1E, left and right). In this region parallel circular grooves run around the fused lateral lips (Fig. 1F, left and lower right drawing). Originating from the labrum, flexible setae-like

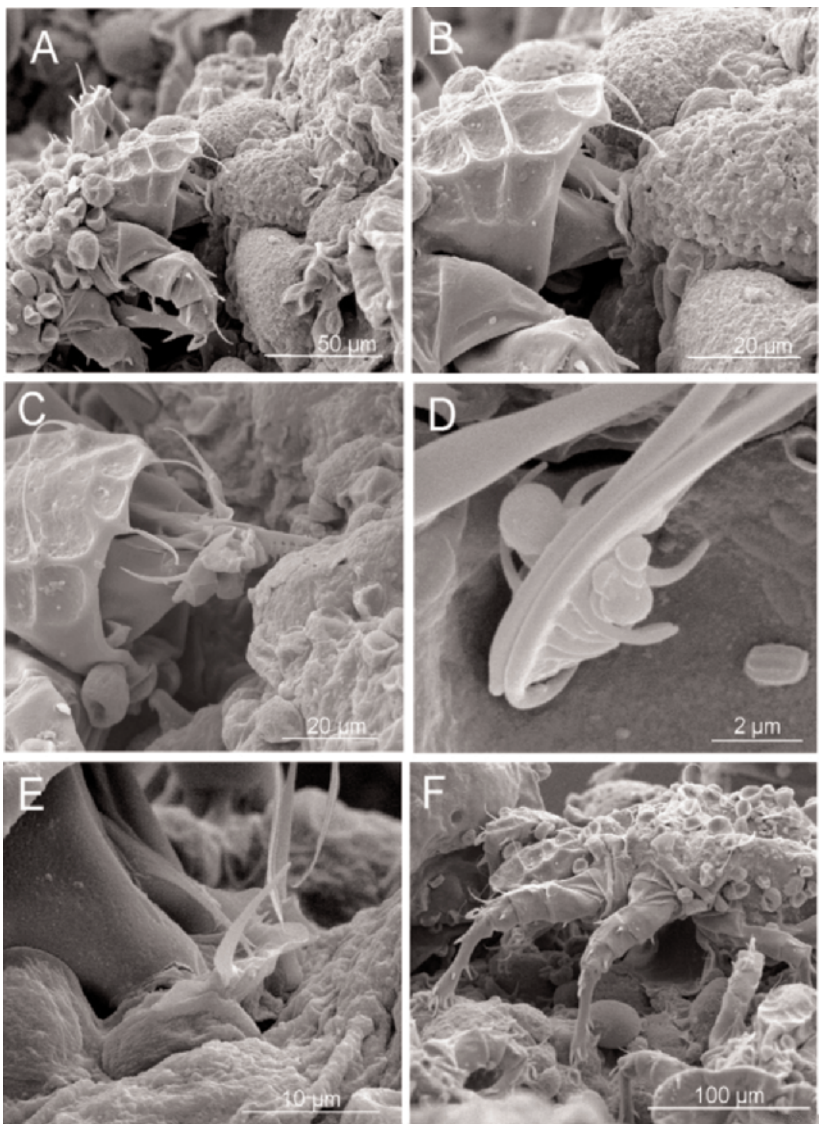

Figure 2 SEM pictures of *Histiostoma feroniarum*, which is closely related to *Histiostoma* sp. from the *Opuntia* habitat. (A) Specimen presses the laterally directed distal pedipalps against the heap of substrate. The distal chelicerae are drilled into the substrate to scrape out food particles. (B) Magnification of the same object. (C) Alternating cheliceral movements scrape microorganisms from the substrate. (D) Seta-like processes, ventral of the distal digitus fixus, hold food particles. (E) Alternative way of feeding: distal pedipalp segments function to push microorganisms into heaps in front of the gnathosoma. (F) Male fixed during walking on the surface of a microorganism film.

processes with unknown function are directed downwards. Fused lateral lips, chelicerae, and pedipalps are close to each other in that area and due to the grooved structure of the lateral lips, cheliceral movements and the corresponding friction food particles are macerated. In the distal area of the mouthparts the pedipalps diverge and form a rounded dorsal opening that facilitates the piling up of collected microorganisms (Fig. 1E).

Walking during the feeding process

In most histiostomatids legs are longer than the maximum width of the propodosoma between legs I and II (Fig. 2F). During locomotion they lift the soma above the substrate (Fig. 2F). Steps are asynchronous. In contrast, legs of *B. opuntiae* are about half as long as the maximum width of the propodosoma and, given these proportions, distinctly shorter than in other species. During walking the ventral body stays in touch with the substrate. The mite can burrow into muddy habitats or swim (more rarely) in watery media. Legs I and II move synchronously during these activities, but in opposite directions. Legs II move anteriorly while legs I move posteriorly (Fig. 1D, Ca) over legs I (Fig. 1Cb). Legs II remain in this position until the distal tarsi of legs I touch their trochanters. Then, legs I slowly move anteriorly again and legs II move backwards slipping over legs I (Fig. 1Cc) which ultimately reach the anterior position (Fig. 1Cd). Subsequently the whole movement cycle starts all over again. This mode of locomotion is termed 'leg-crossing' by the author. Corresponding to the movements of the first legs, legs III and IV perform a similar but less conspicuous 'leg-crossing', where legs IV clasp over legs III.

DISCUSSION

Most histiostomatid species cannot be cultured syntopically in the same dishes under laboratory conditions (water-agar with rotting potato pieces as food), even though they may originate from quite different field habitats. Hence, it is assumed that they are potential food competitors. They probably end up in different habitats due to the association with insects or other arthropods that are ecologically specialized on these habitats. *Bonomoia opuntiae* as well as the *Histiostoma* sp. under study are strictly specialized on *Opuntia*, possibly due to the phoretic relation with hitherto unknown insect carriers. To date, it is not known whether the cactus was colonized by these species after its import to the Mediterranean region or before.

Based on comparative studies, the feeding and locomotory behaviour of *B. opuntiae* were classified as apomorphic. In an earlier study (Wirth, 2006) the mouthpart morphology of *B. opuntiae* was also found to be apomorphic (Wirth, 2006).

Because the *Opuntia* habitat differs from other transient habitats of histiostomatids, such as animal dung, it is hypothesized by the author that the unusual (derived) feeding behaviour of *B. opuntiae*, the cheliceral clasp organ, and the novel composition of the food source are favoured by selection to avoid food competition with *Histiostoma* sp. in cactus. It is striking to observe that eggs of *B. opuntiae* and *Histiostoma* sp. are laid close to each other, a phenomenon that is unexpected, when two species are each other's competitors. The chelicera-cleaning behaviour of *B. opuntiae* may also be derived, because there is a need to remove the remains of muddy microorganism flakes. However, the mechanism by which food is partitioned into small pieces remained similar in the species under study. In this study only some aspects of that procedure were studied, so that further detailed observations remain necessary. Seta-shaped processes on the labrum have as yet no known function, but might assist in keeping the pile of microorganisms down and together, thereby preventing food particles from drift into the watery medium. But thus far piles of food looked rather similar in both mite species.

The 'leg-crossing' behaviour of *B. opuntiae* presumably enables faster movements by concentrating more thrust by the first legs. It is assumed that this behaviour is mainly used to move into a muddy substrate and only secondarily to perform swimming movements, because the mites were found much more often in the muddy substrate.

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Assessment of the usefulness of eight DNA fragments for phylogenetic studies within the family Phytoseiidae

M.-S. Tixier, S. Guichou & S. Kreiter

Ecole Nationale Supérieure Agronomique / Institut National de la Recherche Agronomique, Unité d'Ecologie animale et de Zoologie Agricole, Laboratoire d'Acarologie, 2 Place Pierre Viala, 34060 Montpellier cedex 01, France.
E-mail: tixier@supagro.inra.fr

To determine the suitability of several molecular markers for taxonomic studies on the family Phytoseiidae, 5 nuclear and 3 mitochondrial DNA fragments were sequenced for several populations, species, genera, and sub-families. DNA sequences were obtained experimentally or taken from Genbank. Lowest nucleotide divergence was found for the two 18S fragments. No variability was observed between populations of one species (*Neoseiulus californicus*), between species of one genus, and between two genera of one sub-family. Variation between two sub-families was very low (0.6-1.2%). Poor variability was observed for the two 28S fragments between and within sub-families. Variability was higher for sequences of the fragment ITS-5.8S (intra-genus, 3-7%; inter-genera, 17-20%; inter-subfamily, 29-33%). Variation between intra-species populations was very low for this DNA fragment. Higher nucleotide divergences were obtained for the mitochondrial fragments. For the 12S fragment, the nucleotide divergence rate between two *Neoseiulus* species was 10%, 25-33% between genera of the same sub-family, and 27-33% between two sub-families. For the fragments of COI, variability between species of a single genus was ca. 20%. Nucleotidic divergence of species belonging to two genera was 26-36% within, and 26-43% between sub-families. Intraspecific variation was low (0-0.4%). The 18S and 28S regions seem to be too conserved for phylogenetic analysis within Phytoseiidae, and would be more suitable for deeper phylogenetic analysis. The mitochondrial fragments are too divergent for robust alignments and phylogenetic analysis between genera of the family, and would be more suitable to assess within-genus systematic studies and species discrimination. The ITS-5.8S fragment seems adequate for phylogenetic analysis within sub-families. However, nucleotide divergence is too high for assessing relationships between sub-families.

Key words: Phytoseiidae, taxonomy, molecular markers, mt-DNA, nuclear DNA

The family Phytoseiidae consists of small mesostigmatic mites widely distributed all over the world (McMurtry & Croft, 1997). They are well known for their predatory action on mite pests. Nowadays, more than 1,980 species and 89 genera are described, included in three sub-families (Amblyseiinae, Phytoseiinae, and Typhlodrominae) (Chant, 1993; Chant & McMurtry, 2003a,b, 2004a,b, 2005a,b; Moraes et al., 2003, 2004; Ragusa, 2003; Kreiter & Tixier, 2006). This family is unusual in the degree of controversy over its basic systematics. The few phylogenetic analyses (Chant et al., 1980; Chant, 1993, Chant & McMurtry, 1994) are based on morphological characters and emphasize great rates of homoplasy bringing out numerous questions on the validity of the actual classification.

To resolve contradictions arising from these different taxonomic approaches, molecular phylogenetic analysis would be useful. To do this, a preliminary determination of the suitable DNA markers is required. Indeed, the process of choosing a region that is likely to be appropriate for a particular systematic question is perhaps the most critical step in any phylogenetic analysis (Hillis & Dixon, 1991). If a region is evolutionarily too conserved, there is no information allowing to separate the taxa. On the other hand, too variable regions would lead to alignment and robustness problems, since alignment of positional homologues is an assumption of any phylogenetic analysis (Hillis & Dixon, 1991). The aim of this study was to test the suitability of eight DNA markers (five nuclear and three mitochondrial fragments) for both phylogenetic studies and species discrimination.

MATERIAL AND METHODS

Markers used

Numerous molecular markers have been used in entomology (Loxdale & Lushai, 1998), but only few in acarology (Navajas & Fenton, 2000; Cruickshank, 2002). Mitochondrial DNA (mtDNA) has been widely used in taxonomic and population studies of insects (Simon et al., 1994; Roehrdanz & Degrugillier, 1998) and of some mites (Navajas et al., 1996; Navajas & Fenton, 2000; Toda et al., 2000, 2001; Otto & Wilson, 2001; Cruickshank, 2002; Evans & Lopez, 2002). Furthermore, the many copies of mtDNA facilitates molecular approaches (Harrison, 1989; Loxdale & Lushai, 1998). Regions of rDNA are particularly likely to yield informative data for almost any systematic question, some – like the 18S and 28S regions – being useful for assessing deep phylogeny, and the ITS for closer taxa (species identification and phylogeny) (Hillis & Dixon 1991; Navajas et al., 1996; Gotoh et al., 1998; Navajas & Fenton, 2000).

Specimens studied

Two sub-families and nine genera were included in the analysis. Three genera belong to the sub-family Typhlodrominae, six to the Amblyseiinae (Table 1). Sequences of 15 species were analysed (10 species of Amblyseiinae, five Typhlodrominae). The sequences of the 12S and 28S fragments were taken from the Genbank database, the others were obtained experimentally (Tables 1 and 2). The taxonomic levels studied for each DNA fragment are indicated in Table 1.

DNA extraction

DNA was extracted from eggs, when possible, or from starved females in order to avoid contamination from ingested prey. The CTAB extraction method was used as described by Tixier et al. (2004).

DNA amplification and electrophoresis

The primers used to amplify and the thermal cycling conditions of PCR are presented in Table 3. PCR was performed in a total volume of 25 µl, containing 2 µl mite DNA, 1 µl DNTP (2.5 Mm for each nucleotide), 2.5 µl Taq buffer, 1 µl of each

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Table 1 Species sequenced for the eight DNA markers studied and geneBank accession numbers for the sequences found in this web database.

Sub-families	Species	28S f1	28S f2	18S f1	18S f2	ITS	COI f1	COI f2	12S
Amblyseiinae	*Amblyseius graminis*			X		X	X		
	Euseius stipulatus				X	X	X	X	
	Iphiseius degenerans								AY099368
	Neoseiulus californicus			X	X	X	X	X	AY099367
	N. cucumeris	AF155081	AF155067						
	N. fallacis								AY099364
	N. picanus			X		X			
	Kampimodromus aberrans			X		X	X	X	
	K. ericinus			X		X	X		
	Phytoseiulus persimilis	AF155089	AF155075						AY099369
Typhlodrominae	*Galendromus occidentalis*								AY099363
	Neoseiuella littoralis				X		X		
	Typhlodromus exhilaratus			X		X			
	T. phialatus			X		X	X	X	
	T. pyri	AF155092	AF155078	X	X	X	X	X	
Intra-species level				X	X	X	X	X	X
Intra-genera level					X	X	X	X	X
Intra-subfamily level		X	X	X	X	X	X	X	X
Inter-subfamily level		X	X	X	X	X	X	X	X

Table 2 Characteristics of the populations of the specimens sequenced.

Phytoseiid species	Populations	Country	Locality	Hostplant
Amblyseius graminis		Tunisia	?	?
Euseius stipulatus		France	Montpellier	*Viburnum tinus*
Kampimodromus aberrans	1	France	Montpellier	*Celtis australis*
	2	France	Montpellier	*Quercus pubescens*
K. ericinus		France	Villeneuvette	*Cystus* sp.
Neoseiuella littoralis		Spain	Tarragona	?
Neoseiulus californicus	Brazil	Brazil	Piracicaba	?
	Chile	Chile	La Cruz	*Phaseolus vulgaris*
	Firenze	Italy	Firenze	*Fragaria vesca*
	France	France	Mauguio	*Solanum melongena*
	Japan	Japan	?	?
	Sicily	Italy	Palermo	*Fragaria vesca*
	Spain	Spain	Valencia	*Fragaria vesca*
N. picanus		Chile	?	?
Typhlodromus exhilaratus	France	France	Restinclières	*Vitis vinefera*
	Italy	Italy	Firenze	*Vitis vinefera*
T. phialatus		France	Restinclières	*Viburnum tinus*
T. pyri		France	Source de l'Euzières	*Vitis vinefera*

primer (100 µM), 0.5 µl Taq (Qiagen, 5 U/µl), and 18.9 µl water. Electrophoresis was carried out on 1.5% agarose gel in 0.5× TBE buffer during 30 min at 100 V.

DNA sequencing

PCR products were sequenced using the dynamic ET terminator cycle sequencing kit. Purification of DNA was carried out with Exosap-IT (Amersham). The sequencer used was the Megabase 1,000 apparatus. All DNA fragments were sequenced along both strands.

Sequence alignment and distances

Sequences were analysed and checked using Mega3.1® (Kumar et al., 2004). They were aligned using ClustalW® (1997) (Higgins et al., 1994). The distance matrix was constructed using the Jukes & Kantor model for the eight genes as the rate transition/reversion is near 1 (0.9).

RESULTS

Nuclear fragments

The 18S rDNA fragment 1 (323 nucleotides aligned). No variability was observed between populations of one species (*Neoseiulus californicus*), nor between species of two genera of one sub-family (Table 4). A very low variation rate (0.9%) was observed between the two sub-families Amblyseiinae and Typhlodrominae (Table 4).

The 18S rDNA fragment 2 (333 nucleotides aligned). No data were available to determine the intraspecific level of variation of this DNA fragment. However, it is supposed to be very low, considering the absence of variation between genera of Typhlodrominae and the 0.6% divergence rate between species of *Kampimodromus* (Table 5). The variation between genera of the same sub-family (Amblyseiinae) ranged between 0.3% (*Kampimodromus* – *Amblyseius*) and 0.9% (*Amblyseius* – *Neoseiulus*). The nucleotide divergence between genera belonging to two sub-families was also very low (0.6-1.2%). Comparing these sequences to data from the web database, the level of divergence between Phytoseiidae and Ascidae (*Cosmolaelaps*) is 2.5% (Table 5).

The 28S rDNA fragments 1 and 2 (374 and 401 bp aligned, respectively). Poor variability was also observed for the two fragments of 28S. Variability between two genera of one sub-family is quite the same for the two fragments considered (2.2 and 2.0%, respectively) (Table 6). For fragment 1,

Table 3 Primers used and PCR conditions for each fragment sequenced.

Fragments	Primers	PCR conditions
18S fragment 1	5'-3' GCAAGTCTAGTGCCAGCAGCC	step1: 92°C 1min
F	5'-3' CAAATCACTCCACCAACTAA	step2: 92°C 15s
		step3: 54°C 45s
		step4: 72°C 1 min
		goto step 2; 30 times
		step 5: 72°C 7 min
18S fragment 2	5'-3' CAAATCACTCCACCAACTAA	step1: 92°C 1min
R	5'-3' TCCGTAGGTGAACCTGCGGA	step2: 92°C 15s
		step3: 54°C 45s
		step4: 72°C 1 min
		goto step 2; 30 times
		step 5: 72°C 7 min
COI fragment 1	5'-3' TGATTTTTTGGTCACCCAGAAG	step1: 95°C 1min
	5'-3' TACAGCTCCTATAGATAAAAC	step2: 92°C 1 min
		step3: 45°C 1min
		step4: 72°C 1 min
		goto step 2; 40 times
		step 5: 72°C 5 min
COI fragment 2	5'-3' GGTCAACAAATCATAAAGATATTGG	step1: 95°C 1min
	5'-3' TACAGCTCCTATAGATAAAAC	step2: 92°C 1 min
		step3: 50°C 1min
		step4: 72°C 1 min
		goto step 2; 30 times
		step 5: 72°C 5 min
ITS-5.8S	5'-3' AGAGGAAGTAAAAGTCGTAACAAG	step1: 92°C 1min
	5'-3' ATATGCTTAAATTCAGGGGG	step2: 92°C 15s
		step3: 50°C 45s
		step4: 72°C 1 min
		goto step 2; 30 times
		step 5: 72°C 7 min

Table 4 Distances of Jukes and Kantor for fragment 1 of the 18S rDNA gene for several species, genera, and sub-families of Phytoseiidae.

	T. phialatus	*T. pyri*	*T. exhilaratus* France	*T. exhilaratus* Italy	*K. ericinus*	*K. aberrans*	*N. picanus*	*A. graminis*	*N. californicus*
Typhlodromus phialatus									
T. pyri	0								
T. exhilaratus France	0	0							
T. exhilaratus Italy	0	0	0						
Kampimodromus ericinus	0.006	0.006	0.006	0.006					
K. aberrans	0	0	0	0	0.006				
Neoseiulus picanus	0.012	0.012	0.012	0.012	0.006	0.012			
N. californicus	0.003	0.003	0.003	0.003	0.009	0.003	0.009		
Amblyseius graminis	0.009	0.009	0.009	0.009	0.003	0.009	0.003	0.012	

Table 5 Distances of Jukes and Kantor for fragment 2 of the 18S rDNA gene for several species, genera and sub-families of Phytoseiidae.

	Neoseiulella litoralis	*Typhlodromus pyri*	*Euseius stipulatus*	*Neoseiulus californicus*	*Neoseiulus californicus* Chile	*Neoseiulus californicus* France
Neoseiulella litoralis						
Typhlodromus pyri	0					
Euseius stipulatus	0.009	0.009				
Neoseiulus californicus	0.009	0	0			
N. californicus Chile	0.009	0.009	0	0		
N. californicus France	0.009	0.009	0	0	0	

divergence between two sub-families ranged between 1.4 (*Neoseiulus cucumeris – Typhlodromus pyri*) and 2.5% (*Phytoseiulus persimilis – T. pyri*). For fragment 2, the distances were smaller and ranged between 0.5 (*P. persimilis – T. pyri*) and 1.5% (*N. cucumeris – T. pyri*).

The ITS-5.8S rDNA fragment (333 bp aligned). The divergence rates between populations of *N. californicus* and *Typhlodromus exhilaratus* were very low and similar for the two species (0-0.6%) (Table 7). The variability between

Table 6 Distances of Jukes and Kantor for 28S rDNA genes (fragment 1 below the diagonal, fragment 2 above the diagonal) for several species, genera, and sub-families of Phytoseiidae.

	Neoseiulus cucumeris	*Phytoseiulus persimilis*	*Typhlodromus pyri*
Neoseiulus cucumeris		0.022	0.014
Phytoseiulus persimilis	0.02		0.025
Typhlodromus pyri	0.015	0.005	

Table 7 Distances of Jukes and Kantor for the 5.8S-ITS fragment for several species, genera, and sub-families of Phytoseiidae.

	K. ericinus	*K. aberrans*	*N. californicus*	*N. californicus* Sicily	*N. californicus* Sicily[1]	*N. californicus* Italy[1]	*N. picanus*	*E. stipulatus*	*A. graminis*	*T. exhilaratus* France	*T. exhilaratus* Italy	*T. phialatus*	*T. pyri*
Kampimodromus ericinus													
K. aberrans	0.07												
Neoseiulus californicus	0.17	0.18											
N. californicus Sicily	0.18	0.18	0.003										
N. californicus Sicily[1]	0.18	0.18	0.003	0.006									
N. californicus Italy[1]	0.18	0.18	0.003	0.006	0								
N. picanus	0.18	0.17	0.04	0.043	0.037	0.037							
Euseius stipulatus	0.2	0.19	0.2	0.2	0.2	0.2	0.2						
Amblyseius graminis	0.18	0.16	0.17	0.17	0.16	0.16	0.16	0.2					
Typhlodromus exhilaratus France	0.29	0.27	0.26	0.26	0.26	0.26	0.28	0.27	0.25				
T. exhilaratus Italy	0.29	0.27	0.26	0.26	0.26	0.26	0.28	0.27	0.25	0.006			
T. phialatus	0.3	0.28	0.25	0.26	0.25	0.25	0.28	0.27	0.26	0.034	0.034		
T. pyri	0.33	0.31	0.27	0.28	0.27	0.27	0.31	0.29	0.28	0.066	0.06	0.07	

[1]*N. californicus* Italy was collected in Firenze (Italy), *N. californicus* Sicily was collected in Sicily (Italy).

Table 8 Distances of Jukes and Kantor for 12S rDNA fragment for several species, genera, and sub-families of Phytoseiidae.

	Iphiseius degenerans	*Neoseiulus californicus*	*Neoseiulus fallacis*	*Phytoseiulus persimilis*	*Galendromus occidentalis*
Iphiseius degenerans					
Neoseiulus californicus	0.28				
Neoseiulus fallacis	0.33	0.1			
Phytoseiulus persimilis	0.25	0.27	0.28		
Galendromus occidentalis	0.36	0.38	0.4	0.34	

species belonging to one genus ranged between 3 (*Kampimodromus* spp.) and 7% (*Typhlodromus* spp.). The nucleotide divergence rates between genera were 17-20% within, and 29-33% between sub-families (Table 7).

The 12S mtDNA fragment (329 bp aligned). The divergence rate between the two morphologically close species, *Neoseiulus fallacis* and *N. californicus*, was 10% (Table 8). This divergence ranged between 25 (*Iphiseius – Phytoseiulus*) and 33% (*N. fallacis – Iphiseius degenerans*) for genera of the same sub-family, and between 33 and 38% between two sub-families (Table 8).

The mtDNA COI fragment 1 (285 bp aligned). The intraspecific variation assessed for *N. californicus* and *Kampimodromus aberrans* was low. Variability between species of one genus was 17% for both *Kampimodromus* and *Typhlodromus* (Table 9). The nucleotide divergence between two genera was 26-36% within, and 26-43% between sub-families.

The mtDNA COI fragment 2 (339 bp aligned). Low intraspecific nucleotide divergence was observed between populations of *N. californicus* (0.3%). For species of a single genus (*Typhlodromus*), divergence was 17% (Table 10). The variation between species of different genera was 22-25% within, and 27-29% between sub-families.

DISCUSSION

It is important to choose a marker with a substitution rate appropriate for its purpose. Furthermore, gene choice is of prime importance because of the high weight of properties of the data and homology inferred by alignment (Simon et al., 1994). Hillis & Dixon (1991) stated that alignments are often ambiguous when paired sequences differ by more than 30% of any given region. Table 11 presents the synthesis of the nucleotide divergences observed at various taxonomic levels.

The regions 18S and 28S are highly conserved inside the Phytoseiidae, which agrees with previous works emphasizing that these fragments are useful for resolving deep phylogenies (Hillis & Dixon, 1991; Cruickshank & Thomas, 1999; Dobson & Barker, 1999; Cruickshank, 2002). Black et al. (1997) used this DNA region for assessing phylogenetic relationships among tick sub-families and observed low substitution rates within sub-families (0.01-0.05) and within family (Ixodidae: 0.03). According to the low divergence levels observed in the present study, the four fragments studied will not give any information for species discrimination, nor for phylogenetic studies, within the family Phytoseiidae. However, the distances observed between Phytoseiidae and *Cosmolaelaps* sp. infer the usefulness of these genes for phylogenetic studies on Mesostigmata.

The ITS1-5.8S-ITS2 fragment showed an adequate rate of variation for discriminating species. This marker has already been used to discriminate between two morphologically close species, *T. exhilaratus* and *T. phialatus*, and to characterize the identity of *Kampimodromus* species (Tixier et al., 2004, 2006). Furthermore, the distance assessed between

Table 9 Distances of Jukes and Kantor for mtCOI (fragment 1) for several species, genera, and sub-families of Phytoseiidae.

	Neoseiulus californicus Brasil[1]	*Neoseiulus californicus* Japan[1]	*Neoseiulus californicus* Japan[1]	*Kampimodromus ericinus*	*Kampimodromus aberrans* 2	*Euseius stipulatus*	*Typhlodromus phialatus*
	Neoseiulus californicus Brasil[1]	*Neoseiulus californicus* Florence	*Neoseiulus californicus* Spain	*Kampimodromus aberrans* 1	*Amblyseius graminis*	*Typhlodromus pyri*	*Neoseiulella litoralis*
N. cal. Brasil							
N. cal. Brasil	0.004						
N. cal. japan	0.004	0					
N. cal. Florence	0.004	0	0				
N. cal. Japan	0.004	0	0	0			
N. cal. Spain	0.004	0	0	0	0		
K. ericinus	0.37	0.37	0.37	0.37	0.37	0.37	
K. aberrans 1	0.37	0.37	0.37	0.37	0.37	0.37	0.18
K. aberrans 2	0.36	0.36	0.36	0.36	0.36	0.36	0.17
A. graminis	0.4	0.4	0.4	0.4	0.4	0.4	0.24
E. stipulatus	0.36	0.36	0.36	0.36	0.36	0.36	0.23
T. pyri	0.38	0.38	0.38	0.38	0.38	0.38	0.26
T. phialatus	0.43	0.43	0.43	0.43	0.43	0.43	0.32
N. litoralis	0.41	0.41	0.41	0.41	0.41	0.41	0.29

N. cal. Brasil							
N. cal. Brasil							
N. cal. japan							
N. cal. Florence							
N. cal. Japan							
N. cal. Spain							
K. ericinus							
K. aberrans 1							
K. aberrans 2	0.04						
A. graminis	0.28	0.28					
E. stipulatus	0.24	0.26	0.3				
T. pyri	0.26	0.26	0.28	0.27			
T. phialatus	0.31	0.32	0.35	0.32	0.17		
N. litoralis	0.29	0.28	0.31	0.29	0.33	0.39	

When two samples have the same name, they correspond to two specimens of a same population (Brasil, Japan).

Table 10 Distances of Jukes and Kantor for mtCOI (fragment 2) for several species, genera, and sub-families of Phytoseiidae.

	Typhlodromus pyri	*Typhlodromus phialatus*	*Euseius stipulatus*	*Kampimodromus aberrans*	*Neoseiulus californicus* Chile	*Neoseiulus californicus* Italy
Typhlodromus pyri						
Typhlodromus phialatus	0.17					
Euseius stipulatus	0.28	0.29				
Kampimodromus aberrans	0.27	0.28	0.23			
Neoseiulus californicus Chile	0.28	0.27	0.25	0.22		
Neoseiulus californicus Italy	0.28	0.28	0.25	0.22	0.003	

Table 11 Nucletotide divergence rate (%) obtained with the eight DNA fragments tested at different taxonomic level.

	COI f1	COI f2	12S	28S f1	28S f2	18S f1	18S f2	ITS
Intraspecific			-		-			
Neoseiulus californicus	0-0.4	0.3					0	0-0.6
Kampimodromus aberrans	4							
Typhlodromus exhilaratus						0		0.6
Interspecific					-		-	
Neoseiulus			10					3.7-4
Typhlodromus	17	17				0		3-7
Kampimodromus	17					0.6		7
Inter-genera	26-30	22-25	25-33	2.2	2	0.3-0.9	0	17-20
Inter-subfamilies	26-43	27-29	27-36	1.4-2.5	0.5-1.5	0.6-1.2	0,9	29-33
Inter-families (Mesostigmata)						2.5		

genera of a single sub-family (17-20%) seems to show its usefulness for phylogenetic analysis at this taxonomic level, which agrees with previous data (Hillis & Dixon, 1991; Navajas et al., 1999b). The nucleotide divergence presently observed is too high for assessing relationships between sub-families. These data agree with the literature, as ITS sequences are under very little selection pressure and can accumulate substitutions very quickly compared to the 18S and 28S rDNA (Hillis & Dixon, 1991). The ITS2 fragment was mainly studied for assessing intra- and interspecific differences, for example for tetranychid mites (Navajas et al., 1994, 1999, 2000; Gotoh et al., 1998) and for the genus *Chorioptes* (*C. bovis* and *C. texanus*) (Ochs et al., 1999).

The mitochondrial rRNA COI genes are known to evolve much more rapidly than the nuclear rRNA genes (Hillis & Dixon, 1991). The two mitochondrial COI fragments tested in the present work are too variable to allow phylogenetic analysis between the genera of the family (26-36% for one sub-family). These fragments would be more suitable for within-genus systematic studies, species discrimination, and intraspecific studies. These results confirm a general lack of usefulness for phylogenetics at the among-family and among-order levels for this gene (Moritz et al., 1987; Simon et al., 1994) and confirm the usefulness of this marker for the International Barcoding Project for discriminating between species. The data from the literature are globally in accordance with the present results. Salomone et al. (2002), for instance, showed a level of divergence of 7.8% beween populations of *Steganacarus carlosi* and of 9.8-15.8% between two close *Steganacarus* species . Similarly, Walter et al. (2003) showed a divergence rate of 15-17% for species of the genus *Stratiolaelaps* (Mesostigmata, Laelapidae). The intraspecific variation was, however, higher than that observed for *N. californicus* in the present paper. This marker has also been used for intraspecific studies of tetranychid mites (Navajas et al., 1994, 1998, 1999b) and for discrimination between morphologically related mites (Navajas et al., 1994; Anderson & Trueman, 2001; Hinomoto et al., 2001).

The intraspecific variation observed in these studies was usually higher than that assessed in the present study. At last, some authors have also used this fragment to infer phylogenetic relationships between families of Prostigmata, despite high interspecific distances (13.5-36.5%) (Soller et al., 2001) and between two sub-families and six genera of Tetranychidae, but with a more adequate nucleotide divergence (5-25%) (Navajas et al., 1996).

The 12S rRNA gene seems to be phylogenetically useful for distant taxa, but can sometimes be problematic for assessing relationships among recently diverged species in insects (Simon et al., 1994). This marker has been little used for Acari. Murrel et al. (2001) used the 12S gene for inferring phylogenetic relationships among ticks, combining these results with other markers (18S, COI, ITS2). The sequences used are from the publication of Jeyaprakash & Hoy (2002). In the present study, the high divergence rates would avoid the use of this gene for inferring relationships between genera within the family of Phytoseiidae. This marker would be suitable for species discrimination, intrageneric and intraspecific studies. However, other studies including sequences of 12S of several populations of the same species have to be carried out.

In conclusion, the 18S and 28S fragments studied are not useful for species and genera differentiation, nor for phylogenetic analysis within the family. However, they could be helpful for resolving deeper phylogenetic relationships, for example among families of Mesostigmata. In contrast, the three mitochondrial fragments were highly variable between sub-families and between genera. These fragments would be helpful for studying within-genus relationships and species discrimination. The fragment ITS1-5.8S-ITS2 would be helpful for assessing within sub-family phylogeny, species discrimination, and genera identification, but does not seem suitable for intraspecific studies, nor for inferring phylogenetic relationships between sub-families.

In order to obtain a greater array of markers useful for taxonomy studies of Phytoseiidae, other genes have to be tested, such as COII, COIII, and 16S mtDNA fragments, and the region D3 of the 28S that was used for differentiating oribatid species (Maraun et al., 2004). The 16S region was also reported as a useful marker for resolving phylogenetic relationships among closely related rhinonyssid species, but not for more distantly related taxa (Rojas et al., 2001). None of the markers tested here seem to be suitable for phylogenetic studies within the whole Phytoseiidae family. Other markers, such as the nuclear gene encoding for the Elongation Factor 1α fragment, the gene Pol II (encoding for the RNA polymerase II), and the gene G6pdh (encoding for the pentose phosphate shunt enzyme glucose-6-phosphate dehydrogenase), will be tested in further experiments, as these different genes have already been reported as good candidates for solving generic or ordinal levels for insect and myriapod phylogenies (Soto-Adames et al., 1994; Damgaard & Sperling, 2001; Regier & Schultz, 2001).

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The genus *Dermanyssus* (Mesostigmata: Dermanyssidae): history and species characterization

L. Roy & C. Chauve
Laboratoire de Parasitologie et Maladies Parasitaires, Ecole Nationale Vétérinaire de Lyon, 1 avenue Bourgelat, 69280 Marcy-L'Etoile, France. E-mail: l.roy@vet-lyon.fr

The genus *Dermanyssus* Dugès, 1834 (Mesostigmata: Dermanyssidae) includes hematophagous mites that are ectoparasites of birds. Over the years, the number of species included varied greatly: 56 or more species have once been classified in this genus, but after the last review by Moss in 1978 only 18 species remained; presently there are 23. Major changes in genus definition drastically reduced the number of species included, involving not only the establishment of synonymies, but also many changes in systematic position based on literature data (Roy & Chauve, 2007). However, in 2007 the species definition is not yet clear. We present an overview of the current systematic position of all 56 species that were once included in this genus. In addition, we review host specificity and geographic distribution of *Dermanyssus* species, and we discuss morphological characters posing problems in species characterization.

Key words: Mesostigmata, *Dermanyssus*, morphological characters, systematics

The genus *Dermanyssus* Dugès, 1834 (Mesostigmata: Dermanyssidae) includes hematophagous mite species that are ectoparasites of birds. One of the species in this genus, *D. gallinae*, the red fowl mite, is economically important in the poultry industry. Its damage consists of downgraded eggs, decreased egg production, anaemia, and even mortality due to exsanguination. *Dermanyssus gallinae* can also transmit diseases, such as avian spirochaetosis, fowl cholera, and salmonellosis (Valiente Moro et al., 2005, 2007). Although the genus harbours species of economic importance, the classification of species in this genus has been in a state of confusion.

Of the species that are currently included within *Dermanyssus*, three had been described before the description of this genus, within the genus *Acarus* (*D. gallinae*, *D. alaudae*, *D. hirundinis*). Dugès described the genus *Dermanyssus* in 1834, including five new species, of which one is a junior synonym of *D. gallinae,* the type-species (Table 1). The precise definition of the genus has long been unclear and became firmly established around the middle of the twentieth century (Roy & Chauve, 2007). The character discriminating Dermanyssidae from other families within the Dermanyssoidea is located on the chelicerae. In the Dermanyssidae, chelicerae are elongated and have much reduced chelae, a morphological feature that seems to be correlated to a hematophagous life style (for more details, see Phyllis, 2006). Macronyssidae, another family in the Dermanyssoidea, also includes obligatory hematophagous species which possess chelicerae that are modified in a similar but different way. Here, elongation concerns the first article, rather than the second as in Dermanyssidae, and the chelae are reduced, but not really atrophied as in Dermanyssidae. The family Dermanyssidae as currently defined includes only two genera: *Dermanyssus* and *Liponyssoides*. The main morphological differences between those two genera are to be found on genu IV (*ad* with 2 setae in *Dermanyssus*, with 3 setae in *Liponyssoides*) and on the sternal shield (roughly crescent-shaped with 1 or 2 pairs of setae in *Dermanyssus*, roughly hexagonal with 3 pairs of setae in *Liponyssoides*). The host range differs widely between the two genera. Whereas *Dermanyssus* includes bird parasites, *Liponyssoides* includes species mainly parasitic on rodents and bats.

In its short and general description of the genus, Dugès (1834) included five species. In 1962, Evans & Till produced a more comprehensive review and included 14 species. In 1968 and 1978, Moss published the next and last reviews and included 18 species. Since 1978, five new species have been described. Although the genus seems to be well defined today, this does not hold for the species within the genus (Roy & Chauve, 2007). A total of 23 species are included in the genus *Dermanyssus* in 2007, but 34 species that had been included at some point, were excluded later (Roy & Chauve, 2007). Thus, overall since 1834, at least 56 species have been included in genus *Dermanyssus* (Table 1).

Although included in the genus, two species have been noted as having an unclear nomenclatural status (*incertae sedis* or *species inquirenda*): *D. longipes* and *D. passerinus* (Evans & Till, 1962; Roy & Chauve, 2007). The type specimens of these two species are in such a bad condition that it cannot be decided whether they are to be synonymized with *D. hirundinis* and *D. americanus* as suggested by Zemskaya (1971) and Moss (1978).

Here, we provide an overview of this genus. Firstly, we present the geographic distribution of *Dermanyssus* species. Secondly, we review their host specificity. Thirdly, we examine the reliability of characters used for species characterization.

GEOGRAPHIC DISTRIBUTION OF *DERMANYSSUS* SPECIES

Concerning the geographic distribution of *Dermanyssus* species, *D. gallinae* is the most frequently collected species

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Table 1 List of the 56 species which are or have been included in the genus *Dermanyssus* in chronological order of their formal description. √, species currently still in the genus *Dermanyssus* (sometimes after renaming).

Species included in *Dermanyssus*	Current position	
D. gallinae (De Geer, 1778)	*Dermanyssus*	√
D. alaudae (Schrank, 1781)	*Dermanyssus*	√
D. hirundinis (Hermann, 1804)	*Dermanyssus*	√
D. avium Dugès, 1834	synonymized: *D. gallinae*	√
D. vespertilionis Dugès, 1834	suppressed[1]	
D. hominis Bory de Saint Vincent?	?	
D. convolvuli Dugès, 1834	?	
D. oribatis Dugès, 1834	?	
D. musculi Koch, 1836[2]	*Steatonyssus* (Macronyssidae)	
D. arcuatus Koch, 1839	*Hirstionyssus* (Hirstionyssidae)	
D. carnifex Koch, 1839	*Hirstionyssus* (Hirstionyssidae)	
D. coriaceus Koch, 1839	synonymized: *D. arcuatus*	
D. lanius Koch, 1839	synonymized: *D. carnifex*	
D. noctulae Koch, 1839	synonymized: *D. arcuatus*	
D. murinus Lucas, 1840	*Steatonyssus* (Macronyssidae)	
D. avium Wagner, 1841	synonymized: *D. murinus*	
D. pipistrellae Koch, 1841	synonymized: *D. arcuatus*	
D. lacertarum (Contarini, 1843)	?	
D. natricis Gervais, 1844	*Ophionyssus* (Macronyssidae)	
D. musculi Johnston, 1849[3]	*Hirstionyssus* (Hirstionyssidae)	
D. flavus Kolenati, 1857	*Ichoronyssus* (Macronyssidae)	
D. glutinosus Kolenati, 1857	synonymized: *I. granulosus*	
D. granulosus Kolenati, 1857	*Ichoronyssus* (Macronyssidae)	
D. ambulans Thorell, 1872	*Haemogamasus* (Haemogamasidae)	
D. richiardii Canestrini & Fanzago, 1877	?	
D. sylviarum Canestrini & Fanzago, 1877	*Ornithonyssus* (Macronyssidae)	
D. hirundinis Berlese, 1889	homonymy; nomen novum: *D. chelidonis*	√
D. longipes Berlese &Trouessart, 1889	*Dermanyssus* (incertae sedis)	√
D. passerinus Berlese &Trouessart, 1889	*Dermanyssus* (incertae sedis)	√
D. albatus Oudemans, 1902	synonymized: *D. arcuatus*	
D. aegyptius Hirst, 1913	*Liponyssoides* (Dermanyssidae)	
D. muris Hirst, 1913	*Liponyssoides* (Dermanyssidae)	
D. sanguineus Hirst, 1914	*Liponyssoides* (Dermanyssidae)	
D. quintus Vitzthum, 1921	*Dermanyssus*	√
D. americanus Ewing, 1922	*Dermanyssus*	√
D. oti Ewing, 1925	synonymized: *D. americanus*	√
D. evotomydis Ewing, 1933	synonymized: *D. gallinae*	√
D. prognephilus Ewing, 1933	*Dermanyssus*	√
D. brasiliensis Fonseca, 1935	*Liponyssoides* (Dermanyssidae)	
D. brevis Ewing, 1936	*Dermanyssus*	√
D. scutatus Ewing, 1936	homonymy; nomen novum: *D. hirsutus*	√
D. chelidonis Oudemans, 1939	*Dermanyssus*	√
D. triscutatus Krantz, 1959	*Dermanyssus*	√
D. grochovskae Zemskaya, 1961	*Dermanyssus*	√
D. transvaalensis Evans & Till, 1962	*Dermanyssus*	√
D. intermedius Evans & Till, 1964	*Liponyssoides* (Dermanyssidae)	
D. gallinoides Moss, 1966	*Dermanyssus*	√
D. faralloni Nelson & Furman, 1967	*Dermanyssus*	√
D. hirsutus Moss & Radovsky, 1967	*Dermanyssus*	√
D. antillarum Dusbabek & Cerny, 1971	*Dermanyssus*	√
D. trochilinis Moss, 1978	*Dermanyssus*	√
D. carpathicus Zeman, 1979	*Dermanyssus*	√
D. nipponensis Uchikawa & Kitaoka, 1981	*Dermanyssus*	√
D. brevirivulus Gu & Ting, 1992	*Dermanyssus*	√
D. wutaiensis Gu & Ting, 1992	*Dermanyssus*	√
D. rwandae Fain, 1993	*Dermanyssus*	√

[1]*D. vespertilionis* has been suppressed by International ICZN (direction 66) under the plenary powers for the principle of priority, but not for homonymy (Melville & Smith, 1987).

[2]The history of *D. musculi* Koch is quite complicated. Oudemans (1936) considered it a junior synonym of *A. musculi* Schrank, which he placed in genus *Steatonyssus* (homonymy and synonymy at the same time). Evans & Till (1966) established that *S. musculi* Schrank is a junior synonym of *Ornithonyssus bacoti* (Hirst, 1913).

[3]The history of *D. musculi* Johnston is rather complicated: it seems to be conspecific to *D. musculi* Koch which is conspecific to *A. musculi* Schrank (see note 2).

and it seems to be found all around the world. Some other *Dermanyssus* species are also cosmopolitan. Examples are *D. hirundinis*, *D. brevis*, and *D. quintus*, which have been reported from both the American and Eurasian continents and are thus not restricted to the New or Old World. Some *Dermanyssus* species stem from just a single and recent record, such as *D. antillarum* (Cuba, 1971), *D. nipponensis* (Japan, 1981), and *D. rwandae* (Rwanda, 1993). Clearly, there are not enough data to make any inference on their distribution.

HOST SPECIFICITY

Dermanyssus species are ectoparasites of birds. Most species do not show host specificity and – in absence of birds as hosts – some species have even been noted as ectoparasites of mammals, such as man or rodents.

Up to 30 bird species, distributed over 12 families and eight orders, are known to be parasitized by *D. gallinae* (Roy & Chauve, 2007) (Table 2). Also, *D. hirundinis* has a rather broad host spectrum, as it has 40 bird species as hosts, out of 18 bird families and nine orders (bird classification based on Peterson, 2007). A few species may be host specific. Examples are *D. alaudae*, *D. quintus*, *D. brevis*, and *D. triscutatus*, each of which has been found on birds belonging to a single family. The most recently described species (*D. rwandae*) might be host specific too (Table 2), but there are not enough data available to prove that this is not a simple by-product of limited sampling.

RELIABILITY OF DISCRIMINANT SPECIES-SPECIFIC CHARACTERS

Traditional systematics

Most of the species-level discriminating characters are based on leg and dorsal chaetotaxy, and on relative length of the peritreme vs. coxal position. Concerning leg chaetotaxy, some authors (Evans & Till, 1962; Moss, 1978) cautioned that there is great intraspecific variation. As for dorsal chaetotaxy and relative peritreme length, characters also seem to be very polymorphic (L Roy, unpubl.).

Major characters: dorsal shield chaetotaxy and relative length of peritreme

An example of the dorsal shield chaetotaxy from a single population of *D. gallinae* is shown in Figure 1. Focusing on the *j* line allows observing some important variations in the dorsal chaetotaxy. Dispositions of setae *J3*, *J4*, and *J5* are shown (two nearly parallel longitudinal lines in *c* or a hexagone in *f* and *i*). Several cases of bilateral asymmetries in setation are found, not only with some asymmetrical dispositions (*e*, *j*), but also with a case of unpaired seta *J3*/*J4* (*d* with only one of them on the right side). Note that such frequent asymmetries have been pointed out by Evans & Till (1962). Moreover, there are major differences in the shape of the dorsal shields shown in Figure 1. The shape of the dorsal shield is important for evaluating some chaetotactic characters: variation in position of *j1* – i.e., on or off shield – seems to be due to shield

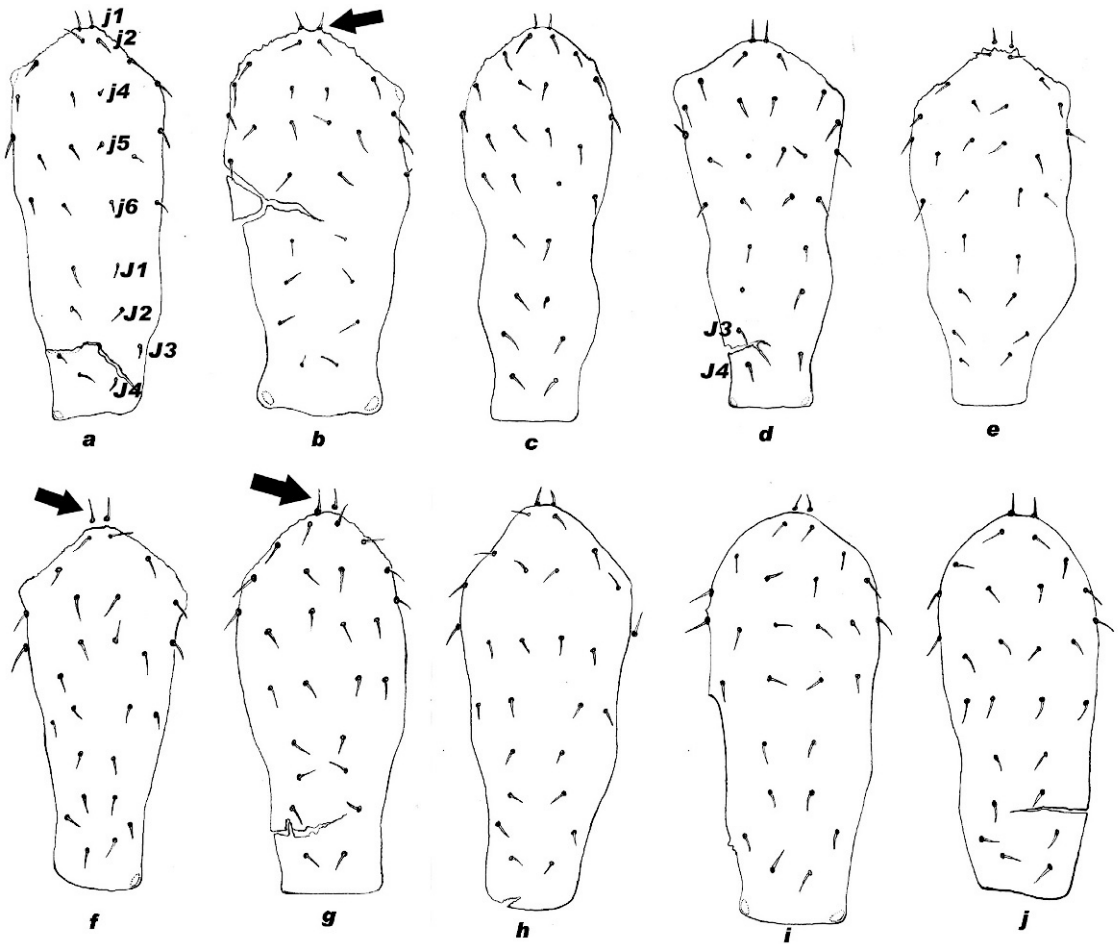

Figure 1 Dorsal shield of 10 out of 20 randomly selected adult females of a cultured laboratory population of *Dermanyssus gallinae*. Setal terminology follows Lindquist & Evans (1965).

Table 2 Host diversity of *Dermanyssus* species (based on literature data), other than *D. gallinae*. Species are in chronological order from left to right. Avian taxonomic groups were checked on http://www.zoonomen.net/avtax/frame.html.

Bird order	Bird family	*D. alaudae*	*D. hirundinis*	*D. longipes*	*D. passerinus*	*D. quintus*	*D. americanus*	*D. prognephilus*	*D. brevis*	*D. chelidonis*	*D. triscutatus*	*D. grochovskae*	*D. transvaalensis*	*D. gallinoides*	*D. faralloni*	*D. hirsutus*	*D. antillarum*	*D. trochilinis*	*D. carpathicus*	*D. nipponensis*	*D. brevirivulus*	*D. wutaiensis*	*D. rwandae*
Passeriformes	Fringillidae				√		√	√															
	Passeridae		√	√	√		√										√				√		
	Sittidae		√				√																
	Alaudidae	√							√											√			
	Muscicapidae		√		√			√											√				
	Paridae		√		√					√									√				
	Hirundinidae		√					√		√	√		√										
	Corvidae											√											
	Certhiidae		√																				
	Motacillidae		√																				
	Vireonidae		√																				
Piciformes	Picidae		√		√	√		√				√		√		√				√			
Strigiformes	Strigidae		√				√																
	Apodidae		√														√						√
Apodiformes	Trochilidae																	√					
	Accipitridae																√						
Ciconiiformes	Alcidae													√									
	Hydrobatidae													√									
Columbiformes	Columbidae		√																				

contour variations. Arrows on Figure 1 show different positions of *j1*, which is always on-shield in *D. gallinae*, but off-shield in *D. gallinoides* according to Moss (1978). Allred (1970) pointed out a similar case in two species of *Ornithonyssus* (Macronyssidae), which are also hematophagous parasites. In these species, the great degree of intraspecific variation in shape and chaetotaxy of the sternal plate imposes major difficulties for species discrimination.

The peritreme is associated with the respiratory organ. It is a groove extending anteriorly from the stigma, which is located near coxa IV. Using a scanning electron microscope, two sclerotized lips can be seen along the groove (Fig. 2). The relative length of the peritreme from coxa IV to coxa III-I is considered to be a taxonomic character discriminating between *Dermanyssus* species, but there are numerous character states and they may overlap (L Roy, based on data from Moss, 1978). The extension of the peritreme varies continuously from coxa IV to coxa III up to coxa I without any distinct gap between species within the genus *Dermanyssus*. There are more than seven possible positions (Fig. 3): peritreme extending to anterior margin of coxa III, and to posterior, middle, or anterior margin of coxae II and I, and various intermediate positions can be observed (L Roy, unpubl.).

Moreover, clearing specimens for slide-mounting and observation with an optical microscope may destroy attachment of the peritreme, so that the soft groove may change length and position, moving inside the podosoma (Fig. 4 illustrates it with a case of asymmetric length). However, the clearing procedure is necessary for unambiguous morphological examination of hematophagous mites.

The high plasticity of these frequently used characters and overlapping character states makes it very difficult to encode them for cladistic analysis.

Other characters

Apart from the major characters above, some others have been used for species characterization. Two characters concerning the dorsal side allow the characterization of two small groups of species and each seems to provide a rather distinct gap between their two states:

– Dorsal setae show marked or no difference in length between 'central setae' and 'peripheral setae' (central setae = *j4-j6* + *J*-series, except *J5*; peripheric setae = *J5*, *z*-series, *Z*-series, *r*-series, *R*-series, *s*-series). 'Central setae' are markedly shorter than 'peripheral setae' in seven species (*D. alaudae*, *D. brevis*, *D. brevirivulus*, *D. hirsutus*, *D. grochovskae*, *D. quintus*, and *D. rwandae*).

– Mesonotal scutella are present or not. They are present in five species (*D. americanus*, *D. antillarum*, *D. transvaalensis*, *D. triscutatus*, and *D. wutaiensis*).

Each of the following three characters defines a single species:

– Ventral neotrichy in the form of a cluster of elongate, simple setae, lateral of the anal shield, is present only in *D. hirsutus*.

– Several inflated setae situated posteriorly on the idiosoma are found only in *D. antillarum.*

– A U-shaped row of very hard and deeply rooted setae on the opisthosomal ventral side is present only in *D. quintus.* In addition, several chitinous apophyses on coxae III and IV and some clawlike setae on trochanters and coxae III and IV, as well as an anal shield, more broad than long, constitute a set of unique characters for *D. quintus*.

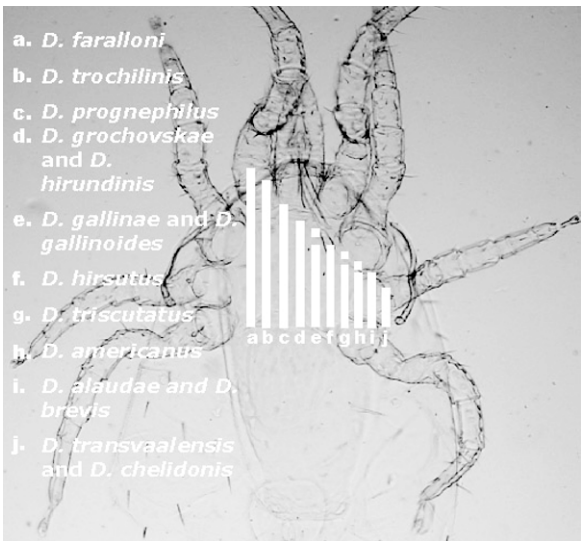

Figure 3 Relative length of peritreme against coxae according to Moss (1978).

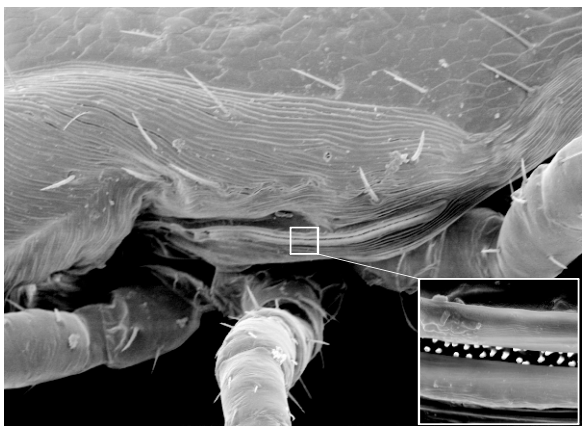

Figure 2 Peritrema in *Dermanyssus gallinae* (scanning electron micrograph).

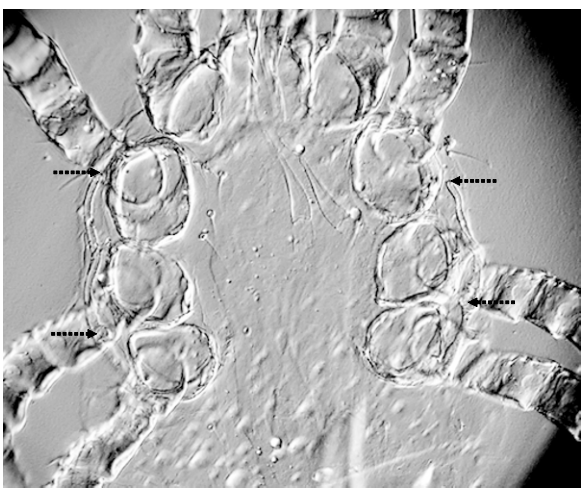

Figure 4 A case of asymmetry in peritrema length.

A new character

Moss (1967) studied the relationships between species within the genus *Dermanyssus* using phenetic tools. He selected mostly morphometric characters in absence of clearly definable morphological characters – a feature he assumed to be an adaptation to the parasitic life style. In addition, he described a character not noted before, i.e., the shape of seta *al1* on the palp genu (distally expanded vs. spike-like). Based on his analysis, two groups of species within the subgenus *Dermanyssus* were distinguished, but, in 1978, the addition of three new species blurred this subdivision. No more exploration of interrelationships between *Dermanyssus* species has been published thereafter.

DISCUSSION

We have argued that the characters hitherto most frequently used for characterization of *Dermanyssus* species are ambiguous: (1) leg chaetotaxy provides characters that vary intraspecifically, (2) dorsal shield chaetotaxy provides variable characters (as we observed in *D. gallinae*) and may lead to misidentification of species (e.g., *D. hirundinis* differs from *D. gallinae* only in the number of setae on the dorsal shield; Evans & Till, 1962), and (3) relative length of peritreme is not distinct enough for species characterization within the genus *Dermanyssus*. Five traditional characters seem to be reliable, but characterize only few species. Moss (1978) revealed a new, non-morphometric and apparently steady character (shape of seta *al1* on the palp genu).

Traditional species-specific characters, i.e., characters used to define new species, do not seem to be stable enough. Moreover, most of these species seem to provide low host specificity and are geographically widely distributed, which contributes to blur the issue. For all these reasons there is doubt about the validity of several *Dermanyssus* species.

We conclude that, even though the genus *Dermanyssus* seems to be well defined, there is not a solid basis for species characterization within this genus. Given that the last review of the genus *Dermanyssus* was incomplete and that five new species have been described since, there is every reason to critically revise the genus. We plan to do this by using cladistic tools. First, we need to test for monophyly of the group of species, to prove that the genus definition is correct. Second, we need to define a set of sufficient characters to discriminate species within this genus – and this seems, a priori, to be the most problematic issue. Finally, because morphological characters are insufficient, we need to add molecular characters to the phylogenetic analysis of the genus *Dermanyssus*.

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Morphology of Acari

First ultrastructural observations on a putative sperm access system in veigaiid females (Veigaiidae, Gamasida)

Gerd Alberti, Antonella di Palma[1], Gerald W. Krantz[2] & Czesław Błaszak[3]

Ernst-Moritz-Arndt Univ, Zool Inst and Museum, J.-S.-Bach-Str. 11/12, D-17489 Greifswald, Germany. E-mail: alberti@uni-greifswald.de; [1]Univ Studi di Foggia, Dipto Scienze Agroambientali, Chimica e Difesa Vegetale, Via Napoli 25, I-71100 Foggia, Italy; [2]Oregon State Univ, Dept Entomology, Cordley 2046, Corvallis, OR 97331-2907, USA; [3]Adam Mickiewicz Univ, Dept Animal Morphology, Umultowska 89, 61-614 Poznań, Poland

Females of Dermanyssina display a reproductive system composed of a region involved in egg production and laying and another involved in sperm reception and likely storage. This second region is called the sperm access system, and it is related to a peculiar sperm transfer mode known as podospermy. Males have the chelicerae modified as gonopods which possess a peculiar process on the movable digit, the so-called spermatodactyl, which is used for sperm transfer. In Veigaiidae, males are rarely, exceptionally, or never found, and some species are considered to reproduce parthenogenetically. Known veigaiid males have spermatodactyls, sometimes of extraordinary length. The conspecific females have so-called spiral organs located behind coxae IV. It is likely that these organs, not known from other gamasid mites, represent the veigaiid sperm access system. In the present study, first ultrastructural details on these peculiar organs are given comparing a bisexual species (*Veigaia* sp.) with two species in which males are extremely rare (*V. nemorensis* and *V. cerva*). Each of these structures is composed of a major tube starting from the opening, a vesicle-like region, and several minor tubes. In general it is considered to be derived from an entapophysis. Muscles attach to the minor tube region. A general similarity to the phytoseiid type of sperm access systems may be noted. But preliminary observations on the whole genital system of both female and male veigaiids also reveal a resemblance to the genital system in Parasitina. Whether these findings may challenge the current status of Parasitina and/or Dermanyssina will require further investigations.

Key words: Dermanyssina, entapophysis, fine structure, Michael's organ, Parasitina, podospermy

Two types of mating behaviour have been described in the Gamasida: tocospermy, where sperm transfer from male to female occurs via the primary genital opening of the female, and podospermy, where sperm transfer occurs via secondary insemination pores located in close association with the bases of the legs (e.g., Evans, 1992; Alberti & Coons, 1999). In the latter case, the primary genital opening is used for oviposition only. This classification into two mating types was shown by Alberti (2002a,b) to be rather superficial considering the important differences among the tocospermous forms. Males of the tocospermous Parasitina have, in contrast to (most) other tocospermous species, modified chelicerae (the movable digit has a spermatotreme), the genital opening is in a presternal position, and the spermatozoa are of the ribbon-type. Parasitine females also have modified genital organs. The ovary is massive and has a nutrimentary and a germinal part, and there is an unpaired oviduct. Thus, these genital structures are rather similar to the large group of podospermous Gamasida frequently classified as Dermanyssina, in which males have spermatodactyls on the movable cheliceral digits, possess a presternal genital opening, and have ribbon-type spermatozoa. The female dermanyssine genital system is quite similar to that of Parasitina, but is in addition provided with a modified nutrimentary organ (lyrate organ) and a sperm access system with a pair of insemination pores typically located close to the leg bases. Thus, the Parasitina are much different from other tocospermous forms which retain a plesiomorphic organization, e.g., females have tubular ovaries and paired oviducts and males have vacuolated spermatozoa (with some exceptions, i.e., Arctacaridae; Alberti & Krantz, 2007).

The family Veigaiidae is a peculiar taxon, in which males of many species are rarely or never found. Known males are provided with a spermatodactyl (Fig. 1b) and a presternal genital opening. Hence, Veigaiidae are frequently placed within the Dermanyssina (e.g., Johnston et al., 1982; Evans, 1992; Norton et al., 1993; Alberti & Coons, 1999; Lindquist et al., 2009), whereas others regard them as closely related to Parasitina (e.g., Karg, 1965, 1993, 2006; Krantz, 1978). The spermatodactyls of certain Veigaiidae may be extremely long, e.g., in *Veigaia paradoxa* they are almost as long as the body of the male (Willmann, 1951; Farrier, 1957; Błaszak & Ehrnsberger, 2001) (Fig. 1c). Females of such species have a pair of peculiar 'spiral organs', which correspond in length with the spermatodactyls [Fig. 1d; see also Błaszak et al. (2006) with respect to *Veigaia leruthi*]. It is suspected that this structure represents at least part of a sperm access system (also called Michael's organ). The ultrastructure of the spermatodactyl of an as yet undescribed veigaiid species has recently been clarified (Fig. 1b; Di Palma et al., 2006). In the present study we have tried to elucidate the suspected sperm access system of the corresponding female.

MATERIALS AND METHODS

Females of the following species were investigated: *Veigaia cerva* (CL Koch), *Veigaia nemorensis* (Kramer) (extracted from leaf litter collected in the surroundings of Greifswald, Germany), and an as yet undescribed *Veigaia* sp. (from leaf litter collected on Mary's Peak, Benton County, OR, USA; see Błaszak et al., in press). Samples were extracted using Berlese funnels. Some specimens were mounted on glass slides using Faure's mixture, and others were macerated with lactic acid and dissected to better expose the putative sperm access system before mounting. Other specimens macerated with concentrated lactic acid were critical point dried in a Balzer's CP-dryer. After removing the dorsal shields, specimens were placed on Alstubs using double sticking carbonated plates. They were coated with Palladium-Gold using a Polaron 7640 Sputter Coater and studied with a Zeiss DSM 940A scanning electron micro-

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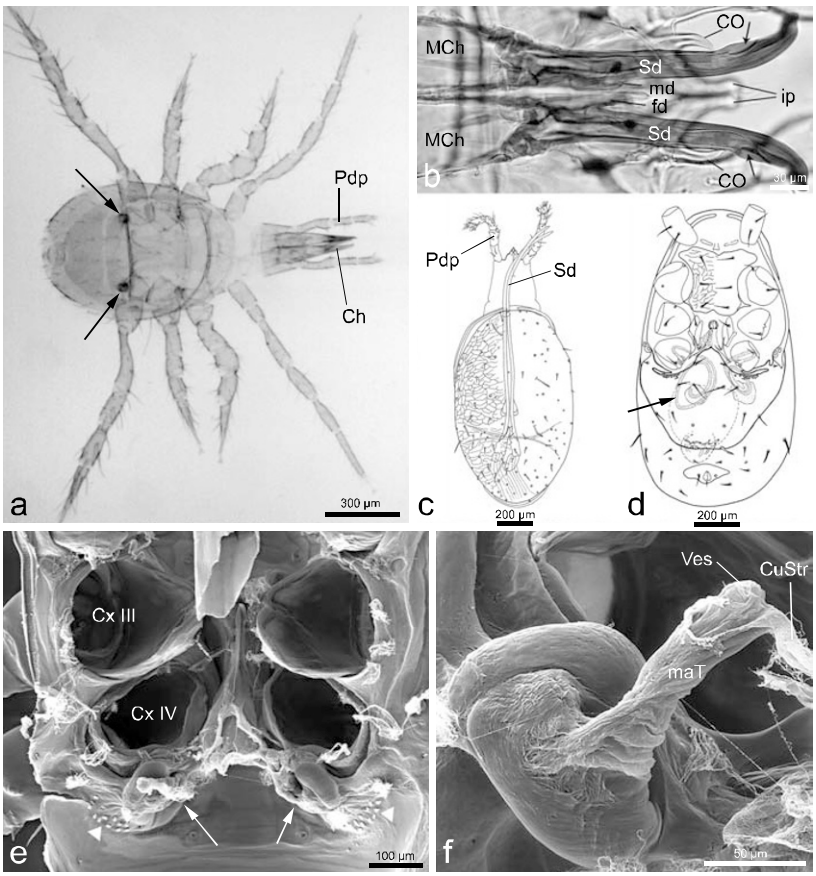

Figure 1 (a) Dorsal view (LM) of female *Veigaia* sp. Arrows point to putative sperm access system. (b) Chelicerae (LM) of male *V. vidae* with rather long spermatodactyls. Arrows point to distal opening of spermatodactyl (from Di Palma et al., 2006). (c) Dorsal view of male of *Veigaia paradoxa* with retracted chelicerae provided with long spermatodactyls. (d) Ventral view of female of *V. paradoxa* with spiral organs (putative sperm access system, see arrow) (c, d from Błaszak & Ehrnsberger, 2001). (e, f) SEM-aspects of a macerated female of *Veigaia* sp. after removing dorsal plates. (e) Overview showing the putative sperm access systems of both sides (arrows). Arrowheads point to openings of dermal glands. (f) Detail showing the structure from a posterior view. Abbr.: Ch, chelicerae; CO, corniculus; Cx III-IV, coxa III-IV; CuStr, cuticular strands; fd, fixed digit; ip, peculiar inner processes on tip of infracapitulum; MCh, middle article of chelicera; md, movable digit; maT, major tube; Pdp, pedipalp; Sd, spermatodactyl; Ves, vesicle-like region.

scope (SEM). Some specimens were cut alive into pieces with a razor blade and prefixed in ice-cold, buffered glutaraldehyde (3.5%). After rinsing in cacodylate buffer (pH 7.4, 0.1M), they were postfixed using 2% buffered OsO_4 and then were embedded in Spurr's mixture (Spurr, 1969). Ultrathin and semi-thin sectioning was performed with diamond knives. The semi-thin sections (400 nm) were stained according to the methodology of Richardson (1961), and the ultrathin sections (70 nm) following that of Reynolds et al. (1963). The semi-thin sections were subjected to light microscopy (LM; Olympus BX60 with an Olympus DP10-digital camera), and the ultra-thin sections were studied with a Zeiss EM 10A transmission electron microscope (TEM).

RESULTS

Light microscopic observations of the mounted specimens show a complex of rather strongly sclerotized cuticle immediately behind coxae IV containing a lumen of complex shape (Fig. 1a). A less sclerotized strand of cuticle extends dorsally from its inner extremity and ends abruptly in a vesicle-like structure. Thin strands of cuticle extend from its adaxial border. The SEM study shows these structures more precisely (Fig. 1e, f). It is evident that the vesicle-like area is not an open structure. The semi-thin sections (Fig. 2) prove that it is really a hollow structure, which opens ventrally immediately behind coxa IV. A narrow channel, termed here the major tube, runs ventrally and turns abruptly to run dorsally. This latter part continues deeper into the body and terminates with the vesicle-like structure. Further, it is evident from the TEM preparations that the first region of the channel has a conspicuously differentiated cuticle which shows different sclerotization in its various parts, with the part running dorsally being more flexible (Fig. 3a). This part is seen in some sections as a hollow tube isolated from the previously described parts, because of a different plane of section and the bending of the tube.

From certain sections, it is evident that a finger-like process projects from the wall of the tube into its interior, so that in some cross sections the process may also be seen within the tube lumen (Figs. 2c, 3b). Levelled with the base of this process, thin cuticular strands extend into the interior of the body (Fig. 3c, d). The strands are connected with muscle cells and hence represent 'tendons'. Remarkably, these cuticular strands include thin hollow channels, here termed minor tubes. The lumina of these tubes are continuous with the lumen of the major tube (Fig. 3c, d). Even more remarkable is that the cuticle in the region just described (referred to as 'vesicle-like' earlier), abruptly becomes extremely thin (Fig. 3c, e). The tissue surrounding this area is vastly different from the epithelium underlying the cuticular structures described thus far. The cells are much bigger and contain numerous dense inclusions. Comparing this structure with that of both the other species shows a generally similar aspect, although the whole structure is generally more stout in *V. nemorensis* and *V. cerva* (Fig. 4). Observations on *V. nemorensis* revealed that the muscles connected to the cuticular strands extend anteriorly and attach to apodemes level with coxae III (Fig. 5).

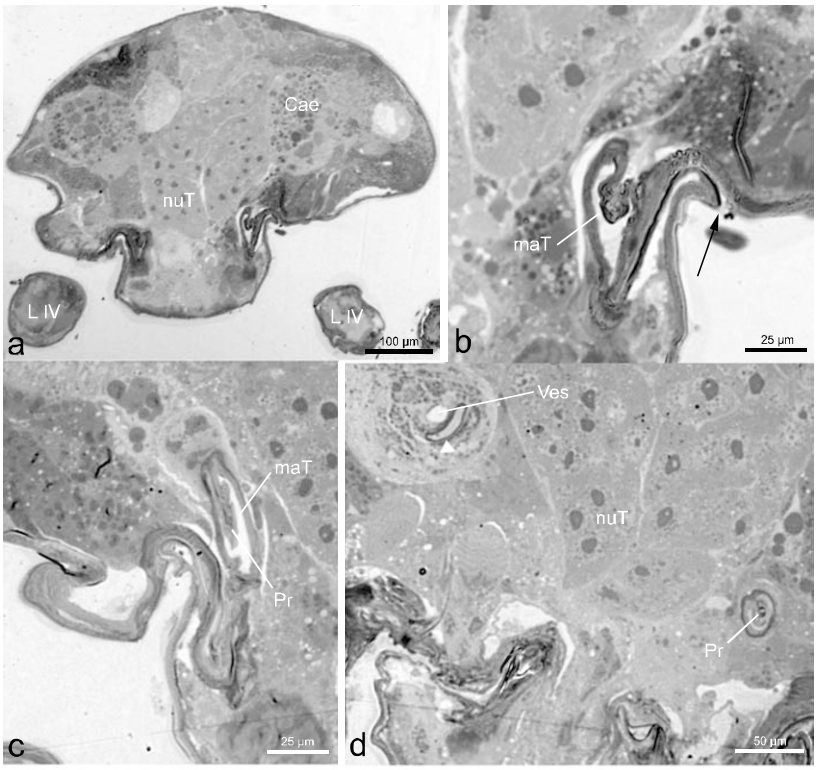

Figure 2 Sections (LM) through female of *Veigaia* sp. (a) Overview. (b) Detail enlarged. Arrow points to opening of sperm access system. (c) The major tube running to the vesicle-like region is evident. (d) The major tube on the right is seen in cross section. In its lumen the cuticular process is cut transversely. On the left, the vesicle-like region is seen. Note modified epithelium around transversely sectioned thin-walled vesicle-like region and piece of thick cuticle of the major tube (arrowhead). Abbr.: Cae, caecum; L IV, leg IV; maT, major tube; nuT, nutritive tissue of ovary; Pr, process; Ves, vesicle-like region.

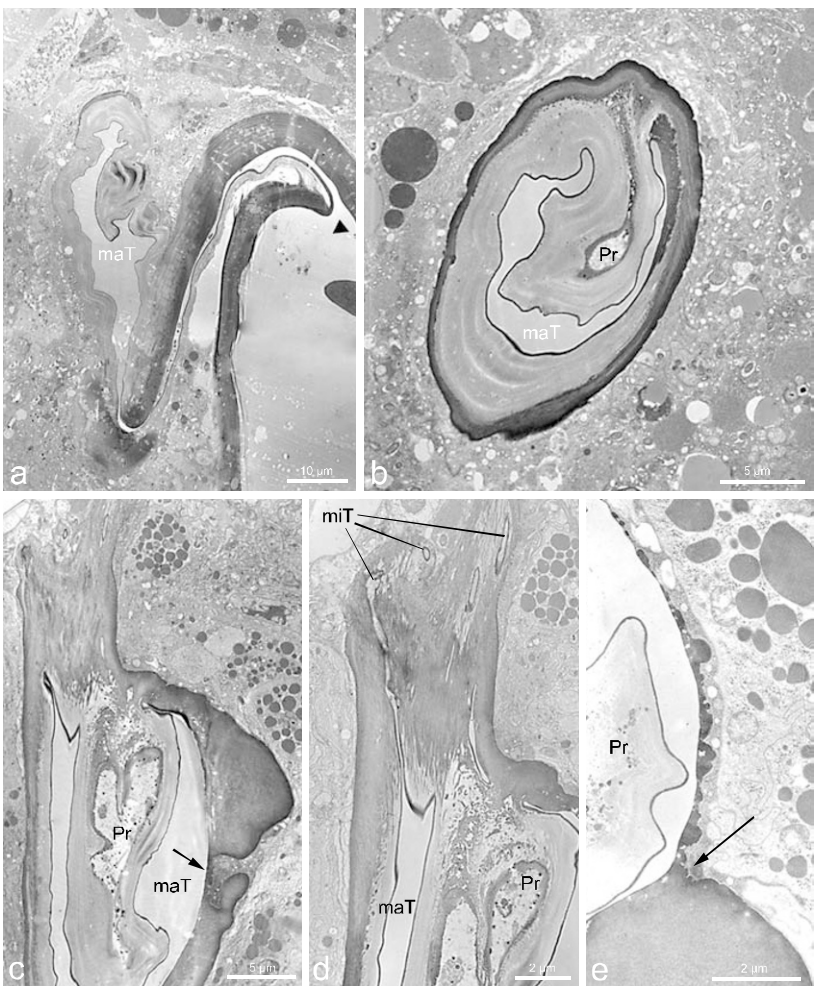

Figure 3 TEM of *Veigaia* sp. (a) Level of opening (arrowhead) of the system. Note different sclerotization of major tube. (b) Major tube sectioned more interiorly (proximally) showing cuticular process reaching into the lumen of the tube. (c) Proximal end of major tube close to vesicle-like region with slender part to which muscles attach. Arrow points to region where cuticle is very thin. (d) Similar aspect as in c showing connection of lumen of a thin, minor tube with lumen of major tube. (e) The cuticle of the major tube abruptly becomes very thin in the vesicle-like region (arrow). Note dense inclusions in the cells underlying this thin cuticle. Abbr.: maT, major tube; miT, minor tubes; Pr, process.

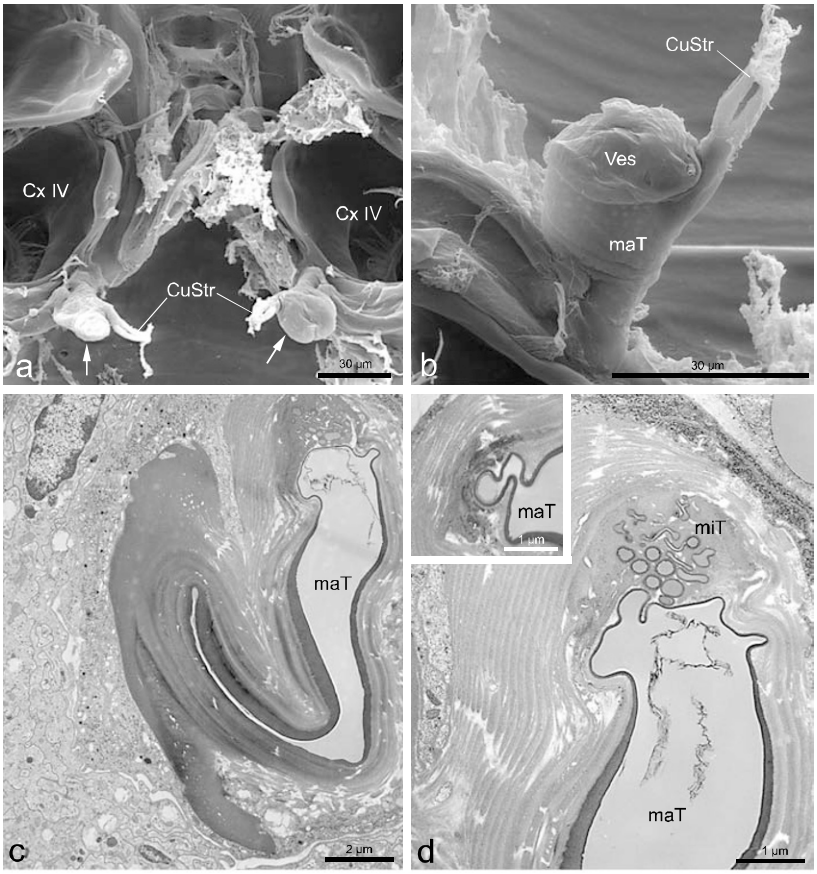

Figure 4 Putative sperm access system of *Veigaia nemorensis*. (a, b) SEM (compare Fig. 1e, f). (a) Overview. Arrows point to sperm access systems. (b) Detail demonstrating more stout aspect of the structures compared with *Veigaia* sp. (c, d) TEM (compare Fig. 3). (c) Major tube close to external opening (lower part of figure) and vesicle-like region (upper part of figure). (d) Region close to vesicle-like region with minor tubes. Inset: Minor tube lumen continuous with lumen of major tube. Abbr.: CuStr, cuticular strands; Cx IV, coxa IV; Ves, vesicle-like region; maT, major tube; miT, minor tubes.

DISCUSSION

We believe that the described structure represents a sperm access system since it evidently complements the male spermatodactyl, as has been conspicuously shown in other veigaiid species having a well developed spermatodactyl and a correspondingly developed spiral organ (Błaszak & Ehrnsberger, 2001). The rare males of *V. nemorensis* have a rather short spermatodactyl, whereas those of *Veigaia* sp. are provided with slightly longer spermatodactyls (Farrier, 1957; Karg, 1993; Di Palma et al., 2006). The strongly sclerotized portion of the major tube from which the less sclerotized part extends also shows a tendency to spiral in our less extremely equipped species. Since the structure is hollow and evidently serves as an attachment site for muscles, it represents an entapophysis. Thus, if this interpretation is accepted, we can conclude that the sperm access system evolved from entapophyses, at least in the Veigaiidae. The possibility of evolution from glandular systems can very likely be excluded, although a row of dermal gland openings close to the posterior margin of coxae IV is typical of Veigaiidae. We suggest that the spermatodactyl is pushed into the tubular part (major tube) of the system, which may be facilitated by its partly flexible cuticle.

Spermatozoa are released and may reach the body cavity either after the cuticle of the 'vesicle' has been penetrated by the action of the spermatodactyl, or by their own activity. The possibility that the thin tubes (minor tubes) mentioned above would allow migration of the sperm cells can be excluded. We found spermatozoa in the ovary that were rather large and of a very complex structure (Fig. 5d). It is hard to imagine that they could pass through the extremely thin minor tubes. Interestingly, the spermatozoa are similar to those found in inseminated females of Parasitina (Witaliński, 1975; Alberti, 1980; Alberti et al., 2000). Our findings thus have several general implications:

1. A sperm access system is likely to have evolved from an entapophysis.

2. Spermatozoa probably penetrate through a thin cuticular region of such a sperm access system (compare problems of understanding the passage of sperm in *Phytoseiulus* females; Alberti & Di Palma, 2002; Di Palma & Alberti, 2002).

3. The sperm access system of Veigaiidae is to a certain degree similar to that of *Phytoseiulus persimilis* (Alberti & Di Palma, 2002: Di Palma & Alberti, 2002). There is a thick major tube (= major duct) and a region with a projecting process (corresponding to an atrium), there are thin minor tube(s) starting from this region (corresponding to the minor duct), and an area with modified cuticle and modified epithelium: the vesicle-like region (corresponding to the vesicle; if the latter region of thin cuticle in *Veigaia* were inflated, it would be equivalent to a real vesicle). Although located differently (*Veigaia*: insemination pores behind coxae IV; *Phytoseiulus*: pores between coxae III and IV) and without showing a connection with muscles, it is possible that the phytoseiid-type of sperm access system (and the laelapid type?) may also have evolved from entapophyses. The plesiomorphic function of these entapophyses and their muscles must be elucidated.

4. Preliminary personal observations on veigaiid sperm structure, male chelicerae, and the female genital system (not shown in detail here) and those of Parasitina seem to support a close relationship between Parasitina and Veigaiidae (Karg, 1965, 1993, 2006).

5. Whether these findings may challenge the status of Parasitina and/or Dermanyssina requires further investigation.

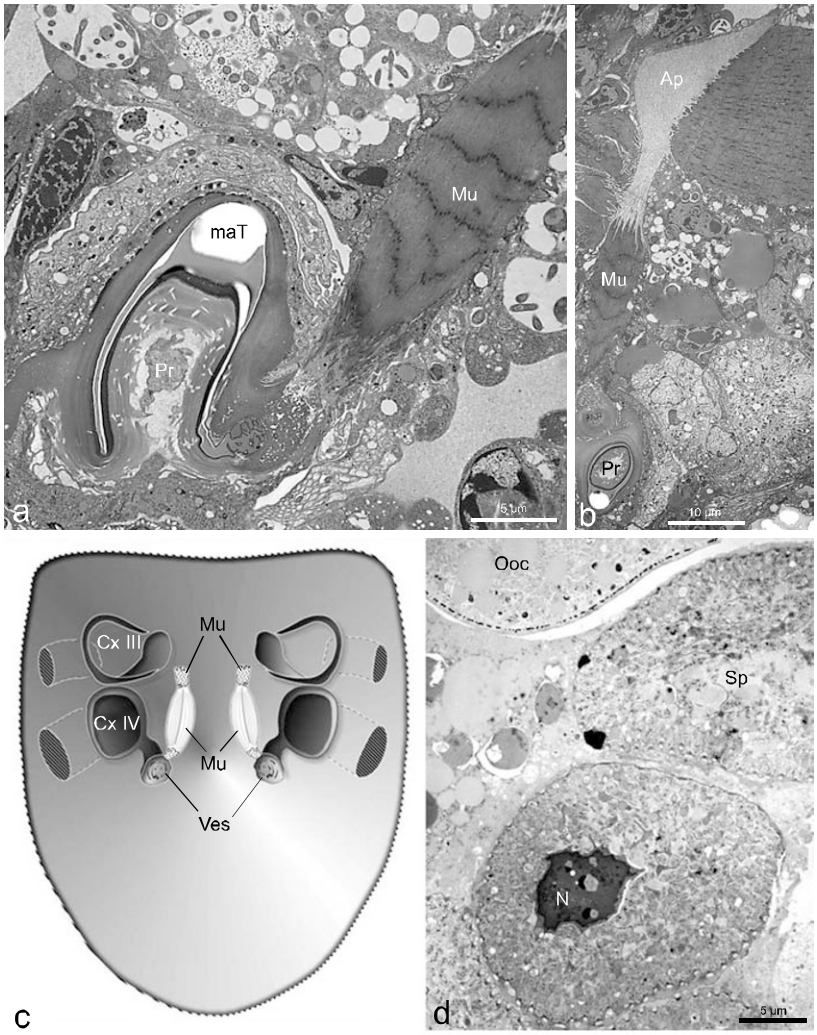

Figure 5 (a) TEM of *Veigaia nemorensis* showing a muscle attaching to region where minor tubes are located. (b) The muscle connects the structure with an anteriorly located apodeme. (c) Schematic reconstruction showing location of main components (acc. to *V. nemorensis*). (d) TEM of female *Veigaia* sp. showing sperm cells close to an oocyte. Abbr.: Ap, apodeme; Cx III-IV, coxa III-IV; maT, major tube; Mu, muscle; N, nucleus of sperm cell; Ooc, oocyte; Pr, process; Sp, sperm cell; Ves, vesicle-like region.

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Comparative ultrastructure of the integument in adult mites of the Parasitengona and its phylogenetic implication

Andrew B. Shatrov
Zoological Institute of the Russian Academy of Science, 199034, St.-Petersburg, Russia. E-mail: chigger@mail.ru

The integument of adult *Hirsutiella zachvatkini* and *Euschoengastia rotundata* (Trombiculidae), *Platytrombidium fasciatum* and *Camerotrombidium pexatum* (Microtrombidiidae), *Piona conglobata* (Pionidae), and *Teutonia cometes* (Teutoniidae) (water mites) were investigated by transmission and scanning electron microscopy. The soft integument of adult trombiculids is built up of specific epithelial respiratory tissue, provided by long, branched mechanoreceptor setae organized as neotrichia functioning as a plastron. The seta pits with clear cuticle are surrounded by cuticular folds with tightly packed ridges composed of an electron-dense substance. The underlying epidermal tissue is formed of separately scattered compact epidermal cells intermingled with large polymorphic so-called 'intra-epithelial cells' with clear cytoplasm devoid of organelles. The integumental folds with the intra-epithelial cells filled with metabolic and sorptional water are thought to function as air gills, which selectively absorb and transport oxygen from the outside to internal tissues. In microtrombidiids, also an internal cuticular meshwork is expressed, formed of thick electron-clear strands immersed into the epidermis and crossing at right angles. This meshwork is separated from the external cuticle, comprising a thick lamellar procuticle covered by thick electron-light epicuticle. Flat uniform epidermal cells contain numerous pigment granules. In water mites, a very thick lamellar procuticle penetrated by pore canals is covered by a thick dense epicuticle. Setae are arranged following the orthotrichous type. The epidermal layer underlying the cuticle consists of flat uniform epithelial cells devoid of pigment. The organization of the integument in stocks of Trombiculoidea and Trombidioidea appears strongly apomorphic, whereas it is plesiomorphic in water mites.

Key words: Epidermis, soft cuticle, ultrastructure, Trombiculidae, Microtrombidiidae, Pionidae, Teutoniidae, Parasitengona

Organization of the integumental tissue in arthropods and, particularly, in mites, composed of the epidermis and secreted cuticle (Neville, 1975; Alberti et al., 1981; Filshie, 1982; Hackman, 1982; Hepburn, 1985; Locke, 1985; Alberti & Coons, 1999), reflects the main strategy in their interaction with the environment (Beament, 1961) and serves at the same time as an indicator of the phylogenetic distance between branches of the evolutionary tree. The integument of representatives of the Parasitengona is poorly investigated and the data on its structure and functions are summarized in Alberti & Coons (1999) and Shatrov (2000).

To fill this gap in our knowledge, the integument of adult *Hirsutiella zachvatkini* (Schluger) and *Euschoengastia rotundata* (Schluger) (Trombiculidae), *Platytrombidium fasciatum* (C.L. Koch) and *Camerotrombidium pexatum* (C.L. Koch) (Microtrombidiidae) (soil mites), as well as *Piona conglobata* (C.L. Koch) (Pionidae) and *Teutonia cometes* (C.L. Koch) (Teutoniidae) (water mites) were investigated by means of transmission and scanning electron microscopy.

MATERIALS AND METHODS

Adults of the soil and water mites were collected in various regions of North-Western Russia. Trombiculids were captured as fully fed larvae leaving their natural hosts (bank voles), and adult mites were afterwards attained in a laboratory culture.

For transmission electron microscopy (TEM), adult mites were initially fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2-7.4) for 2-4 h. After immersion into the fixative fluid, animals were carefully pierced with tiny insect pins for a better penetration of the fixatives. Mites were then washed in several changes of 0.2 M phosphate buffer, postfixed in 2% osmium tetroxide in phosphate buffer for 1-6 h to overnight, dehydrated in ethanol and acetone series, and finally embedded in an araldite mixture. Serial ultra-thin sections both in transverse and longitudinal planes were made on an LKB-III ultramicrotome and, after staining with uranyl acetate and lead citrate, examined with Tesla BS-500 and LEO-900 TEMs at 60-90 kV. For preliminary and general observations, semi-thin sections were stained with toluidine blue and investigated under Amplival and Leica DMLS-2 light optical microscopes.

For scanning electron microscopy (SEM), mites that had been subject to alcohol fixation and an alcohol and acetone treatment were dried at the critical point of carbonic acid in a Hitachi HCP-2 vacuum evaporator, covered with a platinum layer in an Eiko IB-5 apparatus, and examined with a Hitachi S-570 electron microscope at 20 kV.

RESULTS

Soil mites

Trombiculidae

The soft integument of adult trombiculid mites is built up of very specific epithelial respiratory tissue and is provided with long, branched mechanoreceptor setae organized as a type of neotrichia (Fig. 1) (Shatrov, 2000). The round seta pits with centrally located seta bases are surrounded by wave-shaped cuticular folds bearing tightly packed irregular ridges (Fig. 2).

The seta pits and bases are formed of a poorly lamellate, clear procuticle covered with a thin electron-light epicuticle with pore canals (Figs. 3, 4). Towards the surrounding cuticular folds, the procuticle is substituted by an electron-dense substance secreted by the epidermal cells and filling the ridges being covered by the epicuticle (Fig. 4). The overall width of the cuticle in adult trombiculids is ca. 1-1.6 μm.

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The underlying epidermal tissue is formed of separately scattered compact epidermal cells intermingled with large polymorphic so-called 'intra-epithelial cells' with the cytoplasm devoid of distinct organelles (Figs. 3, 5). In the integumental folds, apart of the seta pits, these cells frequently become optically empty and are supposedly filled with metabolic water providing the necessary air-water balance and respiration.

The integumental folds with the intra-epithelial cells filled with metabolic and sorptional water are thought to function as real air gills (Fig. 6), which selectively absorb and transport oxygen from the outside to internal tissues. In general, the neotrichous integument is expected to function as a typical plastron (Hinton, 1968; Mill, 1985) retaining a particular air volume above the cuticle among setae (air bubble) and, in the absence of tracheae, playing a role in skin respiration, especially under unfavourable environmental conditions.

Microtrombidiidae

The strongly ridged extensible soft cuticle of microtrombidiid mites is armed with numerous neotrichous setae of different form and shape in different species (Figs. 7, 8). The setae extend from the vertically protruding high cuticular pedestals (bowls) (Fig. 8).

The most characteristic feature of microtrombidiids is the internal cuticular meshwork, which is formed of thick electron-clear fibrillar cuticular strands (Figs. 9, 10) (Alberti & Coons, 1999), immersed into the epidermis and crossing at right angles (Fig. 9). This internal cuticular meshwork is revealed totally separated from the 'external' cuticle (Figs. 10, 11).

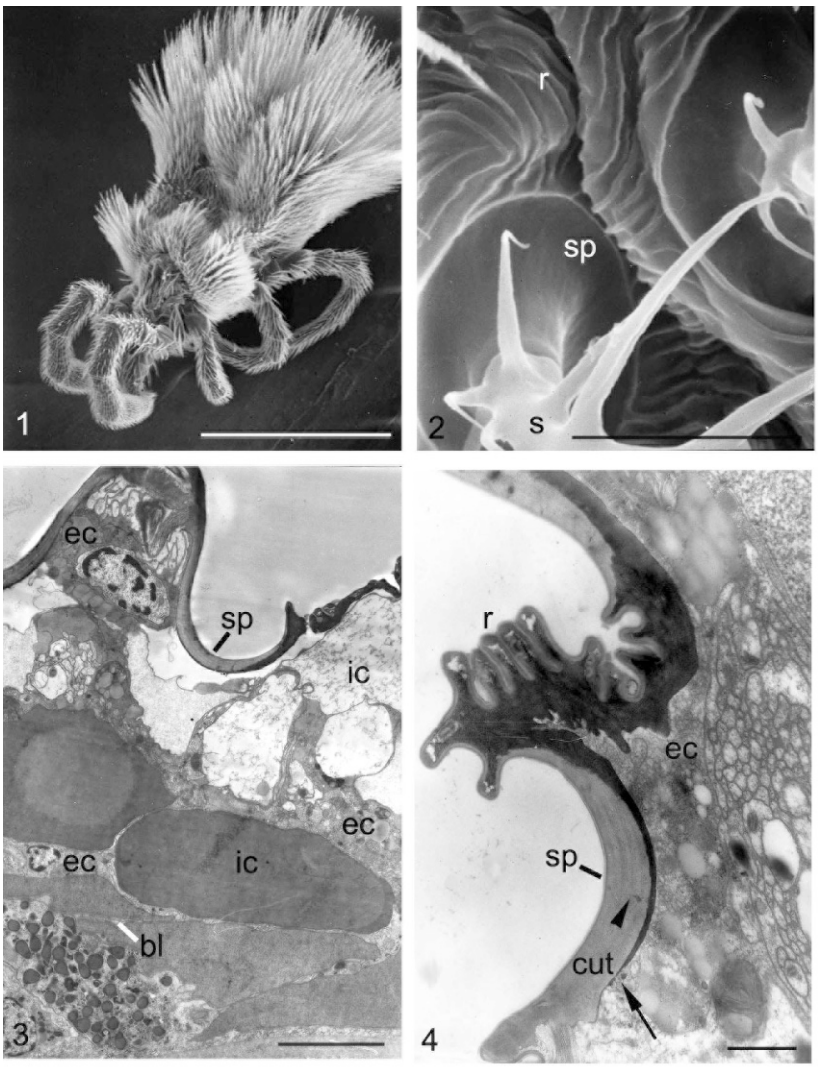
The external cuticle is composed of a thick, weakly lamellar procuticle with fibers tightly packed in basal portions and more loosely packed in the apical zone beneath the epicuticle (Figs. 11, 12). The width of the procuticle varies from 1 to 2.4 µm. The procuticle is penetrated by pore canals and covered by a thick electron-light epicuticle around 0.2 µm in width (Fig. 12). Flat uniform epidermal cells contain numerous pigment granules (Figs. 11, 12). Due to the presence of a tracheal system, gas exchange does not take place through the integument in microtrombidiids.

Water mites

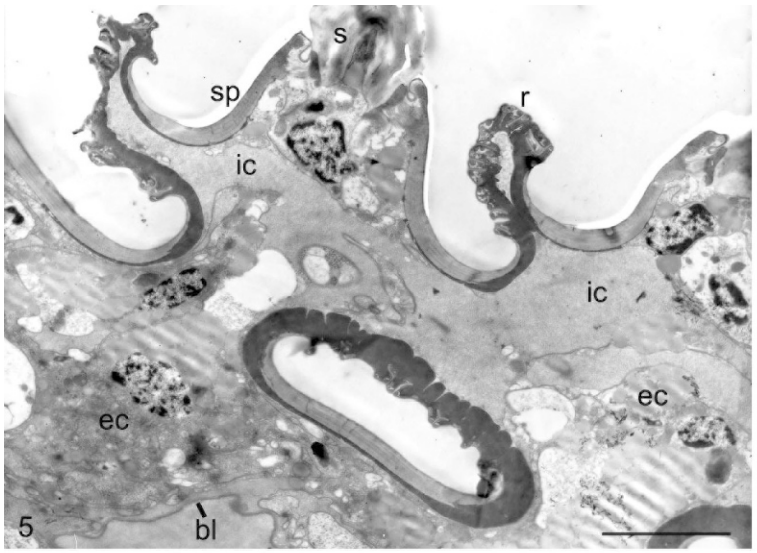
In contrast to the above soil mites, setae in water mites are arranged according to the orthotrichous type (Fig. 13). Externally, the integument is mostly flat (like in *T. cometes*) or somewhat ridged in irregular transverse or longitudinal rows (as in *P. conglobata*) (Fig. 14).

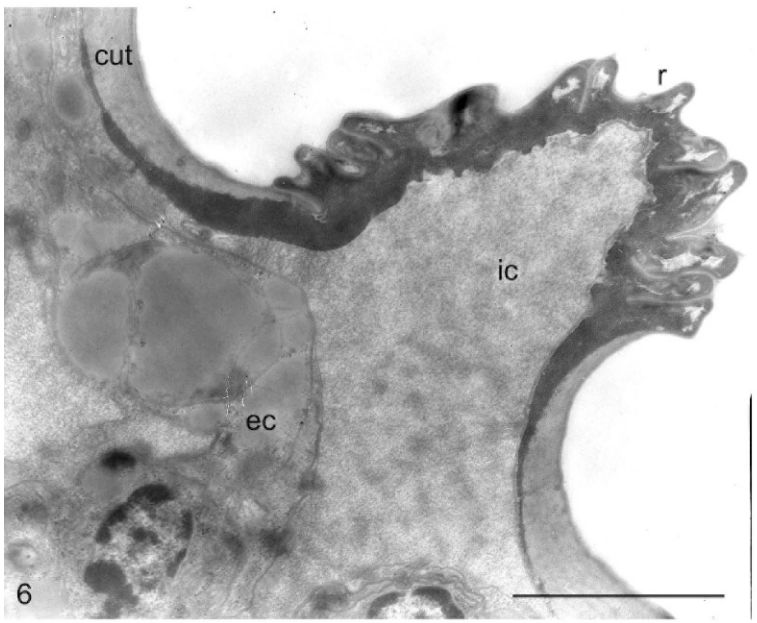
Pionidae

An extremely thick procuticle (9.2-10.4 µm) (Fig. 15) is strongly lamellate in the basal portions (Fig. 17) and homogeneous in the apical zone (Fig. 16), forming irregular ridges seen on the body surface of *P. conglobata* (Fig. 14). The procuticle is



Figures 1-4 Integument of adult *Hirsutiella zachvatkini* (Trombiculidae). (1) General view of adult mite with neotrichous setae; SEM, scale bar: 0.5 mm. (2) External view of integument with seta (s) located in seta pit (sp), and ridges (r) on the integumental folds; SEM, scale bar: 10 µm. (3) Cross section of integument illustrating compact epidermal cells (ec) and polymorphic intraepithelial cells (ic) underlying wavy cuticle with seta pit (sp) surrounded by cuticular folds; bl, basal lamina; TEM, scale bar: 3 µm. (4) Cuticular fold armed with ridges (r) and filled with an electron-dense substance instead of normal cuticle (cut) of seta pit (sp); ec, epidermal cell; arrow, secretion of the electron-dense substance; arrow head, pore canal; TEM, scale bar: 1 µm.





Figures 5-6 Integument of adult *Hirsutiella zachvatkini* (Trombiculidae), TEM. (5) Part of the integumental tissue of the dorsal body wall with seta pit (sp), seta base, and seta (s) surrounded by cuticular folds bearing ridges (r). Note separately scattered epidermal cells (ec) intermingled with large polymorph intraepithelial cells; bl, basal lamina; scale bar: 3 µm. (6) Supposed air gill with intraepithelial cell (ic) and cuticle formed of an electron-dense substance and armed with ridges (r); cut, cuticle of seta pit; ec, epidermal cell; scale bar: 2 µm.

penetrated by pore canals reaching a thin electron-dense epicuticle of 0.09 µm width covering the procuticle (Fig. 16).

Teutoniidae

A very thick lamellar procuticle of ca. 3 µm width with slightly wavy basal border comprises a thick endocuticle and a thinner exocuticle, which both are penetrated by pore canals (Fig. 18). The procuticle is covered by an electron-dense epicuticle with various thicknesses (0.3 µm on average), flat in *T. cometes* from the external aspect. The epidermal layer underlying the cuticle consists of flat uniform epithelial cells devoid of pigment granules and tightly adjoined to both the cuticle and the internal tissues (Fig. 18). The same arrangement is observed in *P. conglobata* (Figs. 15, 17).

DISCUSSION

These results clearly indicate that the organization of the integument in Trombiculoidea and Trombidioidea is strongly apomorphic, whereas in water mites it reveals some plesiomorphic primitive condition. Thus, the branches of Trombiculoidea and Trombidioidea may be considered as groups more early derived from the ancestral Parasitengona than water mites which secondarily returned to water and retained some primitive organization. Nevertheless, it may be supposed that each of these branches is of monophyletic origin, and also that they all are paraphyletic phyla derived in asynchrony from the ancestral group of the Parasitengona that shared some primitive conditions.

The nature and sources of the so-called 'intra-epithelial' cells in trombiculids are still unclear. There may be two explanations for their origin: (1) it is a group of resident epithelial cells strongly modified to function in water balance and respiration, or (2) it is a group of peripheral haemocytes or other cell types (particular type of fat body cells) that have migrated through the basal lamina and are incorporated into the epidermis. In any case, the organization and function of the integumental tissue in deutonymphs and adult mites of trombiculids is considered to be unique (Shatrov, 2000) and needs to be studied in further detail.

Acknowledgements

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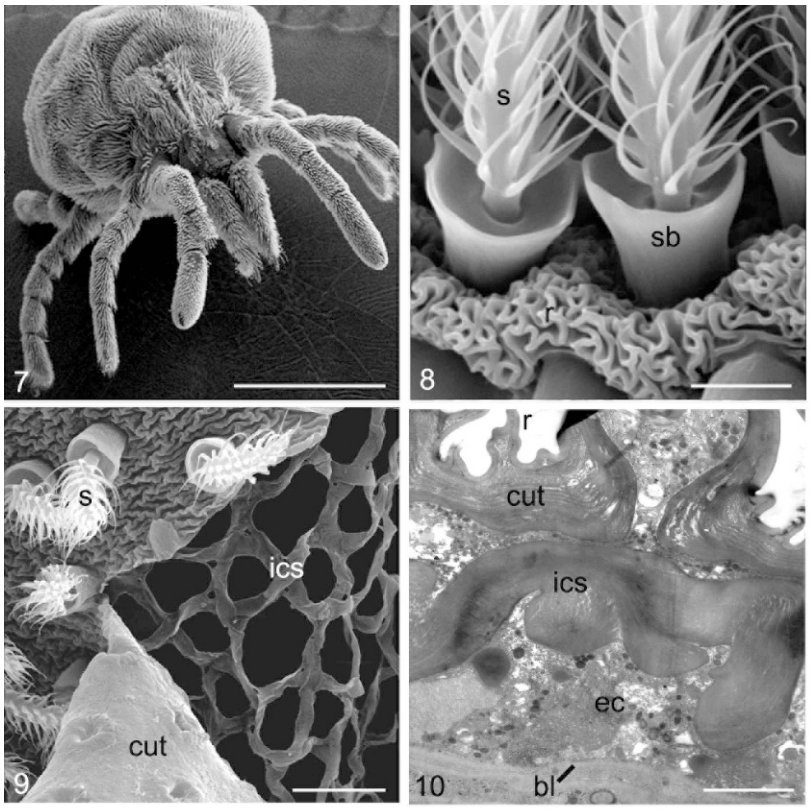
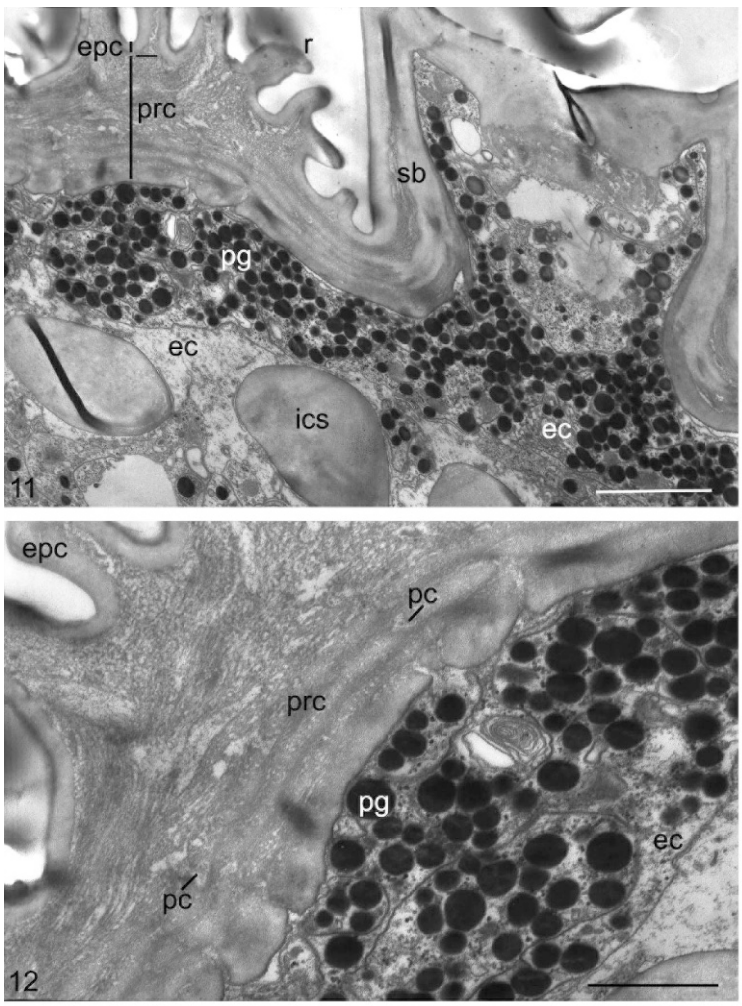
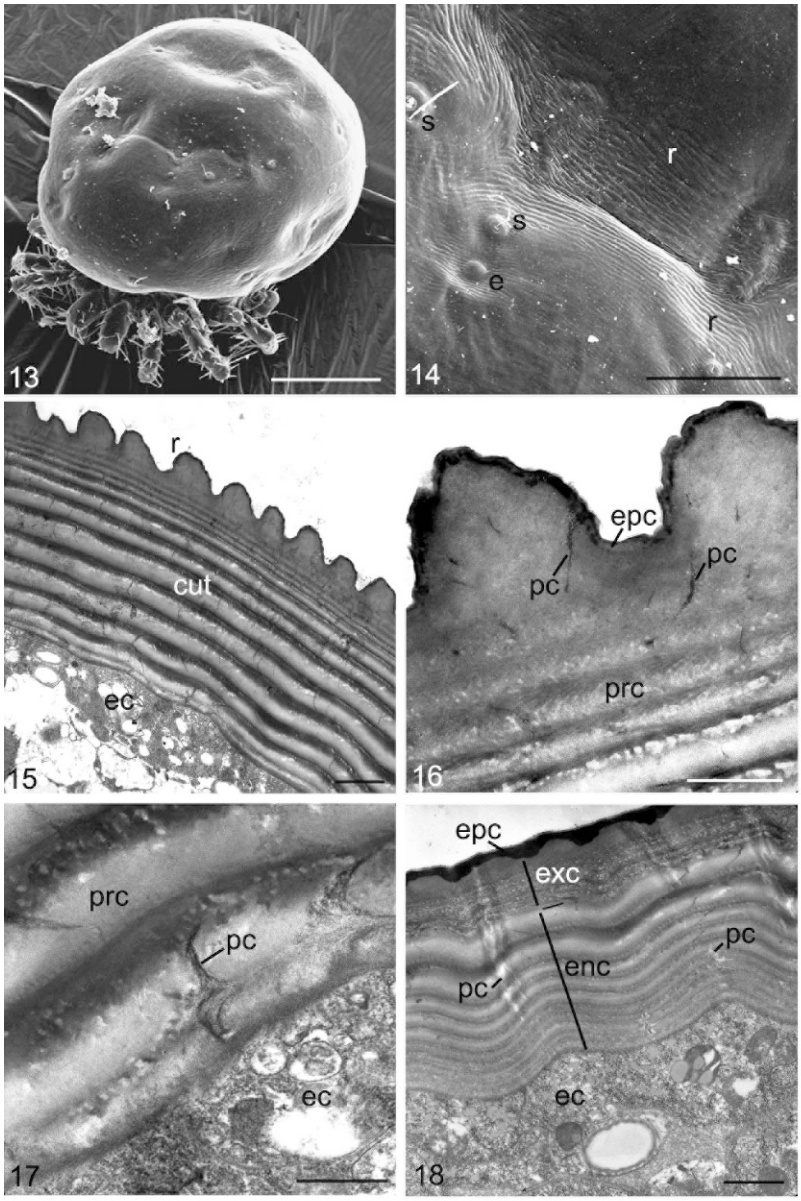


Figures 7-10 Integument of adult *Platytrombidium fasciatum* (Microtrombidiidae). (7) General view of adult mite with neotrichous setae; SEM, scale bar: 0.5 µm. (8) Setae pedestal (bowl) (sb) with seta (s) protruding from the intensively ridged cuticle (r); SEM, scale bar: 5 µm. (9) Removed 'external' cutical (cut) armed with setae (s) exposing internal cuticular meshwork of cuticular strands (ics); SEM, scale bar: 10 µm. (10) Cross section of integument demonstrating 'external' cuticle (cut) with ridges (r) and internal cuticular strands (ics) immersed into epidermal cells (ec) underlain by basal lamina (bl); TEM, scale bar: 3 µm.



Figures 11-12 Integument of adult *Platytrombidium fasciatum* (Microtrombidiidae), TEM. (11) Cross section through the integument showing 'external' cuticle composed of procuticle (prc) and epicuticle (epc) forming ridges on the surface (r). Note seta bowl and internal cuticular strand (ics) disposed within epidermal cells (ec) provided with pigment granules (pg); scale bar: 2 µm. (12) Procuticle (prc) penetrated by pore canals (pc) covered with epicuticle (epc) and underlain by epidermal cells (ec) provided with pigment granules (pg); scale bar: 1 µm.



Figures 13-18 Integument of adult water mites *Piona conglobata* (13-17) and *Teutonia cometes* (18). (13) General frontal view of adult mite with orthotrichous setae; SEM, scale bar: 0.5 mm. (14) Dorsal aspect showing setae (s), eye (e) and transverse ridges (r); SEM, scale bar: 0.2 mm. (15) Cross section through the integument demonstrating thick strongly lamellar cuticle (cut) with ridges (r) on the surface covered with thin epicuticle; ec, epidermal cell; TEM, scale bar: 3 µm. (16) Apical portion of the cuticle showing procuticle (prc) penetrated by pore canals (pc) reaching thin epicuticle (epc); TEM, scale bar: 1 µm. (17) Basal portion of the cuticle with lamellar procuticle (prc) penetrated by pore canals (pc) and underlain by epidermal cell (ec); TEM, scale bar: 1 µm. (18) Cross section through the integument showing procuticle composed of endocuticle (enc) and exocuticle (exc) and penetrated by pore canals (pc) and covered by dense epicuticle (epc). Note epidermal cell tightly adjoining the cuticle; TEM, scale bar: 1 µm.

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The water mite family Pontarachnidae, with new data on its peculiar morphological structures (Acari: Hydachnidia)

Harry Smit & Gerd Alberti[1]

Zoological Museum, University of Amsterdam, Plantage Middenlaan 64, 1018 DH Amsterdam, The Netherlands.
E-mail: smit.h@wolmail.nl; and [1]Ernst-Moritz-Arndt University, Zoological Institute and Museum, J.-S.-Bach-Straße 11/12, D-17489 Greifswald, Germany

The water mite family Pontarachnidae Koenike is the only family of the water mites (Hydrachnidia) occurring in the marine environment. Two genera of this family are known, *Pontarachna* Philippi and *Litarachna* Walter. Until now, this has been one of the least known water mite families. This paper gives an overview of our knowledge on this enigmatic family. For the first time, ultrastructural details are reported based on a study of *Litarachna communis*. Peculiarities of the cuticle, the so-called wheel-like acetabula, the glandular systems, the digestive system, the dorsomedian excretory organ, and the male genital system are reported.

Key words: Hydrachnidia, *Litarachna*, morphology, *Pontarachna*, ultrastructure

The water mite family Pontarachnidae Koenike is unique among the water mites (Hydrachnidia) in that it occurs in the marine environment. Two genera of this family are known, i.e., *Pontarachna* Philippi and *Litarachna* Walter. The first species of the family was already described in 1840 by Philippi. Despite this early description, the family is one of the least known of the water mites. Virtually nothing is known about their life cycle and food. Moreover, the family has peculiar morphological structures, not found in any other water mite species. The function of these morphological structures is unknown.

This paper gives an overview of our knowledge on this enigmatic family and contributes new data on the morphological structures.

MATERIAL AND METHODS

The material for transmission (TEM) and scanning electron microscopy (SEM) was collected in the marine littoral by HS on 10 May 2005, at Ramatuelle (near St. Tropez), France. After piercing the cuticle with a fine needle, the mites were fixed in 3.5% aqueous solution of glutaraldehyde buffered with cacodylate buffer (pH 7.4, 0.1 M). The specimens were kept in a refrigerator for about 2 h. Subsequently, the solution was diluted with buffer solution (4:1) and mailed to Greifswald for further processing by GA. Specimens were rinsed several times with buffer solution and transferred into 2% buffered OsO_4-solution for postfixation. After about 2 h, the mites were dehydrated using graded ethanols. Some specimens preserved in 70% ethanol were used for SEM analysis.

After dehydration with graded ethanols, these specimens were critical-point-dried (Bal-Tec CPD 030) with liquid CO_2 using amylacetate as an intermedium. The mites were placed on Al-stubs using double-sided carbon-tape. They were then coated with Palladium-Gold using a Quorum Technologies SC7620 Sputter Coater. Specimens were studied with a Zeiss DSM 940A SEM.

Specimens that were prepared for TEM were dehydrated as reported and then transferred into Spurr's medium (Spurr, 1969). Polymerization occurred at 70 °C. Ultrathin sectioning (70 nm) was performed with a Leica ultracut UCT microtome using a Diatome diamond knife. The sections were stained with uranylacetate and lead citrate according to Reynolds (1963) and studied with a Zeiss EM 10A TEM. Semithin sections (400 nm) were placed on glass slides and stained according to Richardson et al. (1960). These sections were used for general orientation (Olympus BX60). Light micrographs (LM) were obtained using an Olympus DP10-digital camera.

SPECIES RICHNESS

One of the first papers giving an overview of the family was by Walter (1925). In this taxonomical paper, Walter described *Litarachna*, the second genus of the family. At this time four species of *Pontarachna* were known and five species of *Litarachna*. In 1957, K. Viets published a key with eight *Pontarachna* and six *Litarachna* species, the latter including one *species incerta sedis*. Smit (2002) reported 13 species of *Pontarachna* and nine species of *Litarachna*. To this should be added one *Pontarachna* species described by Mari & Morselli (1983). With new descriptions published later on (Harvey, 1998; Smit, 2002, 2003, 2007, 2008a,b, 2009; Pešić et al., 2008a,b,c; Wiles et al., 2002) the total number of *Pontarachna* species comes to 22 and the total number of *Litarachna* species comes to 18. Most species have been found in tropical and subtropical seas.

Although most species occur in the marine littoral, a few are known from freshwater habitats. Cook (1986) described the first from a freshwater stream on Tasmania, at a few kilometers from the sea. The specimens from this location came

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from interstitial deposits. A second species, *Pontarachna hoffmannae* Cook, from freshwater habitats in South Africa also came from a location close to the sea (Cook, 1996). Cook (1996) stressed that these two locations were definitely not estuarine. KO Viets (1964), on the contrary, reported *P. hoffmannae* from the Keurbooms River Estuary, indicating that this species lives both in the freshwater part of this river as well as in its estuary. Viets (1964) identified his specimens erroneously as *P. punctulum*.

PLACEMENT IN THE SYSTEM OF WATER MITES

The family Pontarachnidae is nowadays usually placed in the superfamily Hygrobatoidea (KO Viets, 1987). However, Tuzovskij (1983, 1987) placed the Pontarachnidae in its own superfamily, the Pontarachnoidea.

ECOLOGICAL AND LIFE CYCLE DATA

Few ecological data have been published. Mari & Morselli (1983) found *Litarachna communis* Walter occurring up to a depth of 25 m, although most specimens were found between 3 and 5 m. *Pontarachna aenariensis* Mari & Morselli was also most numerous between 3 and 5 m, and was not found below 6 m. Near Ramatuelle (France) *L. communis* was found between algae at a depth of less than 0.5 m (H Smit, pers. obs.).

Mari & Morselli (1983) reported a skewed sex ratio in *L. communis* (more than 80% males), whereas in *P. aenariensis* many more females were present; no deutonymphs were found. However, deutonymphs have been described for both *Pontarachna* and *Litarachna* (Tuzovskij, 1987). Larvae have never been found. In a search for potential hosts, a number of Diptera were collected in the marine littoral at Ramatuelle

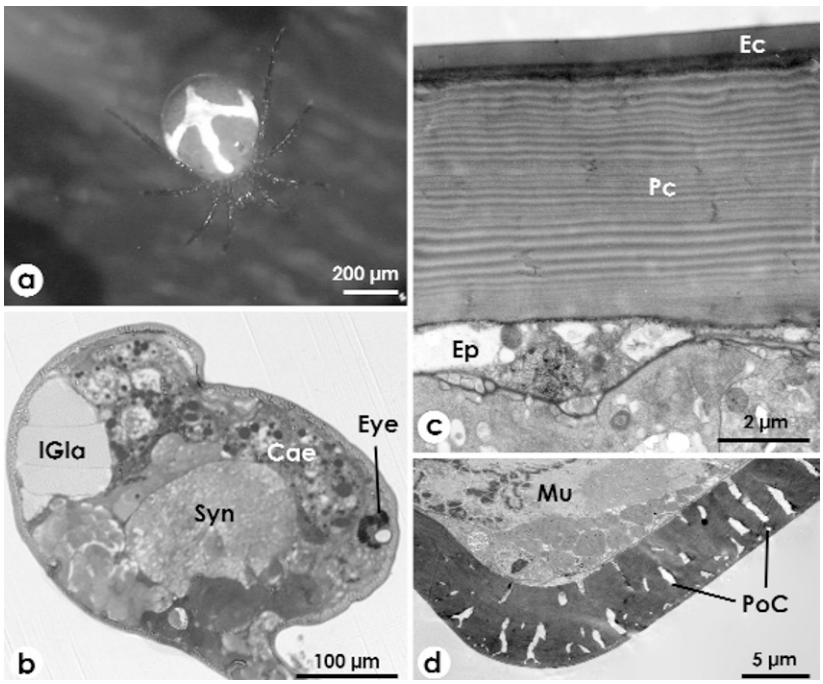

Figure 1 (A) Live pontarachnid mite, *Litarachna halei* from South Australia (Photo by courtesy of Lisa-Ann Gershwin). (B) Oblique longitudinal, parasagittal section (LM) through a male *L. communis.* (C) Cuticle of the weakly sclerotized body parts (TEM). (D) Cuticle of an epimeron (TEM). Abbr.: Cae, caecum; Ec, epicuticle; Ep, epidermis; lGla, lateral gland; Mu, muscle; Pc, procuticle; PoC, pore channels; Syn, synganglion.

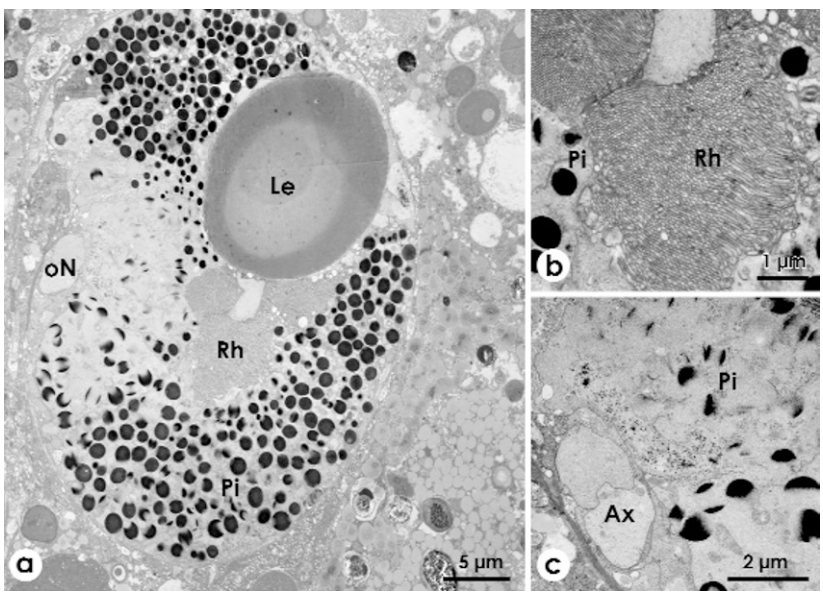

Figure 2 TEMs of a lateral eye of *Litarachna communis*. (A) Overview showing spherical lens sunken under the integument and surrounded by retinula cells providing rhabdomeres and containing pigment granules (compare Fig. 1B). (B) Detail of rhabdome comprised of numerous microvilli. (C) Two axons contributing to optic nerve. Abbr.: Ax, axons; Le, lens; oN, optic nerve; Pi, pigment ganules; Rh, rhabdome.

(Table 1). None of these Diptera, however, were infected by mites. So it remains unknown whether pontarachnids have a larval stage, or whether they forego this stage.

Although most species seem to prefer benthic habitats, a recent observation of *Litarachna halei* (Womersley) (Fig. 1A) from South Australia indicates that this species lives both in benthic habitat as well as in jellyfishes. This occurrence of water mites in jellyfishes has never been observed before.

Table 1 Diptera collected in the marine littoral at Ramatuelle, France, on 10 May 2005.

Family	Number
Anthomyiidae	40
Dolichopodidae	15
Ephydridae	1
Coelopidae	10
Tethinidae	ca. 100
Ceratopogonidae	1

MORPHOLOGY AND FINE STRUCTURE

Cuticle

The integument of *L. communis* consists of a single layered epidermis and a cuticle (Alberti & Coons, 1999) (Fig. 1B-D). Although the epidermis does not show remarkable peculiarities, the cuticle is specific. This refers in particular to the procuticle in most parts of the body (i.e., in the weakly sclerotized parts). In these parts, the procuticle is very densely layered and is almost devoid of pore canals. The epicuticle is quite thick and is homogeneous. Hence, the cuticle seems to be very tight. However, there are some exceptions, e.g., in the epimera (= coxal plates, coxisterna), close to strong muscles, the procuticle does not show distinct layers and contains many pore canals, which are rather wide and may

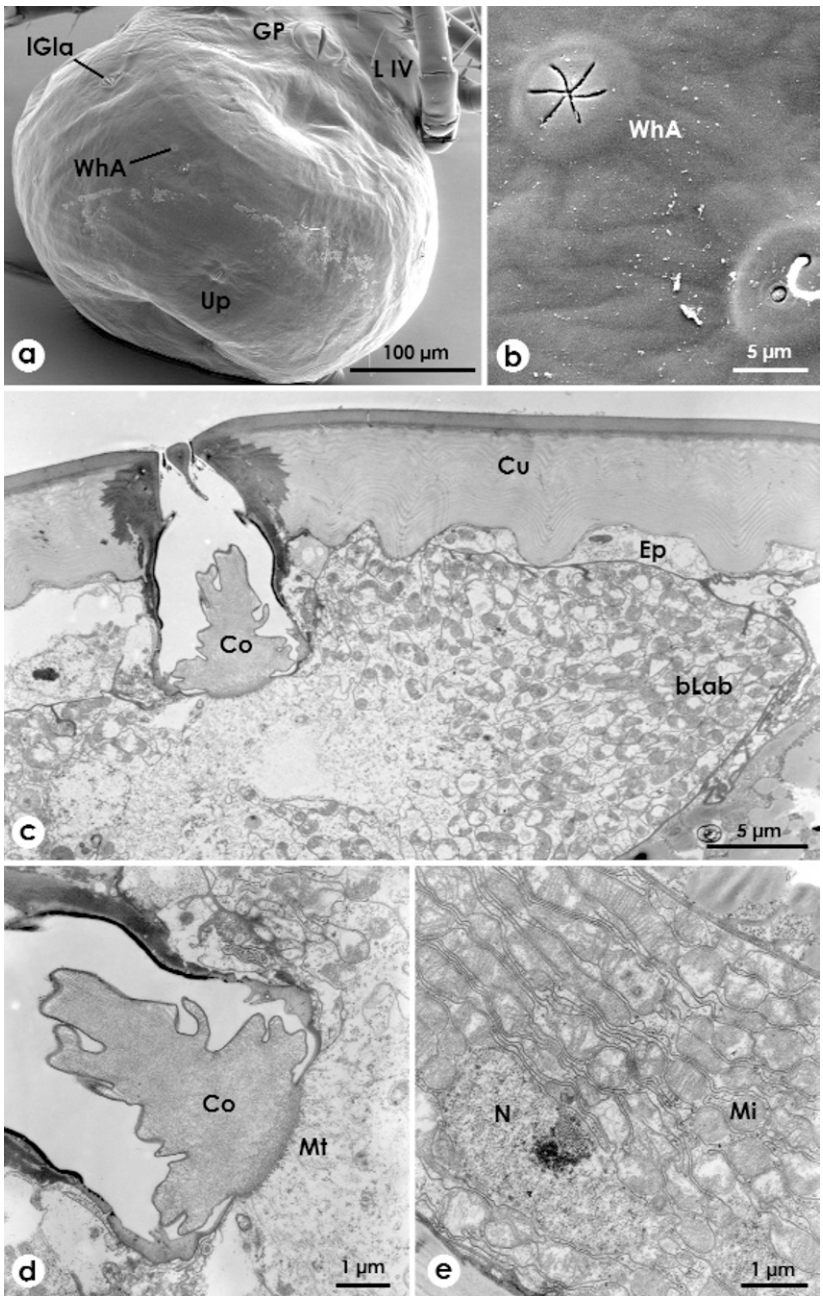

Figure 3 Wheel-like acetabula of *Litarachna communis*. (A) Overview showing position and size of opening of a wheel-like acetabulum in relation to other external structures (SEM). (B) Detail showing radiating folds characteristic of the wheel-like acetabula (SEM). (C) Overview showing wheel-like acetabulum (TEM). Note the modified cuticle in the pore region with a peculiar cuticular cone and basal labyrinth composed of numerous infoldings of the plasmalemma associated with mitochondria. (D) Detail showing modified cuticle with cone (TEM). Note numerous microtubules in the cytoplasm. (E) Detail of basal labyrinth dominated by membranes and mitochondria (TEM). Note inconspicuous nucleus. Abbr.: bLab, basal labyrinth; Co, cone; Cu, cuticle (weak integument); Ep, epidermis; GP, genital pore; LIV, leg IV; lGla, pore of lateral gland; Mi, mitochondria; Mt, microtubules; N, nucleus; WhA, wheel-like acetabulum.

branch towards the exterior. They terminate under the epicuticle which looks like that of other parts of the body. Thus, the cuticle of the epimera seems to be more rigid (strongly sclerotized) on the one hand and more permeable (likely adapted to gaseous exchange) on the other.

Eyes

Lateral eyes are evident due to their dark pigmentation in the live animal (Figs. 1A,B, 2). Each eye is composed of a spherical, cuticular lens which is deeply sunken under the surface of the body and is surrounded by retinula cells which form rhabdomeres directed against the lens. These cells also contain numerous dense pigment granules. The axons of the retinula cells form an optic nerve.

Wheel-like acetabula

Pontarachnidae have structures which are not found in any other water mite species. They appear as rounded structures with radiating spokes. Cook (1996) discussed this morphological structure extensively. He called them wheel-like acetabula, but was uncertain whether or not they were true acetabula. These structures are found both in the weakly sclerotized integument and on the postgenital sclerite in the female of *Pontarachna* species. In the male of *Pontarachna* and in *Litarachna* species they are only found in the weakly sclerotized integument. Males of *Pontarachna* species have small and larger wheel-like acetabula. The number of wheel-like acetabula in the integument is variable. Smit (2003) could not find wheel-like acetabula in two West-Australian *Pontarachna* species.

We studied these wheel-like structures in specimens of *L. communis* collected at Ramatuelle. The term coined by Cook (1996) refers to the cuticular structure which is characterized by small radiating folds surrounding a cuticular depression. Sections through this structure show that the depression widens and contains a cuticular cone-like elevation of peculiar structure (Fig. 3). In contrast to the surrounding weakly sclerotized cuticle it is not built of parallel layers of procuticle cov-

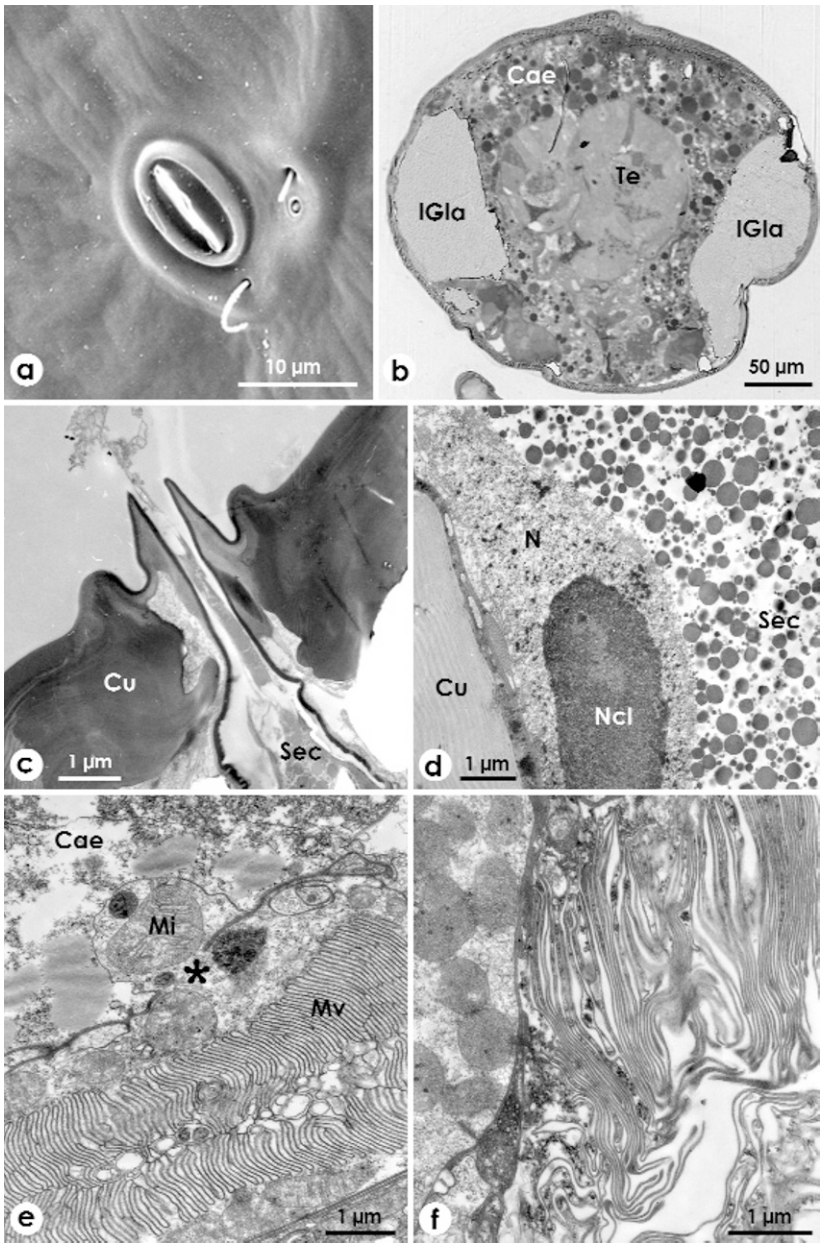

Figure 4 Glandular systems of *Litarachna communis*. (A) SEM of opening of a large lateral gland (compare Fig. 3A). (B) Transverse section (LM) showing extension of lateral glands (compare Fig. 1B). (C) Section through opening of lateral gland (TEM). (D) Detail of gland showing peripheral cytoplasm of one cell with large nucleus containing conspicuous nucleolus (TEM). The remainder of the cytoplasm contains secretion droplets. (E) Detail of a transversely sectioned tube of the coxal gland (TEM). Note conspicuous microvilli and large mitochondria. Asterisk indicates a region where the basal lamina of the caecal epithelium is interrupted and part of the epithelium of the tubule is in direct contact with the caecal cell. (F) Detail of the bladder of the coxal gland (TEM). Note very flat and highly folded epithelium. Abbr.: Cae, caecum; Cu, cuticle; lGla, lateral gland; Mi, mitochondrion; Mv, microvilli; N, nucleus; Ncl, nucleolus; Sec, secretion; Te, testis.

ered by thick, homogeneous epicuticle (see above). Instead, it is made of fibrillar material loosely arranged within an electron-lucent matrix. This material is covered by a very thin electron-dense layer, likely corresponding to the epicuticular layer. This layer may be covered by an electron-dense, diffuse material which is also found at the walls of the depression and within the small opening leading to the external surface. The base of this cone is connected to the weakly sclerotized cuticle by a very thin cuticle, apparently composed only of 'normal' epicuticle. There is also a small fold surrounding the base of the cone. The underlying (probably only few) cells directly contact the cone. These cells are very large and extend under the integument occupying quite a large area. They are characterized by numerous large mitochondria with many cristae which are almost completely occupying the cell body. The cells are specifically structured by numerous basal infoldings of the plasmalemma. A nucleus is rarely found. It shows some heterochromatin and a distinct nucleolus.

Glandular systems

As in other actinedid mites there are several glandular systems in Hydrachnidia, e.g., podocephalic glands (including tubular coxal glands and acinous glands), infracapitular glands (also called salivary glands), and tracheal glands (e.g., Vitzthum, 1943). In contrast to terrestrial Actinedida, freshwater mites are known to possess a number of dermal glands (e.g., Alberti & Coons, 1999). In contrast to coxal glands (Alberti & Storch, 1977), most of the other glands of Hydrachnidia have not yet been studied by electron microscopy. Because the amount of material was limited, we could study these glands only to a limited degree and hence the following is a preliminary assessment.

There are apparently different types of dermal glands. A pair of large glands was found at the level with the anterior wheel-acetabula, but more laterally (Figs. 1B, 4A-D). The openings of these glands differ in structure from the wheel-acetabula. They are slit-like and their cuticular borders are

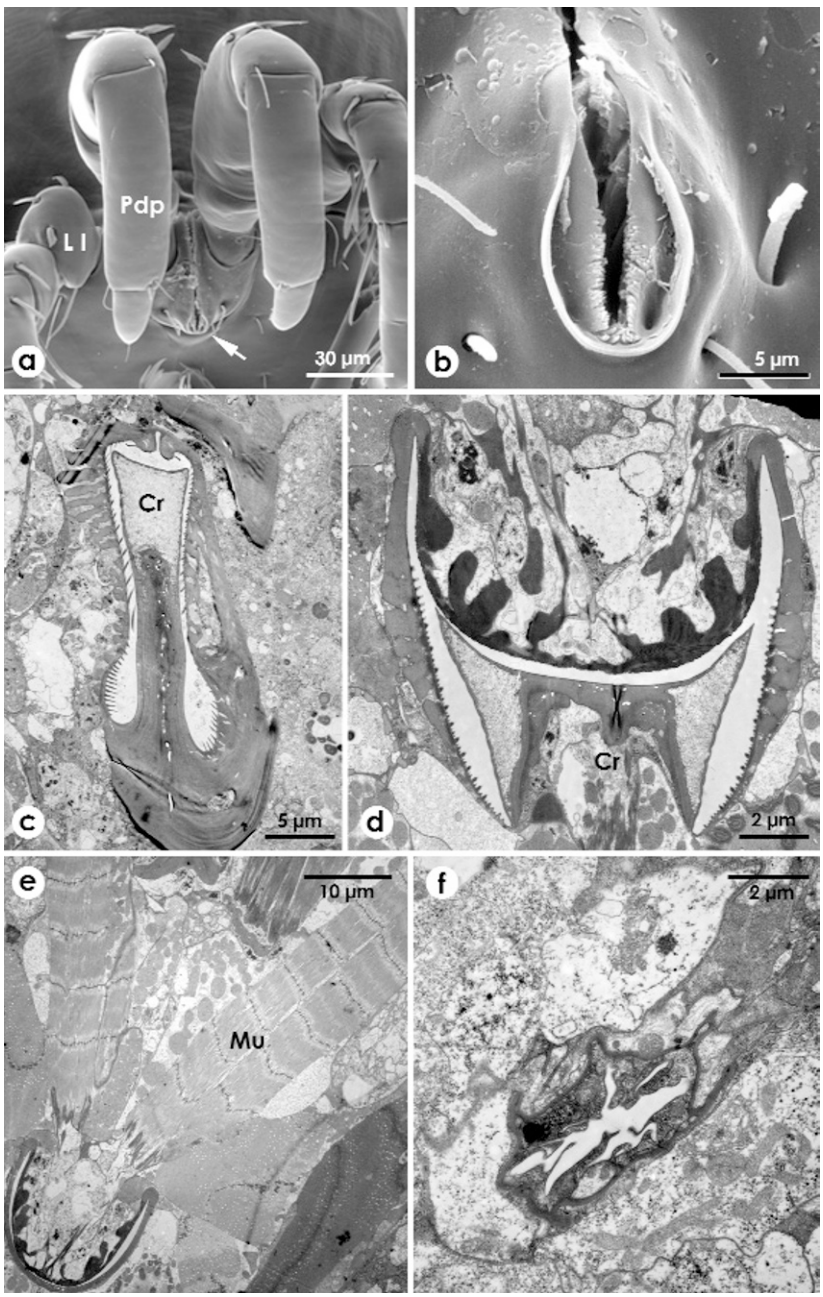

Figure 5 Digestive system of *Litarachna communis*. (A) Fronto-ventral view of the gnathosoma (SEM). Arrow points to tip of infracapitulum. (B) Tip of infracapitulum with opening of preoral cavity surrounded by peculiar fringe (SEM). The chelicerae are completely sunken into the infracapitulum. (C) Anterior part of pharynx (TEM, transverse section). Note cuticular differentations and pronounced ventral crest. (D) Pharynx more posteriorly sectioned (TEM, transverse). Ventral crest shallower, dorsal roof of pharynx acquires U-shape. (E) Pharynx even more posteriorly sectioned (TEM). Ventral crest has disappeared. Note strong dilator muscles. (F) Oesophagus passing through the synganglion (TEM, transverse section). Note small diameter and irregular outline of cuticle-lined oesophageal epithelium. Abbr.: Cr, ventral crest; LI, leg I; Mu, dilator muscle; Pdp, pedipalp.

projecting rather than being indented. They do not have the cone described above. Instead they lead into a huge space occupied by myriads of small granules. Cytoplasmic components of cells composing this gland are almost invisible. Their cytoplasm is limited to a thin layer at the periphery of the structure. Here, a nucleus is rarely found. These glands are likely composed of only very few, but very large cells, which are surrounded by a few small muscles.

More frequently, there occur much smaller glands, which are connected to small setae. These glands appear either empty or contain a homogeneous secretion. The glandular epithelium is quite inconspicuous (not shown).

In *L. communis*, a pair of what we think represents long tubular glands, likely the coxal glands, was observed adjacent to the outer surface of the caeca (see below) and apparently forming a loop (it was cut two times on each side in transverse sections) (Fig. 4E,F). The gland was even embedded into basal parts of the caecal epithelium. The sections always showed an extensive microvilli border completely occupying the lumen of the structure. The cells are provided with numerous large mitochondria containing many cristae. The structure is surrounded by a dense basal lamina, which is sometimes interrupted in areas where the gland is in contact with the caecal epithelium. Although we could not yet reconstruct the course of the complete gland, it seems likely that it terminates with a peculiar highly folded part. The folds are very thin plicae, perhaps covered by an extremely thin cuticle. It is likely that this structure is a bladder, which is known to occur with rather similar appearance in certain freshwater mites (Alberti & Storch, 1977). This part of the system continues into a thick cuticle-lined duct.

In the area of the bladder and the cuticular duct, glandular tissue was seen, which likely is part of the acinous podocephalic glands. However, at the moment we have no more details. Another paired gland was found in a dorsal position in front of the ventricle. These latter glands are characterized by an extensive rough endoplasmic reticulum and conspicuous dense secretory granules. These glands could either represent another podocephalic gland or be the infracapitular or salivary glands. More studies are needed to clarify the course of the corresponding ducts. An unpaired tracheal gland is also present and produces a lipid secretion (glands not shown).

Digestive system

The digestive system starts at the anterior tip of the infracapitulum (= subcapitulum). Here, the opening of the preoral cavity (or groove) is located and this is surrounded by a distinct fold. SEM reveals that there is an internal fringe of small papillae (Fig. 5A,B). The chelicerae are completely sunk into the infracapitulum and thus not visible in our SEMs. The preoral cavity and anterior part of the pharynx show a peculiar cross-section (Fig. 5C-E). Its dorsal roof is provided with a dense, sclerotized cuticle to which a tendon is attached. The ventral wall of this region forms a strong crest projecting against the roof with a peculiarly structured cuticle. The crest is bordered by two deep channels, the cuticle of which bears small teeth on its abaxial walls. More posteriorly, the crest becomes more and more shallow and finally, the pharynx gets a cross section which resembles a 'U'. The pharynx is provided with very strong muscles. It is evident that the pharynx has the capacity for efficient sucking and pumping activities. In contrast, the oesophagus is a rather weak structure which passes through the synganglion (Fig. 5E). It is composed of a thin epithelium bearing a very thin cuticle. No muscles have been observed.

The oesophagus should lead into the midgut ventricle, but this is not yet seen. There are two thick caeca extending dorsolaterally to the posterior of the body (Fig. 6). These are composed of very large cells that almost completely fill the caeca, i.e., a lumen is hardly detectable. The cells are filled with the usual organelles, but are dominated by inclusions of very heterogeneous appearance. These are evidently containing nutritional materials. They are developing into huge lysosomes and residual bodies. Furthermore, there are lots

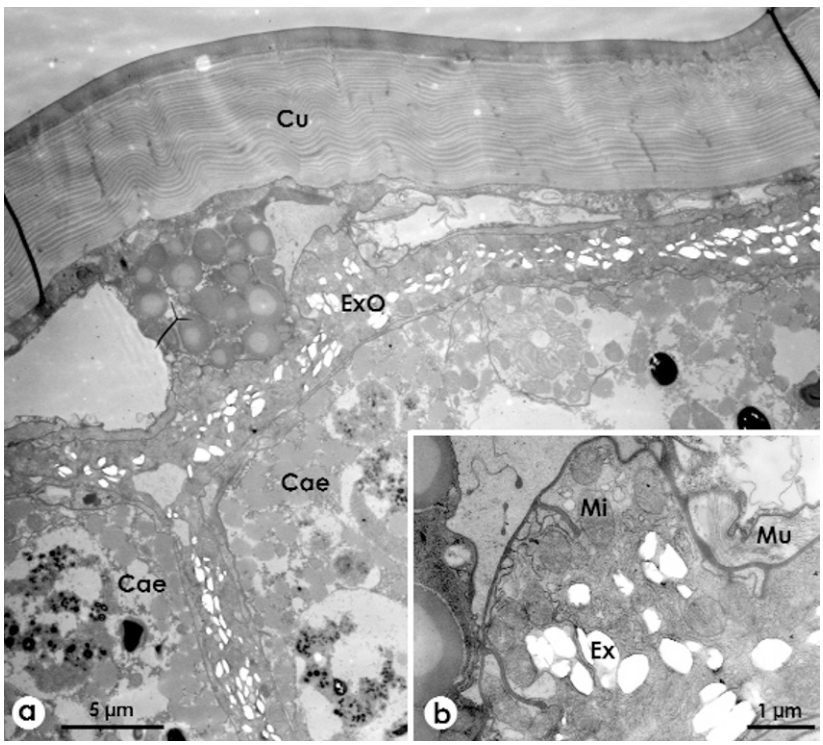

Figure 6 Excretory organ (TEMs, cross section). (A) Overview. The organ is sectioned in the area of its branching (compare Fig. 1A). Note the flat epithelium of the excretory organ and the very large cells of the caeca. (B) Detail showing numerous mitochondria within the epithelial cells and excretory granules (largely lost during sectioning). Abbr.: Cae, caeca; Cu, cuticle (weak integument); Ex, granules of excretion; ExO, excretory organ; Mi, mitochondrion; Mu, muscle.

of lipid inclusions. Hence, these cells are certainly digestive cells with a rather conventional aspect. Secretory cells have not yet been seen (cf. Alberti & Storch, 1983; Alberti & Coons, 1999).

Excretory organ

A dorsomedian excretory organ (the homologue of the postcolon) is present (Fig. 6). It is a long tube which terminates with a uropore (the homologue of the anus) (Fig. 3A). The organ is located close to the dorsal and lateral integument and branches in the middle region of the body. It is composed of a flat epithelium, which shows some basal infoldings of the plasmalemma with adjacent large mitochondria. The epithelium produces a crystalline secretion which is extruded into the lumen of the structure and likely represents guanine. These excretory products are responsible for the bright white cross, that stands out conspicuously in live mites of this group (Fig. 1A).

Male genital system

The male genital system is composed of two very large testes which are located lateroventrally and are composed of two parts, one germinal and one glandular (Fig. 7A-C). The glandular part is composed of very large cells which are dominated by rough endoplasmic reticulum and large nuclei. This tissue certainly contributes to the contents of the testis lumen, although its products are not very visible within the cells. In the lumen of the testis, mature spermatozoa as well as a peculiar secretion containing several different components are present. These components are large, irregularly shaped dense patches, small dense granules, and – most unusually – numerous tubular structures. The sperm cells are spherical and aflagellate, surrounded by a thin secretion sheath. The cells contain a dark compact chromatin body which has a thin extension. This extension indents a vacuole-like structure with electron-lucent contents. Furthermore, there are some inclusions of moderate density. Few, rather large, mitochondria are also present.

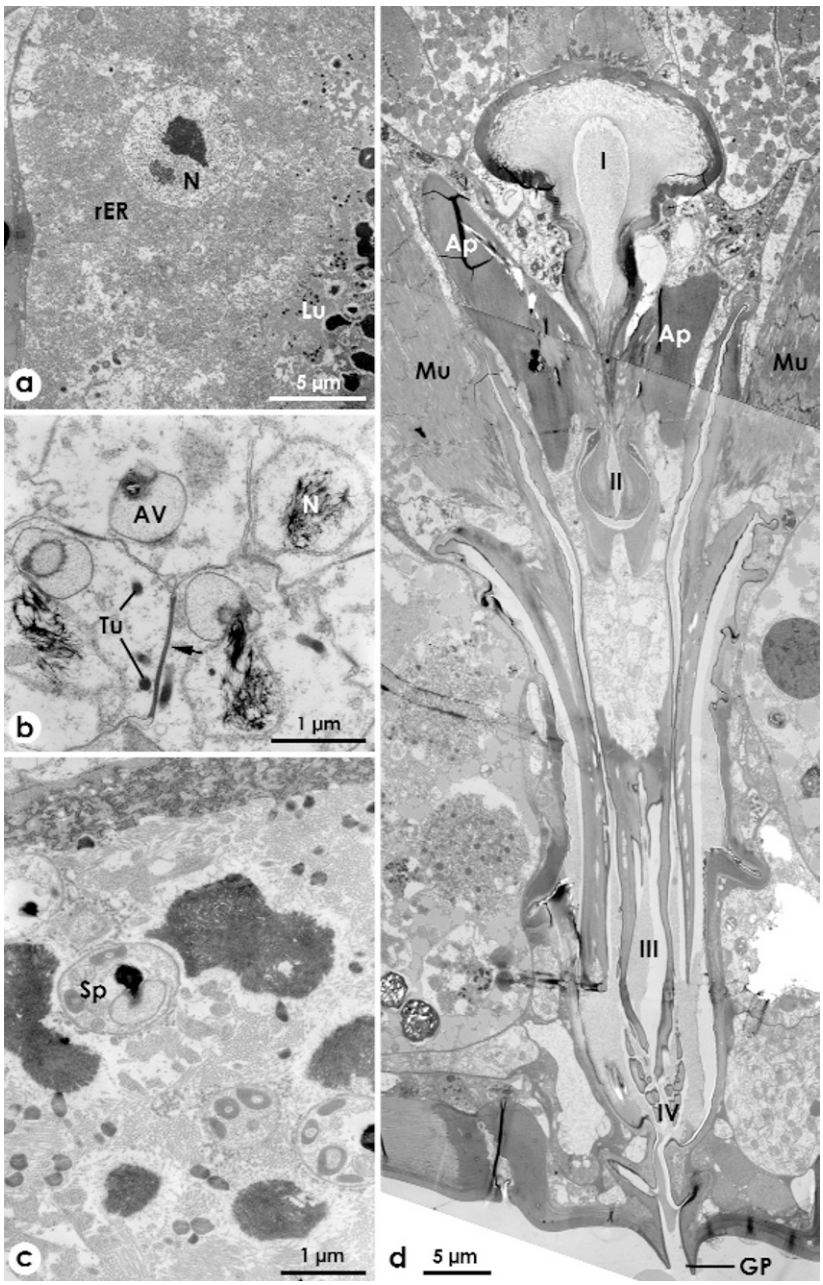

Figure 7 Male genital system (TEMs). (A) Detail from glandular part of the testis. Note large cell full of rough endoplasmic reticulum and with a round nucleus and a conspicuous nucleolus. (B) Germinal part of the testis with late spermatids. Nuclei are partly condensed and in contact with a rather large acrosomal vacuole. Few dense tubes are seen. Note density between two adjacent spermatids (arrow). (C) The lumen of testis contains spherical spermatozoa covered by a thin secretion sheath and a highly complex secretion. (D) Compose figure of the ejaculatory complex. Note complex shape showing roughly four 'storeys' (I-IV) and various apodemes and muscles. Abbr.: Ap, apodeme; AV, acrosomal vacuole; GP, genital pore; Lu, lumen of testis; Mu, muscle; N, nucleus; rER, rough endoplasmic reticulum; Sp, spermatozoon; Tu, tubules.

The testes continue into small vasa deferentia that join to form an ejaculatory duct running through an ejaculatory complex which is oriented perpendicularly towards the genital opening (Fig. 7D). The ejaculatory complex is composed of four vertical 'storeys' of very specific shapes. It is surrounded by strong muscles which are at least partly attached to strong apodemes.

Acetabula (genital papillae) are not present. The genital opening is flanked by the rather thin cuticle of the genital plates.

DISCUSSION

The ultrastructural studies, although still somewhat preliminary, have shown that the general features of actinedid organization are present, as one might expect. This concerns the general organization of the digestive system and the dorsomedian excretory organ. The peculiarities of the pharynx need further study as they may provide clues as to what food is consumed. The cuticle is remarkable as it is almost tight in most parts of the body. This may be an adaptation to the exceptional medium, these mites have conquered to live in. This cuticle is – compared with what is known from other Hydrachnidia – rather similar to that of *Piona* species (Alberti et al., 1981; Alberti & Coons, 1999) occurring in freshwater. Of course, due to our limited knowledge, this cannot be taken as indication of a closer relationship. However, it indicates that these mites need to keep their body surface tight. In contrast, the cuticle of the epimera has numerous wide pore canals. These may help gaseous exchange in the area of the strong muscles that make the legs moving (a tracheal system seems not to occur in *L. communis* or is at least much reduced; Walter, 1925).

A main problem of animals which live in water is osmoregulation. Animals which invaded marine habitats secondarily, are generally hypotonic. Though this is not known from direct measurements in *L. communis*, it likely applies also to this species. However, in *L. denhami* the osmotic concentration of the hemolymph is much higher than in related freshwater mites. Thus the osmotic gradient between the internal and external media is considerably diminished as are the energy demands for osmotic regulation (Witte & Olomski, 1999). In contrast, freshwater-inhabiting animals are usually hypertonic. The latter applies to most of the Hydrachnidia. As was shown by Alberti (1977, 1979), Alberti & Bader (1990), Alberti & Coons (1999), and Goldschmidt et al. (1999), these mites have improved the genital papillae (= acetabula) of the actinotrichid organization as structures through which ions can be actively accumulated from the surrounding medium, thus compensating for losses due to their osmotic properties. Thus, freshwater mites are probably provided with the most complex 'chlorid tissues' (cell complexes which perform active transcellular transport of, e.g., chloride ions) found in the animal kingdom. This complexity is most evident from a highly differentiated cuticular structure. Remarkably, a similar but not identical organization of such structures was found by Fashing (1984, 1988) in some freshwater-inhabiting Acaridida.

Marine inhabiting mites have the opposite problem. Since they are hypotonic, they are in the danger of loosing water and thus being overcharged with ions. Hence, they have to find solutions of preventing ions getting in (making the cuticle tight) and/or getting ions out of the body. In the present study, we can show that the wheel-like acetabula of *L. communis* have properties of transporting epithelia, like the genital papillae (acetabula) of other actinotrichid mites. However, in contrast to all the other aquatic actinedid mites studied until now (including Halacaridae, pers. obs.), the cuticular structures are quite different, in that they are devoid of the pit-like indentations. Instead, we found the peculiar cuticular cone. Hence, we are quite sure that these structures take part in osmoregulation, i.e., in pumping ions from the interior to the exterior. But we are uncertain whether these structures are homologues of the genital papillae (acetabula) of other freshwater mites (or Actinotrichida). Since some Pontarachnidae do not have these structures, it might be that they have evolved de novo in certain species. The study of Witte & Olomski (1999) has shown that *L. denhami* is a very effective hyporegulator. This ability might very well be correlated with the evolution of wheel-like acetabula.

In this respect it would be of much interest to investigate the larva, since the genital papillae (acetabula) occurring in the post-larval stages are usually correlated with similar, but differently placed Claparède's organs. If these would be lacking, this finding would speak against a homology of genital papillae (acetabula) and the wheel-like acetabula. Interesting in this respect is the description of Claparède's organs (urstigmata) in post-larval Pontarachnidae by Tuzovskij (1987). Unfortunately, we could not yet find these structures in our preparations.

Another component of the body which is involved in osmoregulation is the coxal gland, a structure derived from the nephridia. As in other actinotrichid mites, it is part of the podocephalic system. Since our results are still preliminary, we can only state that the close association with the digestive system (caeca) seems to be remarkable. It may help removing water (or ions?) from this system as was suggested for the fluid-feeding spider mites (Alberti & Storch, 1974, 1977; Alberti & Coons, 1999). Of interest concerning this structure is also the presence of a bladder. This was only found in a small number of freshwater mites, e.g., *Arrenurus bicuspidator*, but lacking in *Hydrodroma despiciens* (Alberti & Storch, 1977; Alberti & Coons, 1999).

The eyes may be of interest in possessing a spherical lens sunk into the depth, which occurs also in some other Hydrachnidia and was considered as a derived structure (type II eyes occurring in, e.g., *Piona*, *Arrenurus*, *Atractides*, and others; Schwoerbel, 1967). More observations are needed regarding the arrangement of the rhabdomeres which might also be of significance (e.g., Schwoerbel, 1967; Mischke, 1981).

The male genital system is organized in a way which resembles other Actinotrichida with regards to testis histology (i.e., germinal and glandular parts). The spermatozoa look similar to those of other Hydrachnidia in particular to those of certain Hygrobatoidea (Alberti, 1980; Alberti et al., 2008). However, some differences occur during spermatogenesis and these need scrutiny. The peculiar ejaculatory complex looks similar to that of other Hydrachnidia (Barr, 1972). Hence we expect that these mites use these structures to produce a spermatophore.

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Two novel adaptations for dispersal in the mite family Histiostomatidae (Astigmata)

Norman J. Fashing
Department of Biology, College of William and Mary, Williamsburg, VA 23185-8795, USA. E-mail: njfash@wm.edu

Two unique morphological adaptations for phoretic attachment to arthropod hosts by deutonymphs of the family Histiostomatidae are described. The deutonymphal attachment plate of *Creutzeria* from Madagascar and Seychelles has well developed suckers for attachment to smooth cuticle, as well as modifications that allow for 'grasping'. The conoidal setae are located on the posterior margin with apices modified into trifurcate, claw-like projections. Although the modified conoids are retracted and separated when the sucker plate is relaxed and flat, deutonymphs can fold the sucker plate medially and simultaneously extend the modified conoids. This action causes the medial conoids to merge and interlock their trifurcated tips above a furrow formed from the plate fold. In all probability, these deutonymphs are both entomophilous and pilicolous. The deutonymphal attachment plate of *Ceylanoetus* is enlarged, covering a significant portion of the paraproctal region. Although it has typical conoidal setae, the anterior suckers are greatly reduced and the medial suckers vestigial. The idiosoma has a truncated rear margin bearing flap-like lateral extensions that curve ventrally and surround the attachment organ. When dispersing, a deutonymph wraps the flap-like lateral extensions of its idiosoma around the anterior margin of the beetle host's tibia, thereby completely surrounding it. Although the ridged conoids probably help the deutonymph to hold its position, the ancestral attachment method by means of suckers has been lost in this genus. The term 'crurophilous' is proposed for this unique form of deutonymphal attachment.

Key words: *Creutzeria, Coelanoetus*, Histiostomatidae, deutonymph, hypopus, dispersal

Dispersal by species in the astigmatic mite family Histiostomatidae is typically accomplished through a heteromorphic deutonymphal instar (= hypopus). As in most free-living astigmatic species, histiostomatid deutonymphs are heavily sclerotized and resistant to desiccation, have a greatly reduced gnathosoma without a mouth or mouthparts, and most bear a ventral organ in the paraproctal region utilized for attachment to other organisms. To date, three types of deutonymphs have been recorded for this family: entomophilous, pilicolous, and inert (Evans, 1992).

Most species belong to the entomophilous group, and have a so called 'sucker plate' with setae modified into two pairs of suckers and two pairs of conoids. When dispersing, the suckers are used for attachment to the smooth surface of the arthropod host's cuticle. The pilicolous group is represented by only one genus. Instead of a typical sucker plate, *Fibulanoetus* species have a so called 'clasping organ' that has lateral flaps for clasping and thereby attaching to hairs on their scarabeid beetle hosts (Fain et al., 1980). One histiostomatid species, *Tensiostoma veliaphilum* Wurst et Kovac, has an inert deutonymph (Wurst & Kovac, 2003). Inert deutonymphs have vestigial attachment organs and use passive dispersal. The species is unique among the Histiostomatidae in that it also produces entomophilic deutonymphs that disperse by attaching to water striders (Wurst & Kovac, 2003).

The present paper adds two mechanisms to the diversity of ways by which deutonymphs of species in the family Histiostomatidae attach to their hosts when dispersing.

MATERIALS AND METHODS

Species of *Creutzeria* used in this study came from the following localities and *Nepenthes* (= 'monkey cup', a genus of tropical pitcher plants) species: Cape York Peninsula, Australia [*N. mirabilis* (Lour.) Druce]; Brunei (*N. albomarginata* T. Lobb ex Lindl., *N. ampullaria* Jack*, N. bicalcarata* Hook. f.*, N. gracilis* Korth.*, N. mirabilis*); Dauphin, Madagascar (*N. madagascariensis* Poiret); Mahe, Seychelles (*N. perviellei* Bl.); Singapore (*N. gracilis*); and southern Thailand (*N. gracilis*). Specimens of *Ceylanoetus* near *excavatus* were collected from museum specimens of their phoretic beetle host (*Gondraena* spp.; Hydraenidae) that in turn were collected from southern India, and specimens of *Histiostoma protuberans* Hughes and Jackson were collected from sap flux on an oak tree (*Quercus* sp.) near Williamsburg, VA, USA.

Specimens were cleared in Nesbitt's solution, mounted on microscope slides in Hoyer's medium, and examined under phase contrast and interference microscopy. Deutonymphs of *H. protuberans* (Williamsburg), *Creutzeria* n. sp. from *N. mirabilis* (Cape York Peninsula), *Creutzeria* n. sp. from *N. madagascariensis* (Dauphin), *Creutzeria* near *seychellensis* from *N. perviellei* (Mahe), and *Ceylanoetus* near *excavatus* from India were prepared for scanning electron microscopy (SEM) by dehydrating in ethyl alcohol, drying in a Samdri-PVT-3B critical point dryer (Tousimis), affixing to stubs with double-sided sticky tape, and coating with gold palladium in a Hummer Sputter System (Anatech). SEM microscopy was performed on an AMR-1810. Setal nomenclature follows Griffin et al. (1990).

RESULTS AND DISCUSSION

Deutonymphs of histiostomatid species typically disperse by means of phoresy, utilizing an arthropod that has a similar habitat preference. Like other entomophilous astigmatic species, the attachment organs are located on a rigid, plate-like structure often referred to as a 'sucker plate'. The well developed sucker plate of *H. protuberans* (Figs. 1, 2) is typical of most species in the family Histiostomatidae. Two pairs of suckers more or less surround the anus, and under phase

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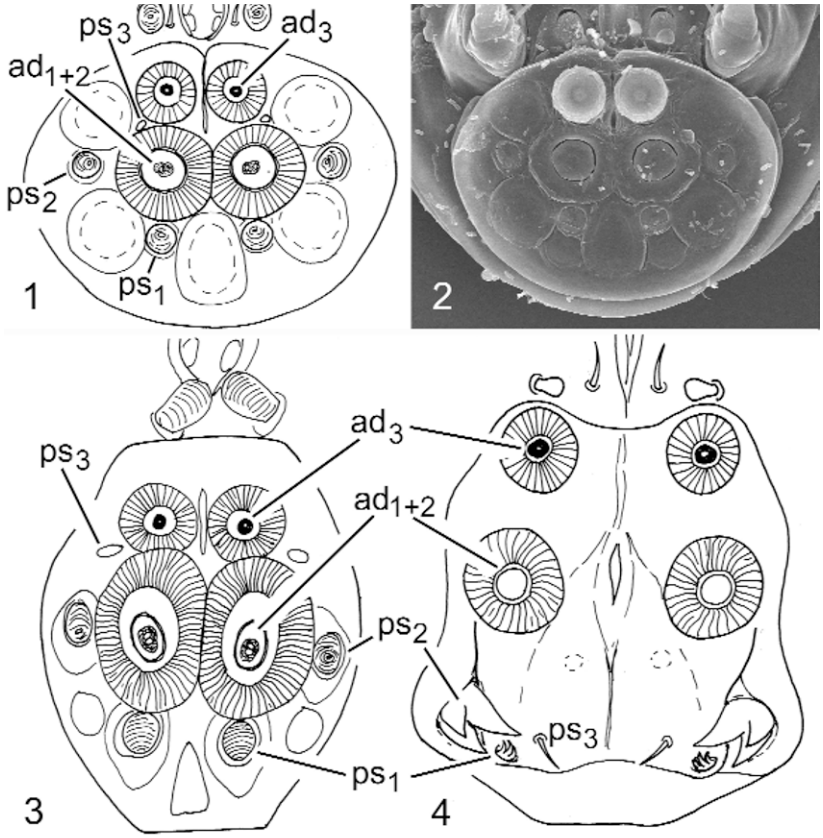
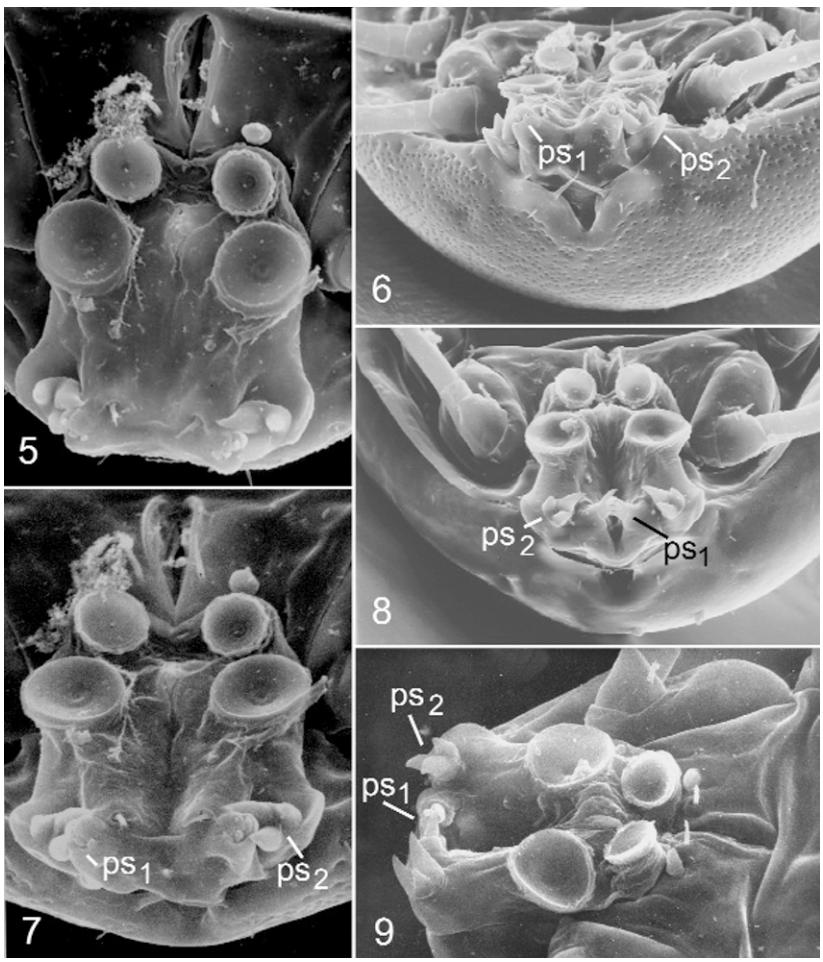


Figures 1-4 Attachment plates of histiostomatid species. (1) Drawing, *Histiosoma protuberans*; (2) SEM photograph, *H. protuberans*; (3) Drawing, *Creutzeria* n. sp. from Australia; (4) Drawing, *Creutzeria* n. sp. from Madagascar. Setal codes are explained in the text.

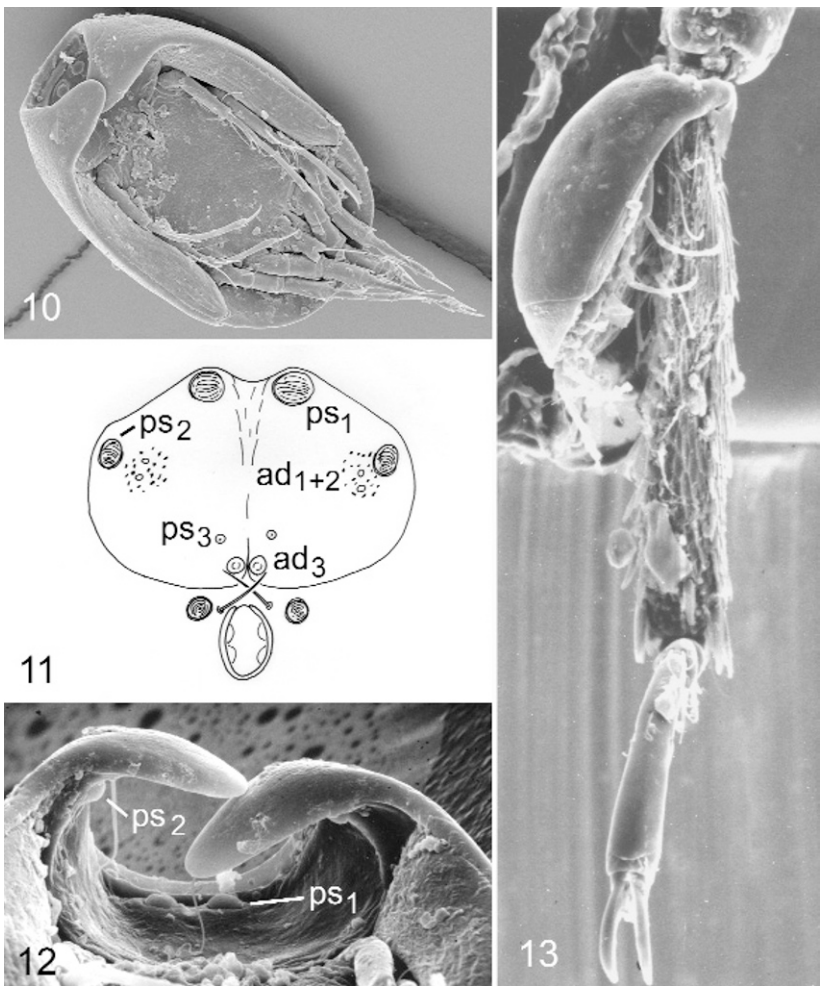


Figures 5-9 SEM photographs of attachment plates. (5) Ventral view of relaxed plate, *Creutzeria* n. sp., Madagascar; (6) Posterior view of relaxed plate, *Creutzeria* n. sp., Madagascar; (7) Tangential view of relaxed plate, *Creutzeria* n. sp., Madagascar; (8) Posterior view of contracted (folded) sucker plate, *Creutzeria* near *seychellensis*; (9) Lateral view contracted (folded) sucker plate, *Creutzeria* near *seychellensis*. Setal codes are explained in the text.

contrast microcopy reveal spoke-like supports radiating from a central ring. The anterior suckers are thought to be derived from setae ad_3, and the median suckers from combined setae ad_1 and ad_2. The attachment plate also has two pairs of conoidal setae, ps_2 located lateral to the median suckers, and ps_1 situated medially and posterior to the median suckers. Setae ps_3, discernible under phase and interference microscopy, is represented only by small alveoli lateral to the junction of the anterior and median suckers. Five oval, ring-like structures can often be seen, and represent apodemes that function for plate rigidity. Retractor muscles inserting on the center of each sucker create a vacuum when contracted, thus providing the suction necessary for deutonymphs to attach to the smooth cuticle of an arthropod host. In their detailed study of the morphology of deutonymphs of *Caloglyphus boharti* Cross, Woodring & Carter (1974) could not determine the function of the conoidal setae. The conoidal setae have a series of concentric ridges that probably grip the host's cuticle, thereby holding the deutonymph in place and preventing shifting.

Species in the genus *Creutzeria* inhabit the fluid-filled pitchers of the insectivorous plant genus *Nepenthes*. Deutonymphs of species from Southeast Asia and northern Australia possess attachment organs that are typical sucker plates, bearing two pairs of well developed suckers and two pairs of ridged conoidal setae (Fig. 3). Like the sucker plates of typical histiostomatids, those on species from the Seychelles and Madagascar contain two pairs of suckers, however they strongly differ in other respects making setal homologies difficult to interpret (Figs. 4-9). The small alveoli representing setae ps_3 are no longer visible on the anterior portion of the plate, but a pair of stout setae, not present on the typical histiostomatid sucker plate and interpreted to be setae ps_3, are found on the posterior margin. Setae ps_1 and ps_2 are no longer conoids, and setae ps_2 are no longer located lateral to the medial suckers. Located on the posterior margin of the plate are two pairs of retractable structures which I interpret as modified conoidal setae ps_1 and ps_2. Both have apices modified into trifurcate, claw-like projections, however setae ps_2 are much larger than ps_1 (Figs. 4-9). Setae ps_2 appear to be only partially retractable, whereas setae ps_1 are almost fully retractable (Figs. 5-7). The attachment plate is elongate and thick and overhangs the posterior margin of the idiosoma. It can be folded medially (Figs. 8, 9), and the rear margin of the idiosoma is indented to facilitate the folded plate (Figs. 6, 8). When the attachment plate is relaxed and flat, trifurcate setae ps_1 and ps_2 are retracted and separated (Figs. 5-7). However, when the sucker plate is folded, the medial trifurcate setae ps_1 merge and interlock their tips above a trough formed in the plate fold (Figs. 8, 9).

Although Beaver (1985) included *Creutzeria* sp. in his food web of the *N. madagascariensis* arthropod community, he did not investigate the biology of the pitcher inhabitants. Ratsirarson & Silander (1996), in a more complete study, found that *Creutzeria* deutonymphs disperse by clinging to



Figures 10-13 *Ceylanoetus* near *excavatus*. (10) Ventral view of idiosoma; (11) Drawing of attachment plate; (12) Attachment plate below wing-like flaps of idiosoma; (13) Deutonymph attached to tibia of the aquatic beetle *Gondraena* sp. (Hydraenidae). Setal codes are explained in the text.

the thorax of adult frit flies (Diptera: Chloropidae). They based this conclusion on only two actual observations of flies harboring phoretic deutonymphs and a positive association between the number of mites and the number of chloropids. Unfortunately the method of attachment used by the deutonymph was not reported and therefore remains unknown. Based on attachment plate morphology, it is possible that deutonymphs have two different methods for attachment to the host insect. While the well developed suckers would allow for attachment to the smooth cuticle of the host thorax, the retractable trifurcate setae ps_1 and ps_2 appear to be modified for grasping and it is possible they are used to grasp or surround a small hair on the host.

The attachment plate and idiosomal shape of *Ceylanoetus* deutonymphs are unique, in fact so unique that they prompted Mahunka (1973) to not only establish a new genus but also a new subfamily for the single, poorly preserved specimen he studied. The ovoid idiosoma tapers posteriorly and has a truncate rear margin (Fig. 10). The venter is concave anteriorly and becomes more so posteriorly. Flap-like lateral extensions on the rear margin of the idosoma curve ventrally and contact in the center (Figs. 10, 12). The attachment organ deviates considerably from the typical entomophilous type of most histiostomatids. It is large, relatively featureless, and extends laterally along the concave walls of the rear idiosomal margin (Figs. 11-12). The anterior suckers (ad_3) are greatly reduced and the medial suckers vestigial, their presence consisting of laterally displaced pigmented areas containing the paired vestigial alveoli of setae ad_1 and ad_2 (Fig. 11). Setae ps_2 are widely separated conoids located on the lateral margins of the enlarged attachment plate, and setae ps_1 are conoids located medially on the posterior margin (Figs. 11, 12). The oval, ring-like structures are not visible, indicating that the apodemes that provide the rigidity necessary for sucker function in entomophilous deutonymphs are not present. The loss of the apodemes is associated with the loss of suckers on the attachment plate.

The genus *Ceylanoetus* is known only from deutonymphs. Specimens of the species used in this study (*C.* near *excavatus*) were collected from museum specimens of their dispersal agent, small aquatic beetles in the genus *Gondraena* (Hydraenidae) that inhabit the pools in splash zones near waterfalls. When dispersing, a deutonymph wraps the flap-like lateral extensions of its idiosoma around the anterior margin of the beetle host's tibia, thereby completely surrounding it (Fig. 13). Rather than using suckers, the deutonymph is secured to the host by the idiosomal extensions. The two pairs of ridged conoidal setae probably help hold the deutonymph in position on the host leg. Much of the ancestral function of the entomophilous attachment organ appears to have been lost in this genus.

Deutonymphs of *Creutzeria* species from Madagascar and Seychelles are unique in that they have attachment plates with two pairs of well developed suckers for attachment to smooth cuticle, as well as conoidal setae modified for grasping or surrounding a hair. In all probability these species are both entomophilous and pilicolous. Although *Fibulanoetus* species also have attachment plates with suckers and structures for grasping, the anterior suckers are quite reduced and the median suckers vestigial (Fain et al., 1980). The reduced size/absence of suckers indicates that deutonymphs are most likely not entomophilous, and they have only been observed attached to hairs. Deutonymphs of *Ceylanoetus* have an attachment organ that places them in an entirely new functional category. Although modified for grasping, deutonymphs do not clasp a hair but rather surround an entire leg segment of the host insect. In addition, they use wing-like extensions of the idiosoma rather than modifications of the attachment plate for this purpose. I propose a new category, crurophilous (= lover of legs), to accommodate deutonymphs that attach to their host in this manner.

Acknowledgements

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'Private matters' of *Sancassania berlesei* (Acaridida, Acaridae): testes, receptaculum seminis, ovary and the location of sperm

Kerstin Klien & Manfred G. Walzl
Department of Theoretical Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria.
E-mail: kerstin.klien@gmx.at

The anatomy of the receptaculum seminis and ovary of female *Sancassania berlesei* (Michael) has been studied by light and scanning electron microscopy. Special attention has been given to the illustration and localisation of sperm in these organs after copulation. Therefore the genital tracts of individually reared females have been dissected after their first copulation. For the illustration of aflagellate spermatozoa in receptaculum seminis, ovary and testes, differential interference contrast microscopy and DAPI fluorescence staining were applied.

Key words: Reproductive system, receptaculum, ovary, testes, sperm, DAPI-fluorescence

"The Acari, noted mainly for their small size, occasionally as a disease vector or simply as pests, have become recognized as an important group for the study of the reproductive mechanisms"

Heinemann & Hughes (1970)

The anatomy of the reproductive system of astigmatic mites has been described at the beginning of the last century by Michael (1901). Since then, several light microscopical studies have been undertaken. One of the species studied relatively well is the stored product mite, *Sancassania berlesei* (Michael). The anatomy of its female reproductive system is generally similar to that described for other astigmatic mites (Nalepa, 1884, 1885; Michael, 1901; Hughes & Hughes, 1938; Hughes, 1959; Rohde & Oemick, 1967; Heinemann & Hughes, 1970; Kuo & Nesbitt, 1970; Witalinski et al., 1990; Walzl, 1992; Witalinski & Walzl, 1995; Alberti & Coons, 1999; Lekimme et al., 2005).

The aim of this article is to supplement and extend previous studies on *S. berlesei* (e.g., Prasse, 1967, 1968; Witalinski & Walzl, 1995) by employing light and scanning electron microscopical (SEM) techniques. Special attention is given to the anatomy of the receptaculum seminis, the ovaries, and the presence of sperm in these organs using Normarski differential interference contrast (DIC) and DAPI (diamidine-2-phenylindole)-fluorescence microscopy.

MATERIAL AND METHODS

Dimorphic males, females, and tritonymphs of *S. berlesei* obtained from a laboratory culture were investigated. Stock cultures were kept in a cupboard warmed at room temperature and maintained in closed plastic vessels on moistened filter paper with bits of fresh yeast as food. To obtain virgin adults of a specified age, quiescent tritonymphs were separated into small chambers (Oberrauch, 1994). Two days after emergence, a male and a female individual were placed together and dissected at various times after copulation (45 min and at 1-h intervals from 1-10 h). Moreover, testes of active male tritonymphs were dissected to elucidate their anatomy.

Receptacula, ovaries, and testes were dissected with two fine sharpened Tungsten needles in spider saline (12.45 g NaCl/l, 0.51 g KCl/l, 0.89 g $CaCl_2$/l, 1.04 g $MgCl_2*6H_2O$/l, 2.383 g HEPES/l, 3 g glucose/l) and fixated for DIC and DAPI-studies in 4% aqueous formaldehyde solution for 24 h. After staining with 0.001% aqueous DAPI-solution for 45 min the organs were cleansed with spider saline 3×, mounted in glycerine, and digitally photographed with a Nikon-Eclipse E800.

Dissected organs intended for SEM and semi-thin sections, were fixated in 0.1 M caccodylate-buffered Karnovsky-medium overnight and post-fixated in 2% aqueous OsO_4 for 4 h. Both primary fixation and post-fixation were carried out at room temperature. Afterwards the specimens were dehydrated with dimethoxypropane (Johnson et al., 1976). For SEM the organs were dried with hexamethyldisilazane (Nation, 1983). The dry samples were mounted on aluminium dishes with Tempfix thermoplastic adhesive (Neubauer Chemikalien-Grosshandel, Münster, Germany) and gold-coated before investigation in a Philips XL 20 SEM.

For sectioning we used ERL as the embedding medium due to its low viscosity. For better resin penetration, embedding was carried out in a vacuum oven. The 1-µm-thick sections were stained following Richardson's method (Walzl et al., 2004). Contrast enhancement of all pictures was carried out using Adobe Photoshop 7.0.

RESULTS

The female reproductive system of *S. berlesei* consists of two functional parts. One functions in mating, sperm storage, and sperm transport. It consists of the bursa copulatrix, the inseminatory canal, the receptaculum seminis, and the ducti conjunctivi. The other part functions in fertilisation and egg formation, vitellogenesis, and transport of the eggs. It comprises the ovaries, the oviducts, the ovipositor, and the oviporus.

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The sperm is introduced by the male copulatory organ (aedeagus) through an insemination pore (bursa copulatrix) reaching the seminal receptacle by a cuticle-lined inseminatory canal after retro-conjugate copulation.

Receptaculum seminis

The receptaculum seminis in *S. berlesei* is a thin-walled, saccular organ, approximately 170 µm long, and it is dorsally situated above the hindgut. It consists of a sclerotized basal part with a median afferent inseminatory canal and paired lateral, efferent and funnel shaped, ducti conjunctivi that lead into the ovaries, as well as to the sac in which sperm is stored (Fig. 1). Three membranes divide the receptaculum in three separate chambers. Only the outermost of these membranes, the receptaculum border, is cellular; the other two are acellular sheaths. The cellular membrane consists of a single-layer epithelium with large oval nuclei, approximately 20 µm long (Fig. 3b).

Spermatozoa and the secretion of the single accessory gland of the male were found only in the chamber bordered by the innermost acellular membrane (Figs. 2, 3a,b). We also found that the receptaculum is expandable and increases its volume after each copulation. Following copulation a spermatozoa package can be found at the entrance to the ducti conjunctivi (Fig. 2) which open into the ovaries. Each of the paired ducti conjunctivi consists of a sclerotized part, the funnel, which is surrounded by large somatic cells. Each ductus conjunctivus measures approximately 40 µm in length, the diameter at the receptaculum is 1 µm and increases to 20 µm at the transition to the ovary.

Sperm

The spermatozoa of *S. berlesei* are approximately 7 µm long. They are characterised by an individual form and absence of a flagellum. The area of the chromatin threads of the mature aflagellate spermatozoa is approximately 2 µm long. This area is not delimited by a nuclear membrane. The chromatin material of the spermatozoa is located centrally or subcentrally in the cell (Fig. 2). With DAPI-fluorescence it is possible to stain not only the large nuclei of the outermost membrane of the receptaculum, but also the chromatin area of the spermatozoa, thus making them readily identifiable (Fig. 3b).

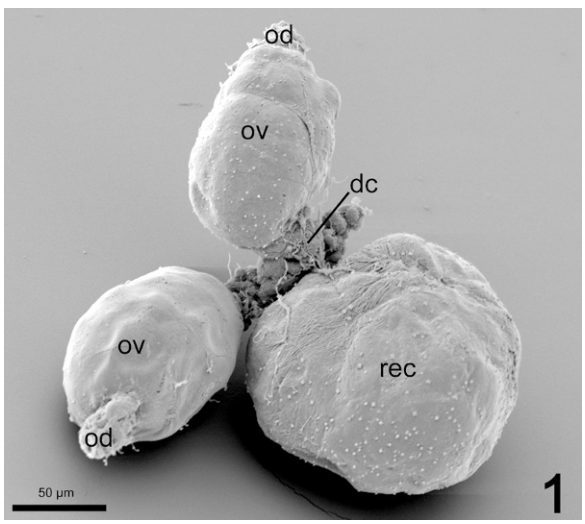


Figure 1 Part of a dissected genital tract of a *Sancassania berlesei* female. Receptaculum seminis (rec), ovaries (ov), ducti conjunctivi (dc), part of oviducts (od). SEM photo.

Ovary

The paired ovaries (ca. 150 µm in diameter), situated at the hind part of the opisthosoma, can be divided into two anatomically different regions: (1) the cortical area, which contains oogonia and growing oocytes, surrounding (2) the central cell – a syncytial tissue, with a nutritive function for the germ cells (Fig. 4). The cortical area is bordered by a flat

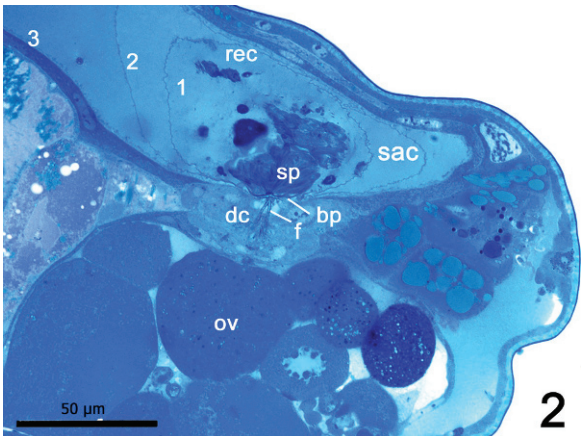
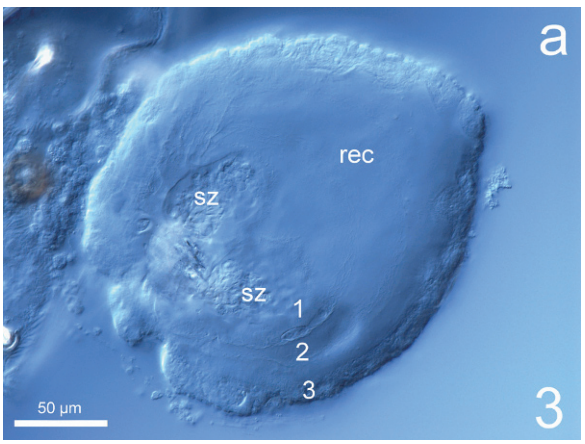


Figure 2 Opisthosoma of an inseminated female of *Sancassania berlesei*, 2.5 h after copulation. The receptaculum (rec) consists of a basal part (bp) and a sac which is composed of three membranes (1-3). Sperm package (sp) at entrance of ducti conjunctivi (dc), funnel (f), ovary (ov). Photo based on semi-thin section.



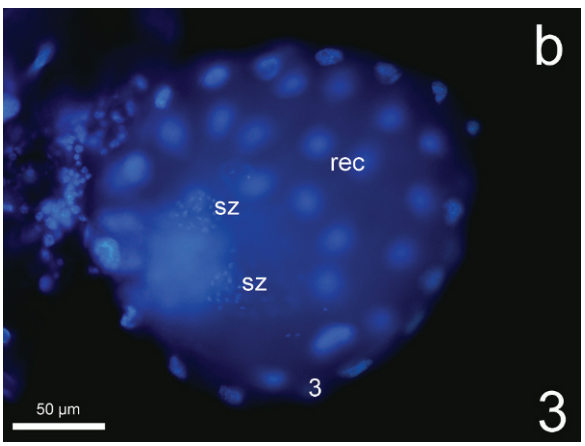


Figure 3 Receptaculum seminis (rec) of an inseminated female of *Sancassania berlesei* using (a) DIC and (b) DAPI methodologies. Spermatozoa (sz) in the innermost chamber. Only the outermost of the three membranes (1-3) is cellular (3).

epithelium of somatic cells. Oocytes of various sizes remain connected with the central cell by plasma bridges (Fig. 4) until they reach the oviduct.

When using DIC-technology only, it is nearly impossible to identify spermatozoa in the ovary (Fig. 5a,b), but with DAPI-fluorescence staining it is possible to identify the area of the chromatin threads of the spermatozoa. Within the ovary, the chromatin material is approximately 2 µm long and can be found next to the oocytes at the entrance of the oviduct (Fig. 5b,c).

Testes

The paired testes (ca. 120 µm in diameter) are characterized by the absence of a nutritive central cell. They cannot be differentiated in a glandular and germinal region, but stages of spermatogenesis are apparent in various regions: the spermatogonia are situated at the periphery of the testis, whereas the spermatocytes are located more centrally and the mature aflagellate spermatozoa are in the region of the vasa deferentia (Fig. 6).

Dissected gonads of male tritonymphs are much smaller (ca. 80 µm in diameter) than testes of adult males. They are filled with spermatogonia and spermatocytes, but contain no spermatozoa (Fig. 7).

The chromatin material of the spermatozoa can be discriminated from the chromatin material of the spermatogonia and spermatocytes by using DAPI-technology (Fig. 8b).

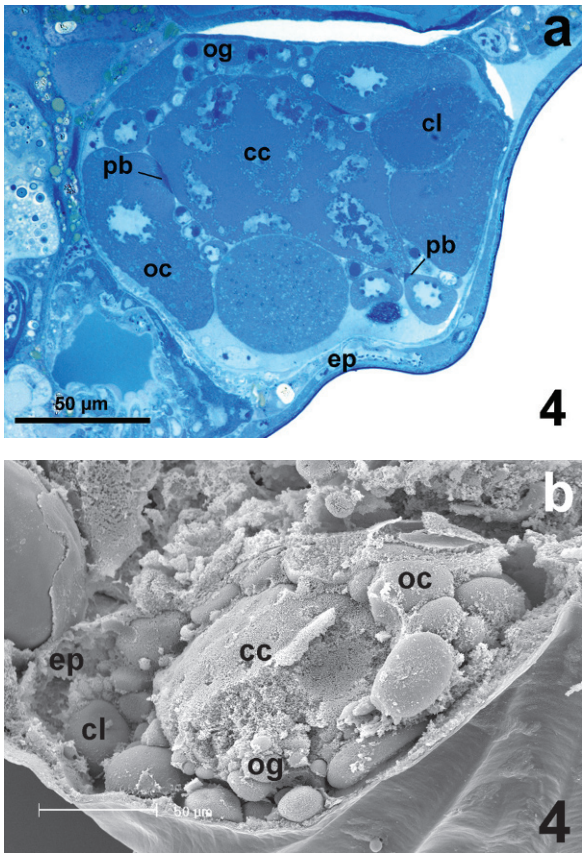


Figure 4 *Sancassania berlesei* ovary composed of two regions: the central cell (cc), to which the oocytes are attached by plasma bridges (pb), and the surrounding cortical layer (cl) consisting of oocytes (oc) ,oogonia (og), and epithelia (ep). Photos based on semi-thin section (a) and SEM (b).

DISCUSSION

One characteristic of the genital tract in astigmatic mites is the presence of a single receptaculum seminis and paired ovaries. The receptaculum seminis in *S. berlesei*, as in other astigmatic mites, consists of a cuticular basal part and a sac for sperm storage. The ovaries are composed of a central cell as observed, e.g., in *A. siro* (Witalinski et al., 1990) or in *Dermatophagoides* spp. (Walzl, 1992), and a layer of growing oocytes surrounding the central tissue.

Spermatozoa (7 µm) must pass the sclerotized part of the ducti conjunctivi (1 µm) to enter the ovaries, but the mechanism by which male gametes reach the oocytes is still

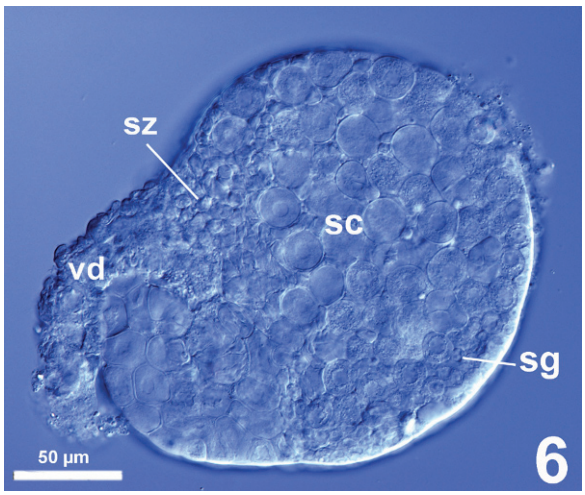


Figure 6 Testis of an adult male *Sancassania berlesei*, showing various stages of spermatogenesis using DIC methodology. Spermatogonia (sg), spermatocytes (sc), and spermatozoa (sz), vasa deferentia (vd).

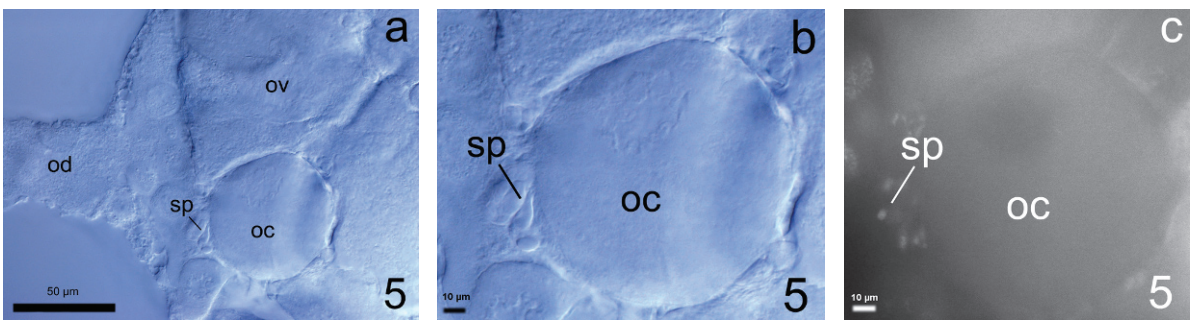


Figure 5 Ovary (ov) and part of oviduct (od) of *Sancassania berlesei* (a) using DIC methodology; (b) detail of oocyte (DIC), and (c) same image using DAPI-technology. Oocyte (oc) with sperm (sp) attached.

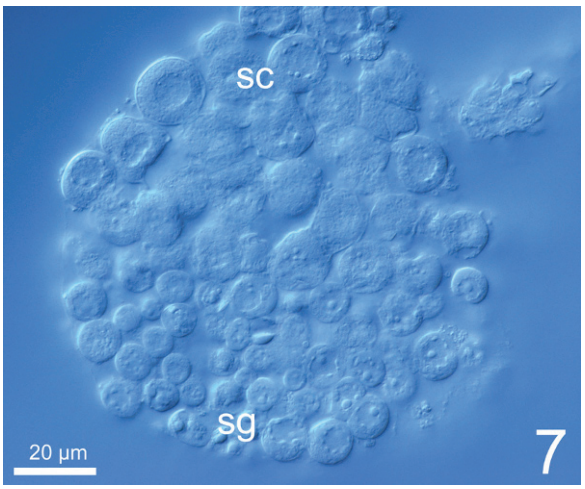


Figure 7 Testis of a male tritonymph of *Sancassania berlesei* with spermatogonia (sg) and spermatocytes (sc) but no spermatozoa, imaged using DIC methodology.

unclear and requires further investigation. We conclude that fertilization occurs in the ovary near the entrance to the oviduct (Fig. 5) and it takes place before the passage of the oocyte through the oviduct and before vitellogenesis, as Prasse described in 1968. A further indication that fertilization takes place in the ovary and not in the oviduct, is that sperm was never found in the oviduct. The development of the oocyte depends on sperm penetration, if unfertilized the oocyte nucleus degenerates (not illustrated).

The differentiation of the testes into different regions, as described by Prasse (1968), could not be confirmed. In agreement with Witalinski & Walzl (1995), a central region with a nutritive function is absent. The male gonads show various stages of spermatogenesis in different regions.

Aflagellate spermatozoa in *S. berlesei* have also been reported in other astigmatic mite families (Reger, 1971; Alberti, 1980, 1984; Witalinski et al., 1990; Walzl, 1992; Alberti & Coons, 1999). According to Alberti (1980, 1984), they are characterised by a polygonal form, the absence of a flagellum and lack of an acrosome. Since the spermatozoa are arranged in packages, it is nearly impossible to separate single spermatozoa using DIC-microscopy, but staining with DAPI enables distinction of chromatin in individual sperm cells (Figs. 5, 8). A typical sperm nucleus is absent, and the chromatin in the sperm is organized into individual threads and is not delimited by a nuclear membrane (Alberti, 1980; Lekimme et al., 2005). Using DAPI-staining no differences were found in the mature spermatozoa whether they were located in the testes, in the receptaculum, or in the ovary (Figs. 3a, 5c, 8b). This study demonstrates that the chromatin material of the mature spermatozoa is 2 µm long and retains the same appearance in all dissected organs.

The genital tract of tritonymphs has yet to be described accurately, and studies of the reproductive system in nymphs are rare (Heinemann & Hughes, 1970), presumably due to the small size of this developmental stage and the difficulties to dissect them. The dissection of male tritonymphs in this study shows that the testes comprise only spermatogonia and spermatocytes. This confirms the assumption that spermatozoa will mature in the quiescent tritonymphs, as well as after moulting into the adult phase. This was why we used only 2-day-old adult mites in the copulation tests.

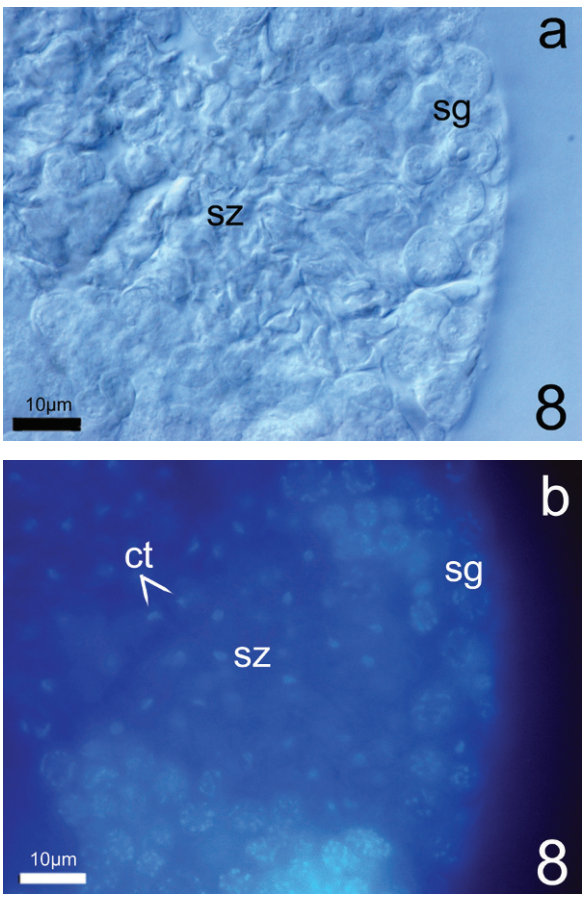


Figure 8 Testis of *Sancassania berlesei*, imaged (a) using DIC methodology and (b) DAPI-fluorescence. Aflagellate spermatozoa (sz), chromatin threads (ct), spermatogonia (sg).

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Biogeography and Biodiversity of Acari

Heterozerconidae: A comparison between a temperate and a tropical species

Beverly S. Gerdeman & Rufino Garcia
Washington State University, Mount Vernon Northwestern Washington Research & Extension Center, WA 98665-9752 USA.
E-mail: mitehunter1@hotmail.com, prostigman7@hotmail.com

A comparative field study was performed between a tropical species and a temperate species of Heterozerconidae (Acari: Mesostigmata). The temperate species, *Narceoheterozercon ohioensis* Gerdeman and Klompen, is a long-lived mite, which shares an intricate relationship with its host millipede, *Narceus annularis* (Rafinesque) (Diplopoda: Spirobolida: Spirobolidae). The phenology between these mites and their millipede host shows coinciding periods of oviposition and mating, with both producing a single generation/year. Although the adults appear to feed on the exudates of the millipedes and are rarely separated, the immatures, developing during the summer, have adapted to a free-living, predatory lifestyle within the millipede 'nest-sites' of fallen logs. In contrast, the relationship between a Philippine heterozerconid, *Allozercon* sp., and its host millipede (Diplopoda: Spirobolida: Rhinocricidae) is less constrained. In these tropical species, both the millipedes and their associated heterozerconids produce multiple generations/year. Millipede mating is no longer a synchronous event and immatures are found throughout the year in localized accumulations of millipede frass, beneath the forest litter.

Key words: Heterozerconidae, Mesostigmata, *Narceoheterozercon ohioensis*, *Allozercon*, Diplopoda, Spirobolida, *Narceus annularis*, Rhinocricidae

Decaying wood provides an array of habitats with moderate temperatures and high humidity. Some of these are natural cavities, others are tunnels cut by insects that leave wood particles and feces behind. A diverse biota of fungi, insects, mites, and other arthropods occupy these cavities. Many social and eusocial orders of insects are common in decaying wood. The complex communities in these relatively stable habitats are sites for the evolution of symbioses. Decaying wood is considered to be the original habitat of Astigmata, mites that possess the largest number of symbiotic associations in the Acari (OConnor, 1982). Symbiotic associations are also common among the Mesostigmata. Within the Mesostigmata, 46 species in nine families are associated with millipedes. The associations range from simple habitat associations such as Podocinidae, which are attracted to the food resources of millipede frass, to complex parasitic relationships like that of *Narceolaelaps* Kethley (Mesostigmata: Laelapidae), an obligate parasite of *Narceus* (Kethley, 1978). However, despite the numerous associations, few mites have rarely evolved strict symbiotic associations with millipedes. Neotenogyniidae is the only family in Mesostigmata known to have an exclusive millipede association.

Heterozerconidae is a family of rarely collected millipede associates. For the most part, adults possess ventral suckers assisting in adhesion to their host millipedes. The adults are commensal and exhibit an obligate association with millipedes, whereas the immatures are free-living, associated with the millipede 'nests' in decaying logs (Gerdeman et al., 2000) or beneath the litter. Although primarily associated with millipedes, a few have become secondarily associated with reptiles and evolved a parasitic relationship (Finnegan, 1931; Lizaso, 1979; Flechtmann & Johnston, 1990).

Narceoheterozercon ohioensis Gerdeman and Klompen may represent the extent of the northern range for Heterozerconidae. Because Heterozerconidae is primarily a tropical family of mites and *N. ohioensis* may not exhibit representative behavior for the family, a concurrent field study of the Philippine species, *Allozercon* sp., was performed for comparison.

METHODS

Temperate study

The study site is located in Little Rocky Hollow, near Gibisonville, in Hocking County, Ohio, USA (39°28'N, 82°32'W). Little Rocky Hollow lies in the unglaciated region known as the 'Hocking Hills'. These uplands mark the southern limit of the Wisconsin glaciers where relict boreal, hemlock-hardwood communities and deciduous communities still persist (Wolfe et al., 1949). Stream-cutting into the resistant Black hand sandstone and conglomerate formed steep valleys and contributes to a neutral calcium-rich soil. The mineral resources provide millipedes with the high calcium requirements necessary for cuticle formation and oogenesis. The abundance of fallen logs and cool moist climate provides a good habitat for the millipedes (Hopkin & Read, 1992). Gaining status as a state nature preserve in 1981, with access by permit only, the secluded study site has provided the protection required for survival by its rare inhabitants.

The host, *Narceus annularis* (Rafinesque), is a long-lived and iteroparous detritivore (Hopkin & Read, 1992). Generally iteroparous millipede species require rotting logs for feeding and oviposition (Blower, 1969, 1970). Millipedes were recovered from logs ranging from 7.5-58 cm in diameter. Millipede preferences for tree species or size were not detected. Millipedes were found under the bark of rotting logs, walking on the forest floor, and in tree holes. Each millipede was checked for mites. Infested millipedes were

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brought back to the lab. Using a dissecting microscope, numbers of male and female heterozerconids and their positions on the millipede were recorded. Representative mites were removed and slide mounted in Hoyer's medium or placed in 70% ethanol as voucher specimens. A few immature heterozerconids were field-collected but most were reared from laboratory cultures. Immature heterozerconid cultures were maintained on a substrate of milled sphagnum in plastic containers at 21 °C. The mite cultures were maintained for 31 months. Millipedes were identified to species using Keeton's morphometric formula (Keeton, 1960), sexed and placed in plastic rearing cages with milled sphagnum. Representative millipede specimens were placed into 70% ethanol. Both mite and millipede voucher specimens are deposited in the Acarology Laboratory, Ohio State University, Columbus, Ohio (OSAL).

Tropical study

The tropical study site was located on the forestry campus of the University of the Philippines at Los Baños (UPLB) at the base of Mt. Makiling (14°7'N, 121°12'E) in Laguna Province on the main island of Luzon. This study site is not protected from human activity as is the Ohio study site. The rainy season begins in June and continues through November with rains decreasing by December. During December and January, the driest and coolest months of the year, the millipedes are inactive.

The host millipede is an undescribed species of Rhinocricidae. The heterozerconid studied is an undescribed species of *Allozercon*. Fain (1989) considered *Allozercon* unrecognizable, and proposed a new name, *Asioheterozercon* for some Malaysian specimens, but preliminary phylogenetic analyses suggest that the few characters that can be coded based on the description of *Allozercon fecundissimus* Vitzthum place this taxon within a highly speciose lineage that includes Fain's '*Asioheterozercon*'. We therefore will use the generic designation *Allozercon* for this new taxon. To determine the phenology of the tropical heterozerconid, 10 infested millipedes were collected each month from September 2000 to September 2001. The immatures were no longer confined to millipede frass in rotten logs as in North America. Instead, they were recovered from piles of millipede frass found beneath forest litter. Millipede frass samples were collected from the same localities and at the same time each month and mite fauna extracted using Berlese funnels. Shield development, evident in the tropical immatures, may explain their successful recovery from Berlese samples, unlike the Ohio immatures, which were never recovered from Berlese samples.

RESULTS AND DISCUSSION

Field collection data

Ohio field collections were made from 1998-2002, with a total of 992 millipedes collected (Table 1). In the Philippines, 120 adult millipedes were collected from September 2000 to September 2001 and 77 immature heterozerconids (24 larvae, 15 protonymphs, 38 deutonymphs) were collected from Berlese samples of millipede frass during the same period (Table 2).

Phenology of a temperate heterozerconid

Heterozerconids are long-lived, averaging at least 1 year. They are obligate temporary associates sharing a commensal relationship with their host. The life cycle of the mites is timed with that of their host (Figure 1). Temperate millipedes become active in April with sustained high temperatures. At this time, the female heterozerconids outnumber the males. Mature females are almost 50% larger than males (n = 2, 1159, and 1234 µm in diameter). They are deep chestnut brown in color, with a conspicuous dorsal dome, which becomes more prominent with egg development. Up to six eggs in a single female have been observed at one time and eggs are laid individually. This is not consistent across all Heterozerconidae; in contrast, 40 eggs were dissected from an undescribed species from Thailand.

Less than half the millipedes in Little Rocky Hollow were infested (Table 1). Total numbers of mites collected/trip were 0-15, except during the mating season at the end of August and early September. The percentage of infested millipedes drops as oviposition nears, because the adult mites leave their hosts to lay eggs. Both mites and millipedes oviposit at the same time, May through June. Freshly laid heterozerconid eggs appear slightly opaque and gelatinous, with no obvious chorion. With age, they turn uniformly white and appear to firm. Development time from egg deposition to eclosion remains undetermined.

Table 1 Ohio field collection data 1997-2002.

1998-1999 and 2000-2001: 234 millipedes collected
Female millipedes (83) possessed 122 male and 178 female mites
Male millipedes (82) possessed 155 male and 137 female mites
Immature millipedes (69) possessed 21 male and 96 female mites
1997-1999 and 2001-2002: 758 millipedes collected
331 millipedes were infested (44%), 427 uninfested (56%)
109 millipedes were immature (13%), 649 adult (87%)
Of total millipedes collected 42% was male, 45% female
Of adult millipedes collected 47% was male, 53% was female

Table 2 Philippine immature heterozerconid field collections, September 2000-2001.

Collecting date	Larvae	Protonymphs	Deutonymphs
Sep 2000	0	0	2
Oct	1	0	2
Nov	3	0	0
Dec	0	0	2
Jan 2001	2	3	5
Feb	5	0	2
Mar	3	4	6
Apr	1	1	3
May	0	0	2
Jun	0	1	4
Jul	5	3	8
Aug	2	2	1
Sep	2	1	1

Temperate developmental biology

A period of summer inactivity by the millipedes, which may involve molting, coincides with immature heterozerconid

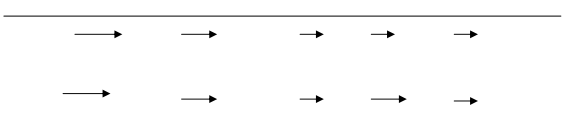

Figure 1 Phenology of *Narceoheterozercon ohioensis* and their host millipede, *Narceus annularis*.

Table 3 Morphological and behavioral differences between immature and adult Heterozerconidae.

Immature	Adult
Unsclerotized	Highly sclerotized
No ventral suckers	Ventral suckers
Hypotrichous, long, curly setae	Hypertrichous, minute setae
Not attracted to millipedes	Highly attracted to millipedes
Never on millipedes	Usually on millipedes
Predatory	May feed on millipede exudates

development in the millipede 'nest sites'. Immatures of Heterozerconidae, including both temperate and tropical species differ dramatically from the adults in behavior and morphology (Table 3).

The Ohio immature heterozerconid developmental times are based on laboratory cultures. The larvae are round in appearance and development for the larval stage requires 2 weeks to over a month. The protonymph resembles the larva in color and shape but has added a fourth pair of legs, more setae, and is 50% larger. Both the larval and protonymphal instars feed actively. The protonymph can last from 18-21 days (n = 2). The deutonymph differs from the previous instars by exhibiting a more elongate shape and burgundy shading of the opisthosoma. The developmental time for the deutonymph requires a minimum of 21 days, which can be extended if conditions are not favorable. Teneral deutonymphs are roughly 50% larger than the protonymph and mature deutonymphs may even be larger than teneral adults. Prior to molting, the deutonymph develops a distinct mid-dorsal depression.

Feeding

The habitat provided by the millipede provides both food and a humid protected site for the immature heterozerconids to develop. The uniform spherical balls of millipede frass create an environment filled with spaces that attract a wide range of predatory mites and other arthropods. Long idiosomal setae may provide the immature heterozerconids with some protection from predators. However the unique interlocking design may actually be more advantageous against crushing and preserving mobility, within the millipede-mediated environment. The interlocking arrangement is formed when dorsal setae *j5* curl posteriorly over the opisthosoma, while setae *Z5* curl anteriorly over the dorsum (Gerdeman & Klompen, 2003). In the tropical species where the immatures develop in the piles of millipede frass rather than in the confines of frass packed behind loose tree bark, the idiosomal setae are not as long as those of the temperate species and the interlocking design was not observed.

Larvae and protonynphs were observed feeding on immature Oribatida and other small mites, as well as immature Collembola (Table 3). Feeding was not observed in deutonymphs. Prey is carried above their bodies with arching pedipalps. Large horn-like corniculi, which only occur in the immatures, assist in grasping their prey. Prey capture was never witnessed. Feeding was not observed in immature Philippine heterozerconids.

Adult heterozerconids appear to feed on exudates produced by the millipedes (Table 3). Adult mites were often observed brushing the millipede's cuticle with their palps. Adult mites have also been observed congregating around wounds on the millipede. In a Philippine species, the mites make broad sweeping movements across the cuticle of the millipede with their gnathosoma, instead of brushing with their palps.

Reproductive biology

Temperate adult heterozerconids appear on the millipedes in late August to early September, coinciding with renewed millipede activity. Newly molted adults are lighter in color and males and females are often difficult to distinguish. With maturity, the females turn chestnut brown, whereas the males exhibit less sclerotization and a lighter coloration. At this time, newly molted heterozerconid adults exhibit their largest yearly densities on the millipedes. Male mites often briefly outnumber the females. The sex ratio of adult mites on male and female millipedes was not significantly different from a 1:1 sex ratio (Table 1). The sex ratio on immature millipedes represented a significant female bias ($\chi^2 = 48.08$, d.f. = 1, $P<0.001$). Immature millipedes would appear to be a poor choice for heterozerconids. Mites on small-diameter, often immature millipedes would frequently fall off their host, especially if it were handled; otherwise mites rarely dropped off the host.

Female mites appeared to exhibit a mid to posterior location preference on the millipede, while males were observed in an anterior site. With onset of the mite-breeding season, this difference became exaggerated, often resulting in a strict division of the sexes on the millipede. Prior to the beginning of the millipede mating season in Ohio, male heterozerconids would briefly outnumber the females on the millipede and as the mating season neared, the mite sex ratio would near 1:1 and strict isolation of the sexes would disappear. Host switching to regulate sex ratio is risky and can result in the inability to locate another host. However, the success rate is improved with increased contact between mating pairs of millipedes.

Autumn observations at the study site indicated mating could be synchronous for millipede residents of a particular log. During the frenzied behavior of millipede mating, mite activity on the millipedes increased, suggesting they were also influenced by the millipedes' chemical cues. Normally contact between the heterozerconid sexes was limited but at this time, the mites ran rapidly across the dorsum of the millipedes with males mounting and dismounting females. Following mating, the adult millipedes seem to disappear abruptly to their overwintering sites. In Ohio, both the logs and the soil beneath logs known to be infested were investigated during the winter but this did not result in the recovery of any millipedes or mites.

The mites do not appear to undergo an overwintering diapause. Laboratory cultures would produce an additional generation in January instead of the single generation/year produced by wild populations. In contrast, Philippine heterozerconids produce multiple, possibly overlapping generations/year (Table 4), with peak numbers of immatures appearing in January, March, and July (Table 2).

Millipede defensive secretions

Millipedes release defensive secretions when they become disturbed. Millipedes in the order Spirobolida, which includes both host millipedes in this comparative study, produce defense secretions composed of benzoquinones (Arab et al., 2003). The Philippine host millipede is capable of shooting these defensive secretions a short distance, whereas the secretions simply ooze from the repugnatorial glands in *N. annularis*. Upon release of the defense fluids, the mites immediately show increased activity and run to areas of safety including the head, collum (segment following the head) and legs. The secretion can also cause the mites to

Table 4 Comparisons of a tropical and temperate heterozerconid/millipede association.

Temperate	Tropical
1 generation/year	2-3 generations/year
Patchy millipede distribution	Random millipede distribution
Millipede aggregation	No millipede aggregation
Cold/dry season	Wet/dry seasons
Millipede frass in isolated pockets of logs	Millipede frass in piles in litter
Millipedes oviposit in logs	Millipedes oviposit in litter
Immature in frass	Immature in frass
Immatures unlike adults in appearance	Immatures unlike adults in appearance
Millipede mating observed only in autumn	Millipede mating observed throughout the year

leave their host. In addition to defense secretions, the millipedes constantly produce exudates from smaller pores on their cuticle. The composition of these liquids is not known. Since the millipedes are capable of selectively releasing defense secretions from repugnatorial glands on specific segments, exudates may also be selectively released through active solicitation by the mites. Brushing the millipede cuticle by *N. ohioensis* may be a form of active solicitation of exudates as food from the millipede.

In order to understand the mites' sensitivity to the defensive secretion, seven adult mites were enclosed in a covered Petri dish with a small quantity of the yellowish defensive fluid. After exposure, they exhibited difficulty in walking and soon died. In a mite-millipede system not only is avoidance of the defense secretions less costly than developing tolerance, it is advantageous. Maintaining a negative response to the defensive secretion encourages the mites to move quickly to safe locations and thus helps prevent accidental separation from their host.

Conclusion

In Ohio, populations of heterozerconids are low compared to those in tropical regions, mainly due to a single generation/year, low mite fecundity, and a limited millipede host range. Numbers of heterozerconids collected in Los Baños, Philippines in 1 year, represented 70% of all heterozerconids collected over a 5-year period in Ohio. The larger populations observed in the Philippines are predicated by weather, resulting in multiple generations/year plus a widespread host range. The distribution of the Philippine millipedes resembles that in North America except they do not aggregate in large groups, perhaps due to the rarity of communal millipede sites, such as fallen logs in the tropical Philippine study site. The strict univoltism of the temperate species forced a synchrony not evident in the Philippine heterozerconids, rendering the temperate species extremely dependent on weather and log resources. These variables may also account for the differences in morphology such as shield development and chaetotaxy of the immatures.

Humidity, the main limiting factor affecting distribution of millipedes (O'Neill, 1968), contributes to the large populations of tropical millipedes. The microclimate of fallen logs in the temperate millipede habitat may substitute for the widespread humidity of the tropics, creating a strict dependency on fallen logs, which rarely persist without protection from human activity, which could lead to extinction.

Data gathered on the biology of the Heterozerconidae revealed an astounding level of dependence by the mite on its host millipede. Some of the behavioral observations presented, are undoubtedly rarely witnessed and therefore chances for duplication may be unlikely.

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Patterns of diversity in the Ceratozetoidea (Acari: Oribatida): a North American assessment

Valerie Behan-Pelletier & Heinrich Schatz[1]

Biodiversity Program, Research Branch, Agriculture and Agri-Food Canada, K.W. Neatby Bldg., Ottawa, ON, Canada K1A 0C6. E-mail: valerie.behan-pelletier@agr.gc.ca; and [1]Institute of Zoology, University of Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria

Represented globally by eight families, 91 genera and about 556 species, the Ceratozetoidea is among the most diverse superfamilies of oribatid mites. Species of Ceratozetoidea occur in forest, grassland, tundra, and semiaquatic habitats. They are found in all biogeographical regions, but are most diverse at mid to high latitudes in the Nearctic, Palaearctic, and Neotropics. The richness of the group in North America allows assessment of patterns of diversity. North America was divided into 12 geographical regions. The ceratozetoid fauna of North America comprises 125 species in 32 genera representing the families Ceratozetidae, Mycobatidae, Chamobatidae, Zetomimidae, Humerobatidae, and Euzetidae. The fauna is dominated by species of *Ceratozetes*, *Trichoribates*, and *Mycobates*, that represent almost 50% of the fauna. No species was found in all 12 geographical regions, and *Pelopsis bifurcatus* and *Punctoribates palustris*, the most widely distributed species in North America, were found in only eight regions; 44% (55 spp.) of the fauna was restricted to one region. The trend of increase in number of species with decreasing latitude was not supported, irrespective of longitudinal zone, and southern latitudes had the lowest number of genera and species. Almost 42% of species are shared with other zoogeographical regions, of which three are cosmopolitan, viz. *Ceratozetes gracilis, C. mediocris*, and *Punctoribates punctum*. Species shared with the Palearctic comprise mainly Amphi-Atlantic, Circumboreal, and Amphi-Beringian faunal elements. Almost 59% of species are restricted to the Nearctic, dominated by species with Carolinean-Austroriparian and East-Beringian distributions. In contrast with the biogeography of other North American oribatid taxa, that of Ceratozetoidea is strongly influenced by Beringia.

Key words: Ceratozetidae, Mycobatidae, Chamobatidae, Zetomimidae, Nearctic, biogeography, distribution, Beringia

Species in the brachypyline superfamily Ceratozetoidea occur in forest, grassland, tundra, arctic, and semiaquatic habitats (Norton & Behan-Pelletier, 2009). In North American forests, species are found both in forest soils and in suspended soils and other canopy microhabitats (Behan-Pelletier, 2000; Lindo & Winchester, 2006). The superfamily is among the few with representation in all biogeographical regions, including Antarctica. The earliest fossil records are from Baltic amber from the Eocene (Labandeira et al., 1997). The superfamily has retained its status since it was established by Jacot (1925), but included families have had a more chequered history. Globally, we recognize about 556 species in 91 genera in the families Ceratozetidae, Chamobatidae, Euzetidae, Humerobatidae, Maudheimiidae, Mycobatidae, Onychobatidae, and Zetomimidae, representing 5.6% of the approximately 10,000 known species of Oribatida (Schatz, 2005; Subias, 2006).

Understanding patterns of distribution underpins systematics and ecology, as the 'current fauna reflects the interplay of phylogenetic history' (Danks et al., 1997). General global patterns of diversity of Oribatida were studied by Maraun et al. (2007), but the only studies of global patterns of diversity for specific oribatid taxa, are those of Niedbała on ptyctimous mites (Niedbała, 1992, 2002). Similarly, there are few publications on the biogeography of oribatid faunas of particular regions of the world, e.g., Galapagos (Schatz, 1998), Central America (Schatz, 2006, 2007), the subantarctic (Block & Stary, 1996; Stary & Block, 1998), Mediterranean region (Bernini, 1984, 1991), British Isles (Luxton, 1996), North America (Reeves, 1998), and Yukon (Behan-Pelletier, 1997).

Although we recognise that many more species await description, the richness of the Ceratozetoidea allows assessment of general patterns of diversity. We use the diversity of this superfamily in North America to address to what extent ecological and historical biogeography have influenced present day distribution of species, and to test hypotheses of species-latitude and species-longitude relationships.

METHODS

North America, north of Mexico, ranges from 26° to 85°N latitude and from 15°W to 173°E longitude and covers ca. 21.5 million km^2. It includes the continental United States, Canada and Greenland. This area was divided into 12 geographical regions arrayed in a grid with three divisions east-to-west by four divisions north to-south following Qian (1998, 2001) (Fig. 1). Regions were based on political divisions, as these are the source of most distribution records, and include: WN (Alaska, Yukon), CN (Northwest Territories,

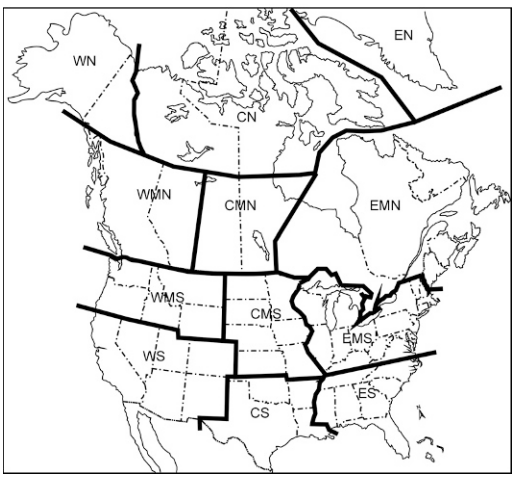

Figure 1 Map of North America north of Mexico showing the 12 regions used in this study.

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Nunavut), EN (Greenland), WMN (British Columbia, Alberta), CMN (Saskatchewan, Manitoba), EMN (Ontario, Quebec, New Brunswick, Nova Scotia, Newfoundland, Maine), WMS (Washington, Oregon, Idaho, Montana, Wyoming), CMS (North Dakota, South Dakota, Nebraska, Kansas, Minnesota, Iowa, Missouri), EMS (Wisconsin, Illinois, Michigan, Indiana, Kentucky, Ohio, West Virginia, Pennsylvania, New York, Vermont, New Hampshire, Massachusetts, Connecticut, Rhode Island, New Jersey, District of Columbia, Delaware, Maryland), WS (California, Nevada, Utah, Arizona, Colorado, New Mexico), CS (Texas, Oklahoma, Arkansas, Louisiana), and ES (Mississippi, Tennessee, Alabama, North Carolina, South Carolina, Georgia, Florida).

The four latitudinal zones (from north to south) are northern (incl. WN, CN, EN), north-middle (incl. WMN, CMN, EMN), south-middle (incl. WMS, CMS, EMS), and southern (incl. WS, CS, ES). The three longitudinal zones (from west to east) are western (incl. WN, WMN, WMS, WS), central (incl. CN, CMN, CMS, CS), and eastern (incl. EMN, EMS, ES).

Taxonomic concepts, especially at the genus level, are still subject to differing opinions in the Ceratozetoidea. In our analysis, we retain as genera many taxa given subgeneric status in Subias (2004, 2006), e.g., *Naiazetes*. The family Euzetidae is retained following Grandjean (1954), *Euzetes* was placed in Chamobatidae by Woas (2002) and Ceratozetidae by Subias (2004). The family Zetomimidae is retained to include the genera *Heterozetes, Naiazetes*, and *Zetomimus* following Behan-Pelletier & Eamer (2003). An analysis of generic and family concepts, per se, in Ceratozetoidea was considered outside the scope of this study, but we recognise, as noted by Woas (2002), that knowledge of immatures will help in establishing relationships.

Data on distribution of genera and species for this study are based on species databases maintained by HS (see Schatz, 2005). These include data on distribution from catalogues listed in Schatz (2005, Table 1; 2006, 2007), Subias (2004, 2006) and Weigmann (2006). The resulting database contains all known species and genera of Ceratozetoidea for North America as of 2008, with their known distribution patterns in zoogeographical regions and subregions.

Data on distribution of species in North America is primarily based on Marshall et al. (1987), with additional data from Behan-Pelletier & Eamer (2004, 2008), and recent papers on ecology of Oribatida in North America (e.g., Hansen, 2000; Lindo & Winchester, 2006; Cianciolo & Norton, 2006).

Zoogeographical Realms used are those outlined in Marshall et al. (1987). North American regions and faunistic elements follow those of Dice (1943) and Scudder (1979). The analysis of distribution patterns in North America follows that of Qian (1998, 2001) and Scudder (1979). The similarity between regions was calculated using the Sørensen similarity coefficient (or Sørensen Index, SI; Southwood, 1978): SI (%) = {2 × C / (A + B)} × 100, where C = number of species in common, and A, B = number of species in region A and B, respectively.

RESULTS

Nature of the fauna

The ceratozetoid fauna of North America comprises 125 species in 32 genera representing six families (Table 1). The family Ceratozetidae is represented by the genera *Ceratozetes* (23 spp.), *Ceratozetoides* (1 sp.), *Dentizetes* (2 spp.), *Diapterobates* (6 spp.), *Edwardzetes* (1 spp.), *Fuscozetes* (5 spp.), *Ghilarovizetes* (1 sp.), *Iugoribates* (1 sp.), *Jugatala* (1 sp.), *Laminizetes* (1 sp.), *Latilamellobates* (1 sp.), *Melanozetes* (5 spp.), *Neogymnobates* (3 spp.), *Oromurcia* (2 spp.), *Sphaerozetes* (4 spp.), *Svalbardia* (1 sp.), and *Trichoribates* (17 spp.); Chamobatidae is represented by *Chamobates* (5 spp.); Humerobatidae by *Humerobates* (2 spp.); Mycobatidae by *Ceresella* (1 sp.), *Cyrtozetes* (2 spp.), *Guatemalozetes* (1 sp.), *Minunthozetes* (1 sp.), *Mycobates* (19 spp.), *Pelopsis* (1 sp.), *Punctoribates* (3 spp.), and *Zachvatkinibates* (7 spp.); Zetomimidae by *Heterozetes* (2 spp.), *Naiazetes* (1 sp.), and *Zetomimus* (4 spp.); and Euzetidae by *Euzetes* (1 sp.).

The number of families, genera, and species in each of the 12 geographical regions is given in Table 2. Comparison with Figure 1 shows that species richness at a regional scale did not decrease with increasing latitude. Among the 12 geographical regions WN, encompassing Alaska and Yukon, had similar species richness to EMN, encompassing Canada east of Manitoba and Maine, and to EMS, encompassing most of the eastern US-states. The south central region (CS), encompassing Texas, Oklahoma, Arkansas and Louisiana, had the lowest species richness.

In terms of regional occurrence no species was found in all 12 regions, *Pelopsis bifurcates* and *Punctoribates palustris*, the most widely distributed species in North America, were each found in eight regions, and *Ceratozetes thienemanni, Diapterobates notatus, Melanozetes sellnicki, Mycobates incurvatus*, and *Heterozetes aquaticus* were each found in only six regions. Forty-four percent (55 spp.) of the fauna was restricted to one region, and 21% (26 spp.) to two regions (Table 1). Similarity indices of pairwise comparisons of regional species composition ranged from 0 to 63.3% (Table 3). Region WMS, encompassing Oregon, Washington, and three other states, was the least similar to all other regions. The highest similarity index (63.3%) was between WN (Alaska, Yukon), and CN (Northwest Territories, Nunavut), two adjacent regions. Similarly, the second highest similarity index was between the adjacent regions CN and EN, encompassing Greenland (Table 3).

The trend of increase in number of species with decreasing latitude was not supported (Fig. 2a), and there was little latitudinal difference in number of genera and species between the South-middle, and Northern latitudes. There was a difference in number of species between longitudinal zones, with the Central zone showing lowest species richness (Fig. 2b).

Similarity in species composition between latitudinal zones decreased with increasing latitudinal separation of the zones (Table 4). The lowest similarity index (SI = 7.9%) was found between Northern and Southern zones (Table 4).

Biogeographic distribution

The biogeographic distribution of each of the 125 ceratozetoid species found in North America is given in Table 1. Forty-two percent of species are shared with other zoogeographical regions, of which three (2.4%) are cosmopolitan (Table 5). Forty percent of species are shared with the Palaearctic (Tables 1 and 5), comprised mainly of Amphi-Atlantic, Circumboreal, and Amphi-Beringian elements. More than 58% of species are restricted to the Nearctic (Table 5). The Nearctic element is dominated by species with Carolinean-Austroriparian, East-Beringian, and Pacific Coastal distributions (Table 5).

Table 1 Known distribution of Ceratozetoidea in political regions of North America (AB Alberta, AK Alaska, AL Alabama, BC British Columbia, CA California, CT Connecticut, CO Colorado, DC District of Columbia, FL Florida, GA, Georgia, IL Illinois, KS, Kansas, KY Kentucky, LA Louisiana, MA Massachusetts, MB, Manitoba, ME, Maine, MI, Michigan, MN Minnisota, MS Mississippi, NB New Brunswick, NC, North Carolina, NF Newfoundland, NH New Hampshire, NJ New Jersey, NM New Mexico, NS Nova Scotia, NT Northwest Territories, NU Nunuvat, NY New York, OK Oklahoma, ON Ontario, OR Oregon, PE Prince Edward Island, QC Quebec, SK Saskatchewan, TN Tennessee, TX Texas, VA Virginia, VT Vermont, WA Washington, WI Wisconsin, YT Yukon, GL Greenland), their distribution in North American geographic regions (as in Fig. 1), their biogeographical affiliation and distribution in biogeographic regions: PAL Palearctic [E East (Central and East Asia, North China, Japan), W West (Caucasus, Europe, Macaronesia, North Africa); OL Oriental (E East, W West, C China Oriental Region); ETH Ethiopian; NEO Neotropical (C Central America, S South America); AUS Australian/Pacific (H Hawaii, Z New Zealand). Boldface indicates the province or state of type locality.

	Distribution in			Biogeographic regions				
Family, species	North America	Geographic regions	Nearctic	PAL	OL	ETH	NEO	AUS
Ceratozetidae								
Ceratozetes angustus (Banks 1947)	**NC**	ES	Carolinean					
C. borealis Behan-Pelletier 1984	AK, **YT**	WN	East-Beringian					
C. cuspidatus Jacot 1939	**NH**, NY, BC, AB, SK, ON, QC, NB, NS, PE	WMN, CMN, EMN, EMS	Trans-Nearctic					
C. enodis (Ewing 1909)	**IL**, KY	EMS	Carolinean					
C. figuratus (Ewing 1909	**IL**	EMS	Carolinean					
C. fjellbergi Behan-Pelletier 1986	**YT**	WN	Amphi-Beringian	E				
C. gracilis (Michael 1884)	AK, YT, BC, AB, SK, MB, ON, QC, NB, NS, NF, NY, VA	WN, WMN, CMN, EMS	Cosmopolitan	**W**E	WE	x	CS	Z
C. inupiaq Behan-Pelletier 1986	**YT**, NU, NT, GL	WN, CN, EN	East Beringian					
C. kananaskis Mitchell 1976	**AB**	WMN	Cordilleran					
C. kutchin Behan-Pelletier 1986	**YT**	WN	East Beringian					
C. longispina Jacot 1936	**NC**, VA	ES, EMS	Carolinean					
C. mediocris Berlese 1908	AB, SK, MB, ON, QC, NB, NS, VA, GA	WMN, CMN, EMN, EMS, ES	Cosmopolitan	**W**E	WCE	x		Z
C. oresbios Behan-Pelletier 1984	**AB**	WMN	Cordilleran					
C. pacificus Behan-Pelletier 1984	**BC**, SK, NY	WMN, CMN, EMS	Trans-Nearctic					
C. parvulus Sellnick 1922	AK, YT, NT, NU, AB, MB, ON, QC, NF	WN, CN, WMN, CMN, EMN	Circumboreal	**W**E				
C. peritus Grandjean 1951	NF	EMN	Amphi-Atlantic	**W**E				
C. spitsbergensis Thor 1934	AK, YT, NU, NT	WN, CN	Circumpolar	**W**E				
C. subaquila (Ewing 1909)	**IL**, NY	EMS	Carolinean					
C. subinconspicuus (Berlese 1908)	**DC**	EMS	Carolinean					
C. thienemanni Willmann 1943	AK, YT, NT, NU, AB, MB, ON, QC, NB, NS, NF, GL	WN, CN, EN, WMN, CMN, EMN	Circumboreal	**W**E			CS	
C. virginicus (Banks 1906)	ON, IL, GA, LA, **VA**, KS	EMN, CMS, EMS, CS, ES	Carolinean-Austroriparian					
C. watertonensis Behan-Pelletier 1984	**AB**	WMN	Cordilleran					
C. zeteki (Ewing 1917)	**IL**	EMS	Carolinean					
Ceratozetoides cisalpinus Berlese 1908	MN	CMS	Amphi-Atlantic	**W**E				
Dentizetes ledensis Behan-Pelletier 2000	**AB**, NS, WI	WMN, EMN, EMS	Trans-Nearctic					
D. rudentiger Hammer 1952	YT, BC, **AB**	WN, WMN	Cordilleran					
Diapterobates humeralis (Hermann 1804)	AK, YT, NT, NU, ON, NS, NF, GA	WN, CN, EMN, ES	Amphi-Atlantic	**W**E	C			
D. notatus (Thorell 1872)	AK, YT, NT, NU, AB, MB, NS, NF, GL	WN, CN, EN, WMN, CMN, EMN	Circumboreal	**W**E				
D. rotundocuspidatus Shaldybina 1970	AK, YT, NT	WN, CN	Amphi-Beringian	**E**				
D. sitnikova Shaldybina 1970	AK	WN	Amphi-Beringian	**E**				
D. unimaculatus (Banks 1906)	**NH**, IL	EMS	Carolinean					
D. variabilis Hammer 1955	**AK**, YT, NT, NU, QC, NF, GL	WN, CN, EN, EMN	Circumboreal	E	W			
Edwardzetes edwardsii Nicolet 1855	**GL**	EN	Amphi-Atlantic	**W**				
Fuscozetes angustus (Banks 1910)	**TX**	CS	Austroriparian					
F. bidentatus (Banks 1895)	MB, QC, **CT**, IL, ME, MN, NH, NY	CMN, EMN, CMS, EMS	Carolinean					

Table 1 Continued

Family, species	Distribution in North America	Geographic regions	Nearctic	Biogeographic regions PAL	OL	ETH	NEO	AUS
F. floridae Jacot 1935	**FL**	ES	Austroriparian					
F. fuscipes (C.L. Koch 1844)	AK, AB, MB, ON, NS, CT, IL, ME, MI, NH, NY, VA	WN, WMN, CMN, EMN, EMS	Amphi-Atlantic	**W**E	E			
F. setosus (C.L. Koch 1839)	QC, NF, VA	EMN, EMS	Amphi-Atlantic	**W**E	W			
Ghilarovizetes longisetosus (Hammer 1952)	AK, YT, **NT**, NU, NS, GL	WN, CN, EN, EMN	Circumboreal	E				
Iugoribates gracilis Sellnick 1944	AK, YT, NT, NU, **GL**	WN, CN, EN	Circumpolar					
Jugatala tuberosa Ewing 1913	BC, **OR**	WMN, WMS	Circumboreal	E				
Laminizetes fortispinosus Behan-Pelletier 1986	**YT**, NT	WN, CN	East Beringian					
Latilamellobates baloghi (Mahunka 1983)	BC	WMN	Amphi-Atlantic	**W**				
Melanozetes crossleyi Behan-Pelletier 2000	**BC**, SK	WMN, CMN	Pacific Coastal					
M. meridianus Sellnick 1929	AK, YT, NT, NU, MB, QC, NY, GL	WN, CN, EN, CMN, EMN	Amphi-Atlantic	**W**E				
M. mollicomus (C.L. Koch 1839)	AK	WN	Circumboreal	**W**E				
M. sellnicki (Hammer 1952)	AK, YT, **NT**, NU, MB, QC, VA, GL	WN, CN, EN, CMN, EMN, EMS	Circumboreal	WE				
M. tanana Behan-Pelletier 1986	**YT**	WN	East Beringian					
Neogymnobates luteus (Hammer 1955)	**AK**, YT, NT, NU, BC, QC, NB, NF	WN, CN, WMN, EMN	Trans-Nearctic					
N. marilynae Behan-Pelletier 2000	**BC**, WA,	WMN, WMS	Pacific Coastal					
N. multipilosus (Ewing 1907)	**IL**	EMS	Carolinean					
Oromurcia bicuspidate Thor 1930	GL	EN	Circumpolar	**W**				
Oromurcia lucens (L. Koch 1879)	AK, NT, NU,	WN, CN	Circumpolar	W**E**				
Sphaerozetes arcticus Hammer 1952	AK, YT, **NT**, NU, NS, NF, VA	WN, CN, EMN, EMS	Circumboreal	E				
S. castaneus Hammer 1955	**AK**, YT	WN	East Beringian					
S. firthensis Behan-Pelletier 1986	**YT**	WN	East Beringian					
S. winchesteri Behan-Pelletier 2000	**BC**, OR,	WMN, WMS	Pacific Coastal					
Svalbardia paludicola Thor 1930	YT, NT, NU, MB, GL	WN, CN, EN, CMN	Circumpolar	**W**E				
Trichoribates austroamericanus Berlese 1910	**DC**	EMS	Carolinean					
T. biarea Gjelstrup & Sølhoy 1994	**GL**	EN	Amphi-Atlantic	**W**				
T. boletorum (Ewing 1913)	**MN,**	CMS	Canadian					
T. copperminensis Hammer 1952	AK, YT, **NT**, NU, BC, VA	WN, CN, WMN, EMS	Amphi-Beringian	E				
T. formusus (Banks 1909)	ON, **NJ**	EMN, EMS	Carolinean					
T. giganteus (Hall 1911)	**CT**	EMS	Carolinean					
T. latincisus (Ewing 1909)	**IL**	EMS	Carolinean					
T. novus (Sellnick 1929)	VA	EMS	Amphi-Atlantic	**W**E				
T. obesus (Banks 1895)	AK, OR, WA, **NY**	WN, WMS, EMS	Amphi-Atlantic	E			C	
T. ogilviensis Behan-Pelletier 1986	**YT**	WN	East Beringian					
T. perfectus (Banks 1896)	**NY**, VA	EMS	Carolinean					
T. persimilis (Banks 1906)	**NH**	EMS	Carolinean					
T. polaris Hammer 1953	AK, YT, **NT**, NU, GL	WN, CN, EN	Amphi-Beringian	E				
T. principalis Berlese 1914	AB	WMN	Amphi-Atlantic	**W**				
T. spatulasetosus Reeves 1967	**NY**	EMS	Carolinean					
T. spinogenulatus (Ewing 1909)	**IL**	EMS	Carolinean					
T. striatus Hammer 1952	YT, **MB**	WN, CMN	East Beringian					
Chamobatidae								
Chamobates cuspidatus (Michael 1884)	QC, NF, VA, GL	EN, EMN, EMS	Amphi-Atlantic	**W**E	W	x		
C. egenus (Berlese 1910)	**FL**	ES	Austroriparian					
C. illinoisensis (Ewing 1909)	**IL**	EMS	Carolinean					
C. pusillus (Berlese 1895)	AK	WN	Circumboreal	**W**E				
C. schuetzi (Oudemans 1902)	AK, VA	WN, EMS	Amphi-Atlantic	**W**E				

Table 1 Continued

Family, species	Distribution in North America	Geographic regions	Nearctic	Biogeographic regions PAL	OL	ETH	NEO	AUS
Humerobatidae								
Humerobates arborea (Banks 1895)	NS, CA, CT, GA, IL, MA, ME, NY	EMN, EMS, WS	Amphi-Atlantic			x		
H. rostrolamellatus Grandjean 1936	QC, NS	EMN	Amphi-Atlantic	**W**E			C	PH
Mycobatidae								
Ceresella reevesi Behan-Pelletier & Eamer 2008	**UT,** WA, OR, CA	WMS, WS	Cordilleran					
Cyrtozetes denaliensis Behan-Pelletier 1985	**AK**, YT	WN	Amphi-Beringian	E				
C. lindoae Behan-Pelletier & Eamer 2008	**BC**, AB	WMN	Pacific Coastal					
Guatemalozetes danos Behan-Pelletier & Ryabinin 1991	CO, **AB**, KS	WMN, WS, CMS	Cordilleran					
Minunthozetes semirufus (Koch 1841)	CA, NS, NF	EMN, WS	Amphi-Atlantic	**WE**				
Mycobates acuspidatus Behan-Pelletier, Eamer & Clayton 2001	**BC**, WA	WMN, WMS	Pacific Coastal					
M. altus Behan-Pelletier 1994	NM, **AB**, CO	WMN, WS	Cordilleran					
M. azaleos Behan-Pelletier 1994	**AB**, OR, BC	WMN, WMS	Cordilleran					
M. beringianus Behan-Pelletier 1994	AK, **YT**, NT, GL	WN, CN, EN	East Beringian					
M. brevilamellatus Behan-Pelletier 1994	**BC**	WMN	Pacific Coastal					
M. conitus Hammer 1952	AK, YT, **NT**, AB, QC, NF, GL	WN, CN, EN, WMN, EMN	Amphi-Beringian	E				
M. corticeus Behan-Pelletier, Eamer & Clayton 2001	**BC**, SK	WMN, CMN	Pacific Coastal					
M. dryas Behan-Pelletier 1994	AK, **YT**, NT, AB, NF, GL	WN, CN, EN, EMN	Trans-Nearctic					
M. exigualis Behan-Pelletier 1994	**NS**, GL	EN, EMN	Amphi-Atlantic	W				
M. flabelliger Berlese 1908	**DC**	EMS	Carolinean					
M. hammerae Behan-Pelletier 1994	AK, **YT**	WN	East Beringian					
M. hylaeus Behan-Pelletier 1994	ON, NB, **NS**, NH, VT, PE, VA	EMN, EMS	Carolinean					
M. incurvatus Hammer 1952	AK, YT, **NT**, BC, AB, QC, NS, NF, CO, NY, GL	WN, CN, EN, WMN, EMN, EMS	Amphi-Beringian	E				
M. occidentalis Behan-Pelletier 1994	AK, **BC**	WN	East Beringian					
M. parmeliae (Michael 1884)	NS	EMN	Amphi-Atlantic	**W**E				
M. perates Behan-Pelletier 1994	AK, NY, YT, **QC**	WN, EMN, EMS	Trans-Nearctic					
M. punctatus Hammer 1955	**AK**, BC	WN, WMN	Amphi-Beringian	E				
M. sarekensis (Trägårdh 1910)	AK, YT, NT, GL	WN, CN, EN	Circumpolar	**W**E				
M. yukonensis Behan-Pelletier 1994	**YT**	WN	East Beringian					
Pelopsis bifurcatus (Ewing 1909)	NU, NT, NS, AB, NH, CT, FL, **IL**, MO, SC, GA, AL, LA, MS, NY, TN, NC, TX, VA, CA	WMN, CMS, WS, CN, EMN, EMS, ES, CS	Trans-Nearctic					
Punctoribates palustris (Banks 1895)	YT, BC, AB, SK, ON, QC, NB, NS, NF, WI, NH, **NY,** NJ, VA, SC, GA, FL, AL, NM, AZ, CO, CA	WN, WMN, CMN, EMN, EMS, WS, CS, ES	Trans-Nearctic					
P. punctum (C.L. Koch 1839)	BC, NB, NF, NY, VA	WMN, EMN, EMS	Cosmopolitan	WE	CWE			Z
P. weigmanni Behan-Pelletier & Eamer 2008	**MO,** ON	EMN, CMS	Canadian					
Zachvatkinibates epiphytos Behan-Pelletier, Eamer & Clayton 2001	**BC**	WMN	Pacific Coastal					
Z. maritimus Shaldybina 1973	YT, AK, BC	WN, WMN	Amphi-Beringian	**E**				
Z. nortoni Behan-Pelletier & Eamer 2005	**BC**	WMN	Pacific Coastal					
Z. quadrivertex (Halbert 1920)	AK, YT, NU, NT, MB	WN, CN, CMN	Amphi-Atlantic	**W**E				
Z. schatzi Behan-Pelletier & Eamer 2005	**BC**	WMN	Pacific Coastal					
Z. shaldybinae Behan-Pelletier & Eamer 2005	QC, ON, **NS,** GL	EN, EMN	East Hudsonian					

Table 1 Continued

Family, species	Distribution in North America	Geographic regions	Nearctic	Biogeographic regions PAL	OL	ETH	NEO	AUS
Zachvatkinibates tetrasklerosus Behan-Pelletier 1988	**CA**	WS	Pacific Coastal					
Zetomimidae								
Heterozetes aquaticus (Banks 1895)	BC, ON, QC, NB, **NY**, NH, GA, FL, AL, MS, LA, OK, CA	WMN, EMN, EMS, WS, CS, ES	Amphi-Atlantic	W				
H. minnesotensis (Ewing 1913)	SK, MB, **MN**, ON, QC, NB, NY, WI	CMN, EMN, EMS	Trans-Nearctic					
Naiazetes reevesi Behan-Pelletier 1996	QC, NY, MS, **AL**	EMN, EMS, ES	Carolinean-Austroriparian					
Zetomimus cooki Behan-Pelletier & Eamer 2003	ON, **AL**, MS, LA, FL	EMN, CS, ES	Carolinean-Austroriparian					
Z. francisi (Habeeb 1974)	BC, AB, ON, NB, **NY**, WI	WMN, EMN, EMS	Trans-Nearctic					
Z. naias Behan-Pelletier 1998	FL	ES	Neotropical				**C**	
Z. setosus (Banks 1895)	ON, NS, **NY**, WI	EMN, EMS	Carolinean					
Euzetidae								
Euzetes globulus (Nicolet 1855)	ON, QC	EMN	Amphi-Atlantic	**W**E				

Table 2 Number of families, genera and species of Ceratozetoidea in each geographical region of North America. Regional division of North America is shown in Figure 1.

Region	WN	CN	EN	WMN	CMN	EMN	WMS	CMS	EMS	WS	CS	ES
No. families	3	2	3	4	3	6	2	3	5	3	3	5
No. genera	17	14	12	18	10	20	6	7	15	9	5	9
No. species	49	30	21	40	18	41	7	7	46	9	5	13

Table 3 Matrix presenting the Sørensen Index (%; lower left) and number of species in common (upper-right) for pairwise comparisons of geographical regions using ceratozetoid species of North America. Regional division of North America is shown in Figure 1.

	WN	CN	EN	WMN	CMN	EMN	WMS	CMS	EMS	WS	CS	ES
WN		25	15	13	10	16	1	0	10	1	1	2
CN	63.3		15	8	7	14	0	1	5	1	1	2
EN	42.9	58.8		4	5	12	0	0	3	0	0	0
WMN	29.2	22.9	13.1		11	16	5	2	13	6	3	3
CMN	29.9	29.2	25.6	38.0		11	0	1	9	1	1	1
EMN	35.6	39.4	38.7	39.5	37.3		0	4	23	5	5	8
WMS	3.6	0	0	21.3	0	0		0	1	0	0	0
CMS	0	5.4	0	8.5	8.0	16.7	0		3	1	2	2
EMS	21.1	13.1	9.0	30.2	28.1	52.9	3.8	11.3		4	4	7
WS	3.4	5.1	0	24.5	7.4	20.0	0	12.5	14.5		3	3
CS	3.7	5.7	0	13.3	8.7	21.7	0	33.3	15.7	42.9		5
ES	6.5	9.3	0	11.3	6.5	29.6	0	20	23.7	27.3	55.6	

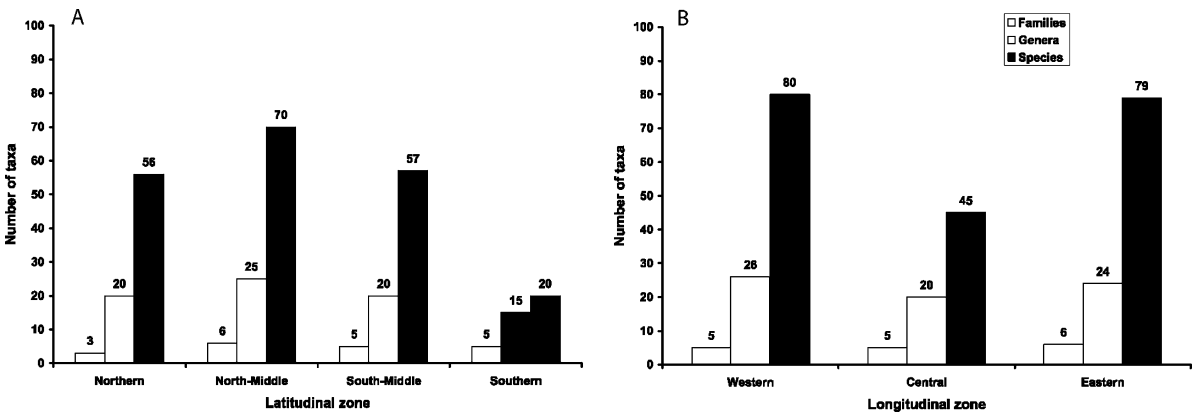

Figure 2 Number of ceratozetoid families, genera and species in each of the four latitudinal zones (A) and in each of the three longitudinal zones (B) in North America.

Table 4 Matrix presenting Sørensen indices (%; lower left) and number of species in common (upper right) for pairwise comparisons of latitudinal zones.

	Northern	North-middle	South-middle	Southern
Northern		29	11	3
North-middle	46.0		33	13
South-middle	19.5	52.0		10
Southern	7.9	28.9	26.0	

DISCUSSION

The Nearctic ceratozetoid fauna represents almost 11% of the 1 117 species of Oribatida known for the region (Schatz, 2005). The fauna is dominated by species in three genera, *Ceratozetes*, *Trichoribates*, and *Mycobates*, representing almost 50% of the fauna. Though there is no global assessment of these genera, they are considered temperate genera (Behan-Pelletier, 1997).

For most groups of organisms taxonomic richness tends to increase with decreasing latitude from the poles towards the equator (Rosenzweig, 1995; Qian, 1998). This trend is correlated with climatic variables such as mean annual temperature, available energy and frost-free days, which tend to increase towards the equator. Other than family richness, the latitudinal gradient demonstrated by North American Ceratozetoidea, is almost the reverse of the norm, with species richness in middle to northern latitudes almost equal, and more than double that of southern latitudes (Fig. 2a). The pattern for Ceratozetoidea somewhat mirrors that of the global oribatid fauna for which 'diversity increases from the boreal to the warm temperate region but not further to the tropics' (Maraun et al. 2007). Generic diversity among Ceratozetoidea also is higher in middle to northern latitudes than in more southern latitudes (Fig. 2a). This reverse gradient is possibly mirrored by other arthropods, such as Plecoptera, some Coleoptera tribes, and some families of primitive Diptera, that show a North-South vicariance (Downes & Kavanagh, 1988). If a similar latitudinal gradient occurs among Ceratozetoidea in the Southern hemisphere, it may indicate a negative impact of high equatorial temperatures in the Cretaceous to the present on the ceratozetoid fauna, as with other insect groups (Downes & Kavanagh, 1988). To date, only one Neotropical species, *Zetomimus naias*, is known from North America (Table 1), and ceratozetoid species in Central America are mainly known from high elevations (Schatz, 2007).

There was a distinct pattern in longitudinal richness, with species richness in the Central zone almost half that in the Eastern and Western zones (Fig. 2b). Longitudinal patterns of taxonomic richness within a continent can be related to continentality (Qian, 1998). Most of the geographical regions with lowest ceratozetoid species richness – WMS, CMS, WS, and CS – are areas of low annual precipitation, comprising the semi-arid prairies and desert regions of North America (Scudder, 1979) (compare Figs 1 and 2b). The limited ceratozetoid fauna in these regions suggests that these taxa are intolerant of extended periods of low moisture and high temperature.

The Nearctic biogeographical component of this ceratozetoid fauna is 58% of species (Table 5). This is low in comparison with North American species of *Carabodes*, for which almost 89% are Nearctic (Reeves, 1998), but is comparable to that of ptyctimous Oribatida, for which the Nearctic fraction is about 70% (Niedbała, 2002). Among the non-Nearctic component of the fauna, Amphi-Atlantic species dominate, reflecting land-bridge connections to North America during the Tertiary (Matthews, 1979). The Circumboreal and Circumpolar elements may also mirror these Tertiary connections. However, a significant component (8%) of the ceratozetoid fauna is Amphi-Beringian, occurring in both East and West Beringia, an area from east of the Lena River in Russia to Yukon. These species, along with the 10.4% of species that are inhabitants of East Beringia, i.e., Yukon and Alaska, are inhabitants of an area that was periodically unglaciated for long periods during the various advances of Pleistocene glaciations across northern North America (Matthews, 1979). Furthermore, the Bering land-bridge was an extensive land-mass during periods of the Tertiary and Quaternary (Matthews, 1979). The Bering land-bridge was an essentially treeless steppe extending eastwards from the Palearctic, and most ceratozetoid species found in East and West Beringia today are inhabitants of tundra or dry steppe (Behan-Pelletier, 1997). This biogeographic pattern is also seen in some Lepidoptera (Lafontaine & Wood, 1997), and other insect groups (Danks et al., 1997), but is essentially absent in carabodid and ptyctimous Oribatida, possibly because of their habitat requirements (Reeves, 1998; Niedbała, 2002).

Table 5 Analysis of the North American ceratozetoid fauna.

Distribution type		No. species	% of total species
Amphi-Atlantic		22	17.6
Circumboreal		10	8.0
Amphi-Beringian		10	8.0
Circumpolar		6	4.8
Cosmopolitan		3	2.4
Neotropical		1	0.8
Sub-total		*52*	*41.6*
Nearctic	Carolinean and Austroriparian	28	22.4
	East-Beringian	13	10.4
	Pacific Coastal	11	8.8
	Trans-Nearctic	10	8.0
	Cordilleran	8	6.4
	Canadian	2	1.6
	East-Hudsonian	1	0.8
Sub-total		*73*	*58.4*

Distinct Carolinean and Austroriparian elements combined make up the largest biogeographic component of the North American ceratozetoid fauna (22.4%). In contrast to ceratozetoid species in Beringia, that are associated with dry tundra habitats, these species are essentially endemic to the southeastern forests of North America, and probably evolved along with Mesophytic forests during the early Tertiary (Matthews, 1979). During the Wisconsinan glaciations, populations of these species apparently inhabited Pleistocene refugia with temperate conditions well south of the maximum extent of the Laurentide Ice Sheet. Resolution of whether this Carolinean and Austroriparian fauna evolved as a result of a North Atlantic vicariance, or is more closely related to the fauna of east Asia awaits phylogenetic analysis. However, this biogeographic pattern is similar to that of the genus *Carabodes* and Carabodidae in general (Reeves, 1998) and ptyctimous mites (Niedbała, 2002). These biogeographic elements indicate that the superfamily Ceratozetoidea is more ancient than its fossil history suggests, and may have been evolving since the early Tertiary in North America.

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Mites occurring in the canopy of Sitka spruce growing in Ireland

Julio Arroyo, Patrick Neville[1] & Thomas Bolger
School of Biology and Environmental Science, University College Dublin, Belfield, Dublin 4, Ireland.
E-mail: julio.arroyo@ucd.ie; [1]Coillte Teo., Newtownmountkennedy, Co. Wicklow, Ireland

Following centuries of deforestation, the area of forest in Ireland is increasing at a rate of between 20,000 and 25,000 hectares annually. However, the vast majority of the afforestation has been with Sitka spruce which is native to North America. In this study we describe the oribatid fauna of a Sitka spruce canopy in Ireland and compare it with the fauna occurring in the canopies of North America. In Ireland, 24 species of Oribatida, representing 20 genera, were recorded from two arboreal microhabitats: canopy (leaves and branches) and epiphytic moss. Two of these species, *Malaconothrus* (*M.*) *egregius* and *Ophidiotrichus tectus*, were new records for Ireland and the latter was the first record of the genus *Ophidiotrichus*. Diversity indices and rarefaction curves demonstrate that oribatid mite diversity is greatest in both canopy and epiphyte samples collected at the upper heights. Over 50% of the oribatids recovered from the canopy were Brachypilina and the assemblage was dominated by *Phauloppia lucorum* and *Camisia segnis*, with the remainder of the species being poorly represented (except *Chamobates schuetzi* and *Eupelops acromios*). Of the 19 species found in the moss, *P. lucorum* and *Zygoribatula exilis* were the most abundant taxa, and *C. segnis* was uncommon. In this microhabitat, 91% of individuals collected were Brachypilina which is not unexpected. Species richness is considerably lower than in similar habitats with native Sitka spruce and arboreal genera, such as *Dendrozetes* and *Scapheremaeus*, were not found in this study.

Key words: Oribatids, canopy, arboreal mites, afforestation, Sitka spruce, Ireland

Forest is the climax vegetation for most of Ireland. Yet, at the beginning of the last century, because of deforestation, only 1.4% of the land area was afforested. Currently government policy encourages afforestation and at present approximately 10% of the land is forested. More than 90% of these forests are plantation forests (Fahy & Foley, 2002) and most of this new forest consists of exotic trees, such as Sitka spruce (*Picea sitchensis*) introduced from North America. This species is chosen as a productive canopy species (Forest Service, 2000). Little is known of the flora and fauna of these plantations (Bolger, 2002) and this is especially true for invertebrate species (Fahy & Gormally, 1998). In Ireland no native invertebrate species can have co-evolved with these trees and it is therefore of interest to know the composition of the fauna.

In the current study we focus on the oribatid mite fauna occurring in the canopy microhabitats of Irish Sitka spruce. Lindo & Winchester (2006) studied oribatid mites in many arboreal habitats, including the bark and trunks of trees, leaf domatia and stems, moss, lichen, and other corticolous epiphytes and in accumulations of organic matter known as suspended soils. These authors found distinctive oribatid communities in soil and canopy habitats. Many oribatids are found in arboreal habitats in both tropical and temperate forests (Behan-Pelletier & Walter, 2000). They show special morphological and physiological adaptations (Karasawa & Hijii, 2004) and several factors, such as the height in the canopy, have been shown to influence population dynamics (Fagan & Winchester, 1999).

In this study we catalogue the oribatid species occurring in the canopy of a relatively old Irish Sitka spruce plantation and compare it with the fauna occurring in North America where this tree species is native.

MATERIALS AND METHODS

Samples were taken in November 2005 from a relatively old Sitka spruce forest (planted in 1925) growing at Baunreagh, Co Laois, Ireland [29°02'N] (stand elevation: 360 m above sea level; soil type: near Gley Soils).

Samples were collected from five randomly selected trees by climbers. The sampling followed protocols developed by Finnamore et al. (2004) and Behan-Pelletier (pers. comm.). Five sections of one branch, approximately 25 cm long and with a similar diameter, were collected in each tree from the top of the living crown (ca. 40 m), mid crown (ca. 38 m), and at the bottom of the living canopy (ca. 36 m). The main epiphytic moss cover was removed and the branches and twigs were bathed in a dilute solution of NaOH for 48 h. The liquid was then filtered and the animals were collected. The samples collected from each branch were combined for each layer within an individual tree. Dry weights of samples were recorded.

Moss (together with some lichens) was collected at each of the heights using a scraper. The samples were variable in size and taken primarily from the junction of the trunk and the selected branches, at the same heights as the samples of the canopy fauna. The moss was mainly *Hypnum cupressiforme cupressiforme*. Animals were extracted by placing the samples in a Berlese funnel for 98 h. Sample dry weights were recorded.

Animals were stored in ethanol + lactic acid. The oribatid mites were slide mounted in Hoyer's liquid and identified to species level using several keys (i.e., Balogh & Mahunka, 1983; Pérez-Íñigo, 1993, 1997; Subías & Arillo, 2001).

Shannon's diversity index (H), Equitability (relative diversity or evenness, J), species abundance, species richness, and rarefaction curves were calculated for all samples using the software Bio Diversity Pro V.2, whereas analysis of variance (ANOVA) of the richness and diversity indexes with height as factor was carried out using Stat Graphics Plus V.

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Table 1 Abundances and species checklist of oribatid mites found in the canopies of five trees (1-5) at three heights (L, lower; M, middle; U, upper). Dry weight (g) recorded for each tree and height is showed in the bottom row.

Tree canopy	L1	L2	L3	L4	L5	M1	M2	M3	M4	M5	U1	U2	U3	U4	U5
Phauloppia lucorum	5	7	18	8	23	2	17	55	7	35	6	15	41	57	28
Chamobates schuetzi	2	4	6	1	21	4	8	11	0	13	4	3	0	3	19
Chamobates sp.	0	0	0	1	4	0	0	2	4	2	4	2	5	1	6
Camisia segnis	16	21	26	46	41	26	58	28	31	44	22	32	15	37	41
Adoristes poppei	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0
Eupelops acromios	1	1	1	1	2	1	7	2	3	0	3	27	3	12	3
Eupelops sp.	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Ceratoppia bipilis	0	1	0	0	0	0	1	0	0	0	0	0	1	1	0
Cepheus latus	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0
Parachipteria sp.	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Platynothrus peltifer	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
Odontocepheus elongatus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Edwarzetes edwarsi	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Total	24	34	52	57	92	34	92	98	46	96	42	81	66	113	97
Dry weight (g)	242.7	416.3	313.2	282.8	434.6	360.7	328.2	465.4	309.8	261.8	370.3	463.6	702.0	563.5	407.8

Table 2 Diversity indexes for both microhabitats (mean ± SD, based on no. individuals/g dry weight).

	Lower layer	Middle layer	Upper layer	$F_{2,12}$	P
Tree Canopy					
Richness	5 ± 0.70	5.2 ± 0.44	6.6 ± 1.14	5.70	0.018
Shannon H'	1.47 ± 0.35	1.499 ± 0.18	1.867 ± 0.21	3.57	0.061
Shannon J'	0.635 ± 0.12	0.636 ± 0.07	0.695 ± 0.10	0.58	0.58
Moss mats					
Richness	2.8 ± 1.09	2.6 ± 0.89	5 ± 4.30	1.29	0.31
Shannon H'	0.956 ± 0.55	0.997 ± 0.55	1.593 ± 1.26	0.86	0.45
Shannon J'	0.568 ± 0.33	0.733 ± 0.25	0.644 ± 0.38	0.32	0.73

4.0. Detrended correspondence analysis (DCA), redundancy analysis (RDA), and canonical correspondence analysis (CCA) were used to examine the relationships between the mite communities and height in the trees using CANOCO V 4.0. (ter Braak & Prentice, 1988; ter Braak & Smilauer, 1998). According to ter Braak & Smilauer (1988), if the length of the gradient (LG) for the first axis in DCA is less than 1.5, the data display a linear relationship with the dominant axis, and RDA should be used in relating species assemblages to environmental variables. If the length is ≥3, the relationship is non-linear and CCA should be used. In all cases, the model used for statistical analysis was a split plot design where heights within a tree were taken to be subplots with trees as plots.

RESULTS

This study has added two new species, *Malaconothrus* (*M.*) *egregius* and *Ophidiotrichus tectus*, to the Irish fauna. In fact, this is also the first record of the genus *Ophidiotrichus* in the country.

Tree canopy

A total of 1,024 individuals representing 15 species were obtained. *Phauloppia lucorum* and *Camisia segnis* were dominant in almost all samples, and they were the only species found in all trees at all heights – the remaining species, with the exception of *Chamobates schuetzi* and *Eupelops acromios*, were poorly represented (Table 1). Of the individuals of *E. acromios* a typical arboreal oribatid, 71.5% occurred in the higher parts of the canopy, where *P. lucorum* had near 0.5% of its abundance. In this microhabitat 52.5% of the individuals belong to the cohort Brachypilina (higher oribatids).

The rarefaction curve for this microhabitat is shown in Figure 1. Diversity was highest in the canopy (Table 2) and species richness showed statistical differences between upper vs. middle heights, and between upper vs. low heights. However, the other diversity indices studied did not show statistical differences between heights (Table 2). RDA (gradient length in DCA = 1.115) showed that the assemblage structure did not vary with height in the canopy.

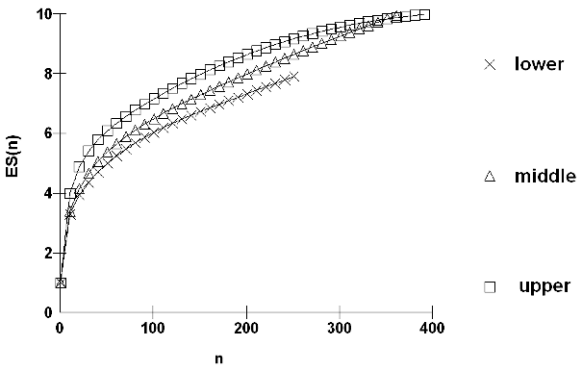

Figure 1 Rarefaction curve for mites from the tree canopy showing the expected number of species (ESn) in a sample of n individuals.

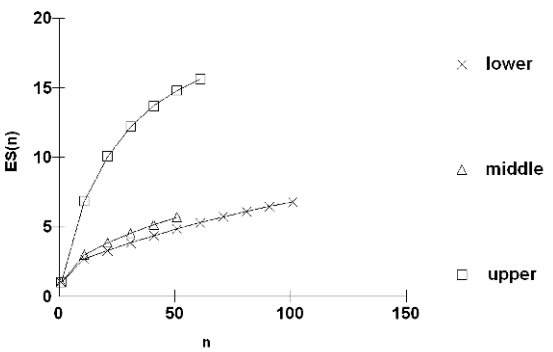

Figure 2 Rarefaction curve for mites from epiphytic moss in the canopy showing the expected number of species (ESn) in a sample of n individuals.

Table 3 Abundances and species checklist of oribatid mites found on epiphytic moss of five trees (1-5) at three heights (L, lower; M, middle; U, upper). Dry weight (g) recorded for each tree and height is showed in the bottom row.

Moss mats	L1	L2	L3	L4	L5	M1	M2	M3	M4	M5	U1	U2	U3	U4	U5
Phauloppia lucorum	12	7	7	3	20	0	8	15	2	15	5	5	3	5	3
Chamobates schuetzi	1	1	0	0	0	0	2	0	1	0	1	0	0	1	2
Chamobates sp.	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0
Camisia segnis	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2
Malaconothrus egregius	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
Liochthonius brevis	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Liochthonius hystricinum	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Liochthonius sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Pthiracarus (*Pthiracarus*) sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
Eupelops acromios	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Ceratoppia bipilis	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Parachipteria punctata	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Zygoribatula exilis	22	0	0	1	30	1	0	1	3	6	2	0	0	0	1
Odontocepheus elongatus	2	0	0	0	0	1	0	0	0	0	6	0	0	0	0
Moriztoppia oreia	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Moriztoppia (*M.*) *neerlandica*	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Ophiodiotrichus tectus	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
Nanhermannia dorsalis	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Medioppia subpectinata	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Total	37	9	7	5	51	2	10	16	7	22	15	6	3	34	9
Dry weight (g)	15.81	7.004	7.019	4.468	17.18	14.63	8.449	3.367	5.209	16.85	8.536	10.00	2.167	4.624	62.19

Moss mats

In this microhabitat 90.6% of the individuals recovered belonged to the cohort Brachypilina. In total, 233 individuals and 19 species were found, but eight of these were found in only one tree. *Phauloppia lucorum* occurred in most of the samples and it represented approx. 50% of the oribatids collected (Table 3). Although there was a general trend for higher diversity in epiphytic moss from higher levels in the canopy, this was not significant for any of the diversity indices calculated (Table 2). This lack of significance reflects the high variation in the diversity recovered from individual trees. However, rarefaction curves show that larger numbers of species were found in epiphytic moss from the upper canopy when the samples from all trees are combined (Fig. 2). DCA showed large variation among samples and the gradient length for the first axis was 3.952. Therefore CCA (using values of individuals/g dry weight) was used to analyse the variation in assemblage structure at various heights. Both the first axis ($F = 3.418$, $P<0.01$) and all axes ($F = 2.027$, $P<0.01$) were significant, indicating large differences between the mites from epiphytic moss high in the canopy and those from lower levels (Fig. 3). More than 70% of the *Zygoribatula exilis* collected were from lower heights, a trend also observed by Wunderle (1992). By contrast, *E. acromios* has only been found in samples from the highest levels in the canopy.

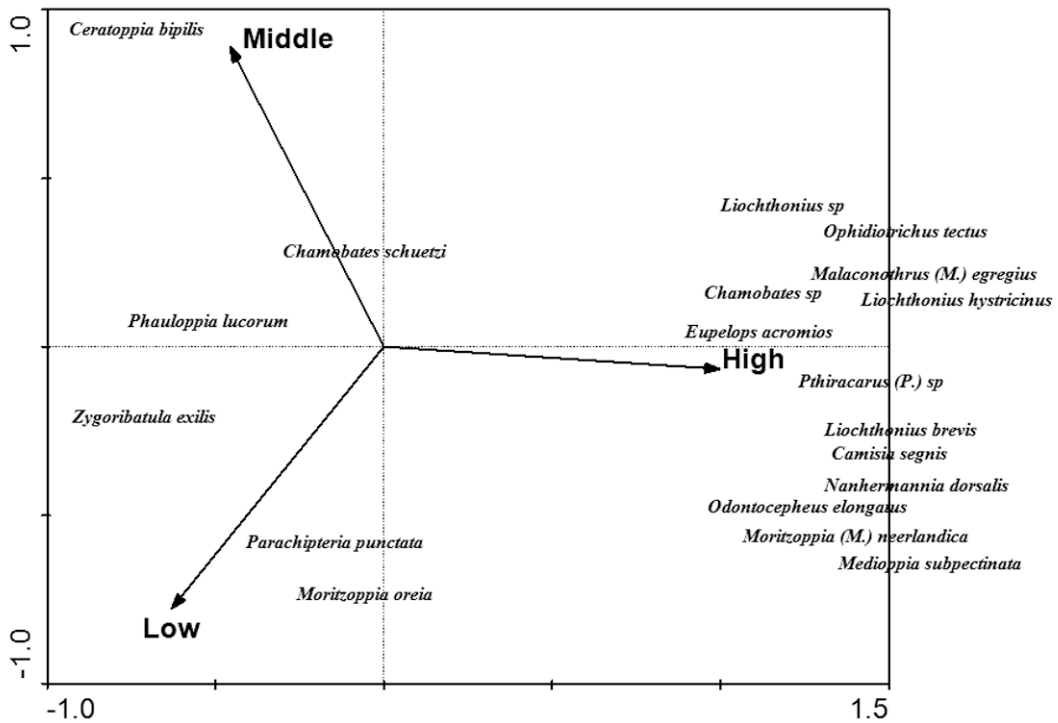

Figure 3 Plot of first two axes of a canonical correspondence analysis out of the mite assemblages recovered from epiphytic moss mats.

Table 4 Species richness (no. species) recovered from arboreal habitats in various studies.

Country	Habitat	Tree	Methods	Richness	Reference
Ireland	Tree canopy	Sitka spruce near 80 years	Washing	13	This study
	Moss mats	Sitka spruce near 80 years	Berlese	19	This study
Canada	Moss / soil (4 / 1 cm)	Sitka spruce near 700 years	Berlese & Malaise	43	Winchester et al, 1999
	Suspended soil	Red cedar trees	Berlese	53	Lindo & Winchester, 2006
Poland	Epiphytes on bark	Scots pine	Berlese	29	Seniczak et al., 1997
Finland	Branches	Oak forests	Branch trap	33	Kopponen et al., 1997

DISCUSSION

The mite fauna occurring in the canopy of Sitka spruce and in moss on the branches of this tree in Ireland was largely unknown. Two species not previously recorded in Ireland were found in this study and the other species recovered are typical canopy species. However, as expected, the fauna is less species rich than faunas of these trees in its native North America or in other native forests in other parts of the world.

Both microhabitats showed low species richness when compared to studies carried out with epiphytic moss, lichens, and branches in temperate forests (Wunderle, 1992). The species richness observed in moss mats of Baunreagh is lower than the value found with arboreal moss samples in Sitka spruce of temperate forests in Canada (Winchester et al., 1999). The richness in the canopy samples is also low in comparison with other studies (Kopponen et al., 1997). The abundance found in higher moss mats is lower than in soil suspended high in the canopy of native red cedar in Canada (Lindo & Winchester, 2006).

Brachypilina (higher oribatids) dominated the catches in both microhabitats (52.5% in the canopy, 90.6% in the moss) due to their resistance to desiccation, as can be inferred from their morphological adaptations (Behan-Pelletier & Walter, 2000; Norton & Alberti, 1997). Nearly all of the lower oribatids found in the canopy were *C. segnis*, a species typically associated with trees. This is similar to the proportion found by Lindo & Winchester (2006).

The results indicate lower species richness in the Irish Sitka spruce than in native species in temperate areas (Table 4). Particularly large numbers of oribatid species (43 and 53, respectively) were found by Winchester et al. (1999) and Lindo & Winchester (2006) from coniferous species in Canada. Whereas most of the species found in large numbers were primarily or strictly restricted to arboreal habitats, several typical arboreal genera, such as *Dendrozetes*, *Dometorina*, or *Scapheremaeus* (Aoki, 1973; Behan-Pelletier & Walter, 2000), were not found in this study which may explain the reduced richness. Species of Damaeidae, which are often seen in arboreal habitats, were not found in Baunreagh, nor were any members found of the family Dendroeremaeidae described from bark and twigs of coniferous and deciduous trees in the north-western part of North America (Behan-Pelletier et al., 2004).

All of these data suggest that the acarine fauna of non-native Sitka spruce stands in Ireland is depauperate. Most frequently found were *C. segnis* and *P. lucorum*, which are usually associated with higher plants and arboreal habitats, respectively. They have been found previously in Ireland (Luxton, 1998), which suggests that there is not an exclusive link between tree species and the mites in them. However, at this point it is not possible to assess the extent to which the low species richness is due to this factor or to the more general history of deforestation, or whether it is a result of the fact that Ireland is an island. These factors need to be teased out for an accurate understanding of the biogeography of the acarine fauna of Irish forests.

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Changes of the oribatid community after a windthrow event

Eileen Kreibich, Coronula Grauf[1] & Sabrina Strauch
Zoological Institute and Museum, University Greifswald, Bachstr. 11-12, 17489 Greiswald, Germany.
E-mail: eileen_kreibich@hotmail.com; and [1]Institute of Biochemistry and Biology, University of Potsdam, Maulbeerallee 2a and 2, 14469 Potsdam, Germany

In December 1999 the winter storm 'Anatol' hit the southern coast of the Baltic Sea and caused damage to forest areas along the coast. On the Darss peninsula in north-eastern Germany the storm destroyed one relatively large forest area and several small ones. Interestingly, mainly spruce trees were affected. The large windthrow area measured about 2.5 ha. This area was chosen to investigate the long-term effects of a windthrow event on the fauna. Repetitive sampling was carried out in the first 4 years after the storm (2000-2003) and in the 6th year (2005), and additional samplings are planned for the 10th and the 15th year. To investigate the soil micro-arthropods, in 2000-2003 samples were taken three times a year, in spring, summer, and autumn, directly under root plates of two fallen spruce trees, in the windthrow area, and in adjacent pine and spruce stands. Altogether 81,023 specimens of oribatid mites had been found in all 4 years. Results show that the overall abundance of oribatid mites decreased from 2000 to 2002 on almost all plots – including the control plot in the fir forest –, before their numbers started to increase again in 2003. On the plots underneath the root plates the abundance of oribatid mites increased continuously over the years as the plots were recolonised. The oribatid mite community on the windthrow area changed over the years, but these were quantitative rather than qualitative changes.

Key words: Oribatid mites, ecology, windthrow, recolonization, species diversity, mite abundance, succession

Severe storms cause windthrows and windbreaks. In primeval forests, windthrow events are known to be very important for forest regeneration (Falinski, 1976; Fischer, 1999), whereas in managed forests they cause severe economical damage. Therefore, in managed forests, the wood is quickly removed and the areas are replanted.

The winter storm 'Anatol' hit the Darss peninsula in north-eastern Germany in December 1999 and caused one relatively large plus several small windthrows in the National Park 'Vorpommersche Boddenlandschaft'. Because the large area was situated in the core zone of the National Park, the chance arose to leave the wood to the natural decomposition processes and to study the long-term effects of these processes on the fauna.

Windthrow events are known to occur quite regularly, but there is little information on their effects on the fauna, especially the soil fauna. For oribatid mites, comparable studies were only carried out on clear-cuttings (Karppinen, 1957; Moritz, 1965). Although there are many similarities between clear-cuttings and windthrows, one of the major differences is the removal of the wood from the former. Therefore, it can be expected that differences emerge in the development of the faunal community, especially concerning xylophagous species.

The storm affected a forest dominated by spruce trees mixed with single pine trees. Interestingly, mainly spruces were affected. The first sampling period took place in the first 4 years after the storm from 2000 to 2003. More samples were taken in 2005 and additional sampling periods are planned for 2009 and 2014.

METHODS

Samples were taken three times a year, in spring, summer and autumn, from 2000 to 2003. Plots were chosen in the windthrow area (WTI, WTII), in the soil that formerly was covered by the root plate of a spruce and was laid open by the storm (RP), and in adjacent pine (Pi) and spruce (Sp) stands.

Of the two plots in the windthrow area, WTI was situated in the centre and therefore in the first year much exposed to the sun, whereas WTII was situated more towards the edge of the area and therefore more shaded than WTI. In the first year the herb layer consisted mainly of grass and a few specimen of common bracken, *Pteridium aquilinum* (L.) Kuhn. From the second year on, the windthrow area was densely covered by common bracken. In the spruce forest no undergrowth was found, whereas in the pine forest the soil was densely covered by grass and common bracken.

The top 5 cm of the soil were sampled with a corer (6.4 cm diameter). Animals were separated from the soil by means of dynamic heat extraction (Macfadyen, 1953) and the oribatid mites were determined to species level when possible (mostly based on Weigmann 2006). The juvenile stages and the Brachychthoniidae were not further identified and were left out of the calculations, because the Brachychthoniidae were difficult to separate from the juveniles of some species, especially the Tectocepheidae. Suctobelbidae, Oppiidae, Damaeidae, and the genus *Phthiracarus* also have not been identified further, because of limited time.

The abundance (no. individuals/m^2) and dominance of each species and plot were calculated. To compare the plots, the diversity (Shannon-Weaver index) and evenness were used (Schaefer & Tischler, 1983), as well as the Renkonen index (Tischler, 1984).

RESULTS AND DISCUSSION

In the course of this investigation 81,023 oribatid mites were found altogether. More than half of these mites (43,020 specimens) were juveniles. The abundance of oribatid mites in

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the plots in the windthrow area does not differ significantly from that in the reference plots (Fig. 1). On the plots in the windthrow area and in the coniferous forests, abundance varies between 81,754 (2002, WTI) and 265,777 ind./m² (2003, Sp). These data match those for coniferous forests in Central Europe (e.g., Moritz, 1965; Sylwestrowicz-Maliszewska et al., 1993; Seniczak et al., 1994; Kreibich, 2004; Kreibich & Alberti, 2006). The only striking difference between these plots is the high number of juveniles in 2001 in plot WTI. Unfortunately, data from the time before the windthrow event are not available. But a possible explanation might be that, as a result of considerable environmental changes, new niches emerged that provided favourable conditions for a higher number of juveniles and that were not yet occupied by adults.

In the first 2 years, the abundance of oribatid mites in the plot underneath the root plate (RP) is significantly lower than in all other plots. This was to be expected because the sampled soil was originally 60 cm below the root plate and therefore only sparsely populated. Only after removal of the root plate by the storm, the soil was laid open and became accessible for colonisation. The oribatid community of RP is constantly changing during the first 4 years after the storm (Fig. 2). The only similarity in all 4 years is that *Tectocepheus velatus velatus* (Michael) is the most common species. In this early stage of colonisation, the community was probably formed by randomly immigrating species from the surrounding area.

The oribatid community in the windthrow area changes over the years (Figs. 3 and 4). The dominance structure in plot WTI becomes more uneven and the species composition changes. On the other side, the dominance structure in WTII is very uneven from the beginning and only in 2002 it is more balanced than in the other years. Especially the xylophagous *Steganacarus striculus* (C.L. Koch) seems to have profited from these changes, e.g., from a higher availability of dead wood. This species occurred in the other plots only recedently. Furthermore, it seems that on plot WTI the number of oppiid mites had decreased rapidly after the windthrow event. In 2000 only ca. 9,000 ind./m² could be found, as opposed to 20,000-40,000 ind./m² in the spruce and pine plots. Over the next 3 years their number rises back to normal. This development might have been caused by the high temperatures and desiccation of the soil in this plot, resulting from the high solar irradiation. In the more shadowed plot WTII the abundance of this species decreases more slowly over the first 3 years, from 36,000 to 9,000 ind./m² in 2000 and 2002, respectively; then its number increases again in 2003. Another species on WTI that seems to be influenced by the event was *T. velatus velatus.* In 2000

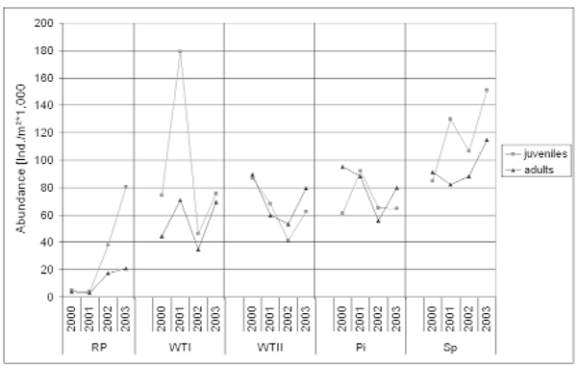

Figure 1 Abundance of adult and juvenile Oribatida on each plot.

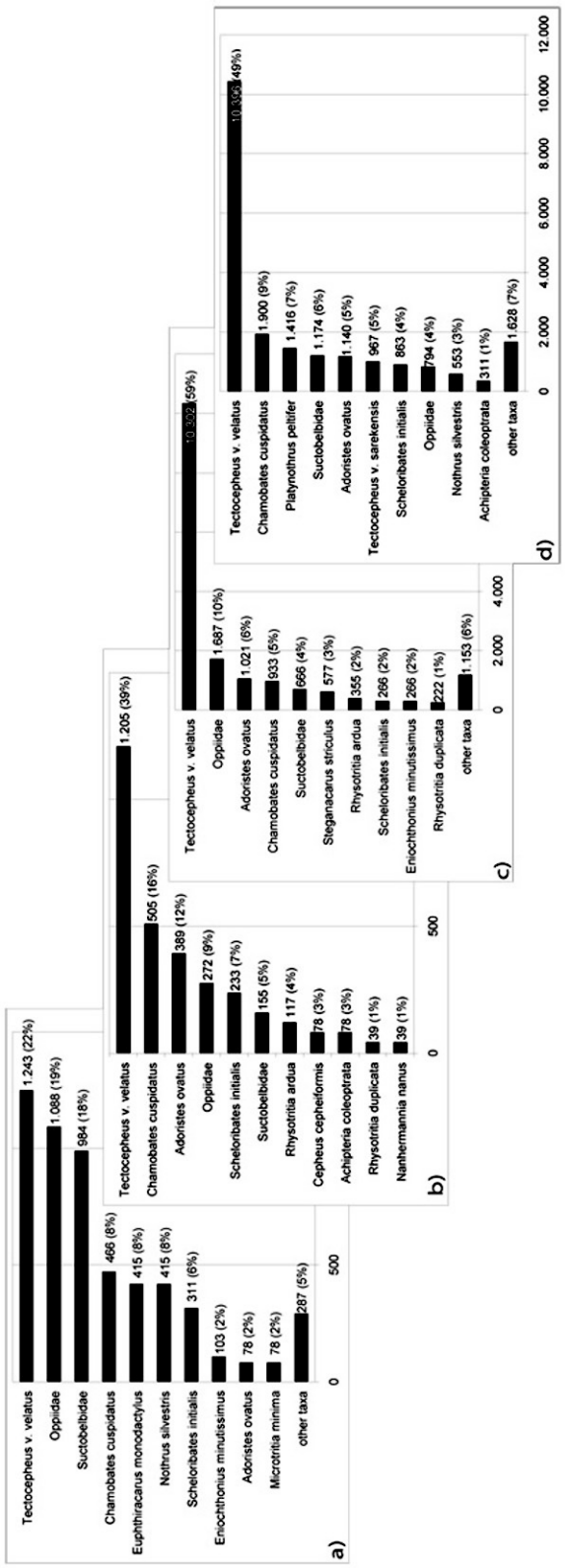

Figure 2 Dominance structure of the Oribatida on plot RP, a) 2000, b) 2001, c) 2002, d) 2003.

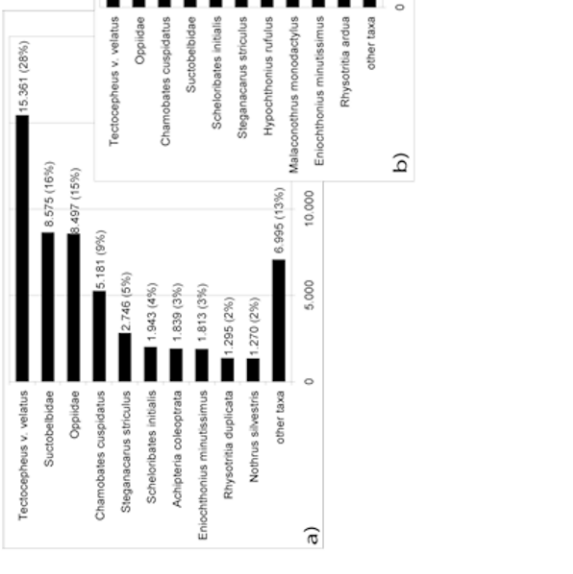

Figure 3 Dominance structure of the Oribatida on plot WT I, a) 2000, b) 2001, c) 2002, d) 2003.

Figure 4 Dominance structure of the Oribatida on plot WT II, a) 2000, b) 2001, c) 2002, d) 2003.

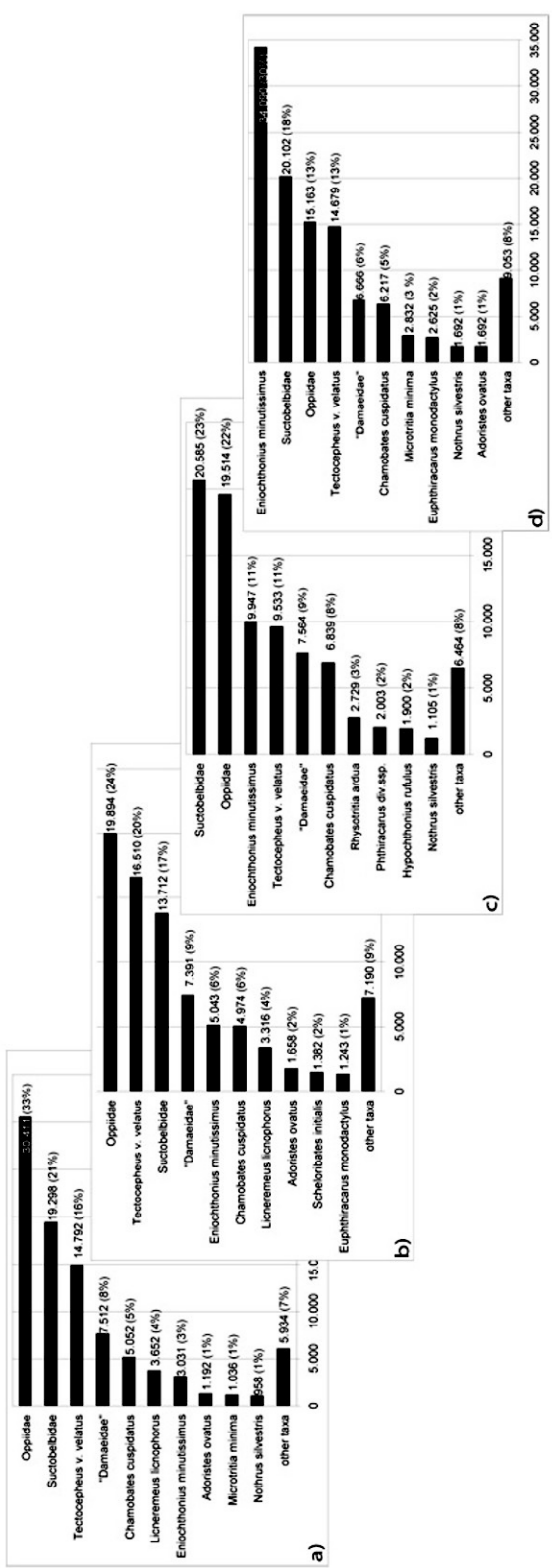

Figure 5 Dominance structure of the Oribatida on plot Sp, a) 2000, b) 2001, c) 2002, d) 2003.

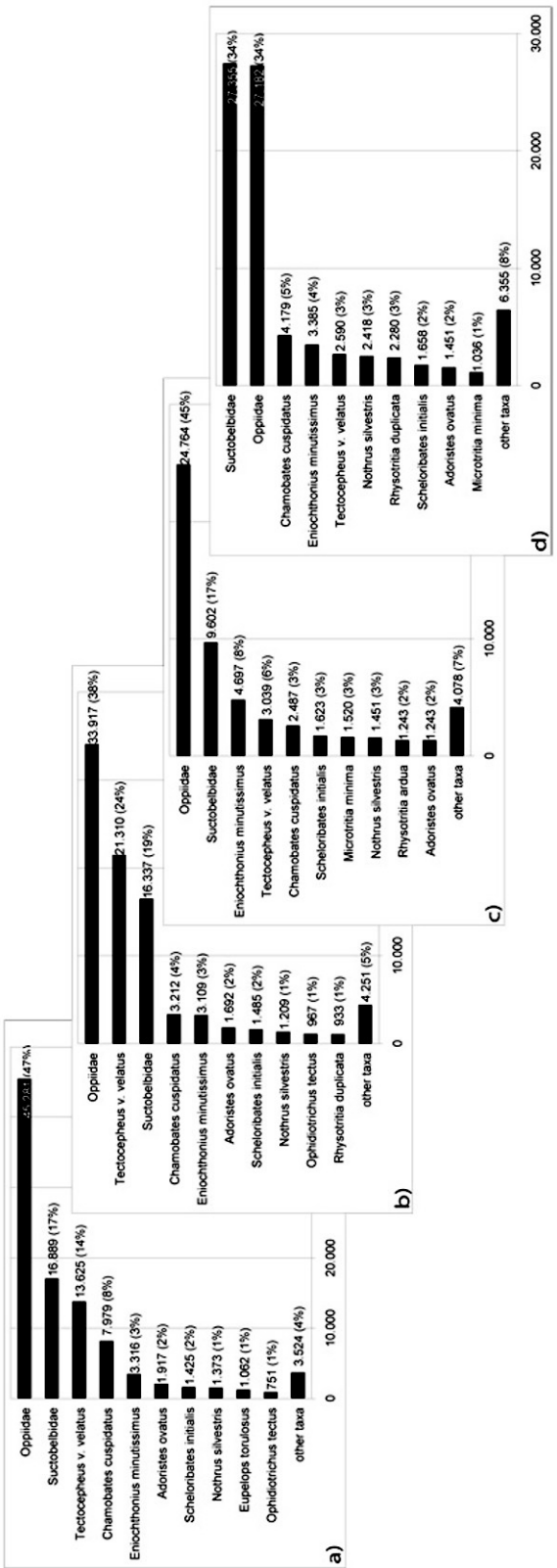

Figure 6 Dominance structure of the Oribatida on plot Pi, a) 2000, b) 2001, c) 2002, d) 2003.

and 2001, it is as abundant as in the reference plots, but its number drops during the next years.

Samples were also taken in a spruce stand (plot Sp), presuming that this resembles the situation before the windthrow event. The dominance structure and species composition of the oribatid mite community in this plot was relatively stable over the years (Fig. 5). One striking change was the distinct increase of *Eniochthonius minutissimus* (Berlese) from 3,000 to ca. 35,000 ind./m² in 2000 and 2003, respectively. This development may have been initiated by a growing anthill on the chosen plot. The accumulation of decaying organic matter in anthills possibly leads to more fungi than in the surrounding area, which may benefit a microphytophagous species like *E. minutissimus*.

Another striking observation is the relatively high abundance of *Licneremaeus licnophorus* (Michael) in the first years. This species presumably lives on trees and on trunks (Weigmann, 2006). Due to the storm, these animals may have fallen off the trees and accumulated in the soil.

As another reference plot, a pine stand (plot Pi) was chosen, because in the windthrow area the pine trees remained standing. Furthermore, common bracken is very widespread on the Darss peninsula and colonises open habitats rapidly. Therefore, it could be expected that the bracken would quickly inhabit the windthrow area. In the chosen pine stand, it is the dominant plant species in the herb layer. The dominance structure and species composition in the pine plot is even more stable than in the spruce forest (Fig. 6). Only the abundance of *T. velatus velatus* fluctuates over the years.

Regarding their dominance identity, the oribatid mite community in the windthrow area still closely resembles that of the spruce forest right after the storm (Fig. 6). Over the next 3 years the community changes such that it becomes more closely related to that of the pine forest. This may be caused by the changing undergrowth. In the beginning, there was only little undergrowth, mainly grass, as can be expected in relatively dense spruce forests. During the next years the area was colonised by common bracken, so that now the undergrowth is like that in the pine forest. These results suggest that the undergrowth has a strong effect on the oribatid mite community.

General conclusion

Karppinen (1957) and Moritz (1965) observed an increase in the abundance of oribatid mites in the year after a clear-cutting. Here, this effect was only seen on plot WTI, in the centre of the windthrow area. At the edge of the windthrow area (WTII), the abundance decreased as it did in the reference plots, probably due to climatic effects. Moritz explains the increasing abundance by the influence of higher temperatures in the open area on the reproduction rate. Plot WTI is more exposed to the sun than the other plot, leading to higher temperatures in the soil. The high number of juveniles in this plot in 2001 could be attributed to the influence of the higher temperature. Later, this plot was densely covered by common bracken, which neutralises this effect. As already observed by Moritz, there are quantitative rather than qualitative changes of the oribatid mite community, that are expressed by a shift in the dominance structure.

Acknowledgements

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Effects of a windthrow event in the forest of the peninsula Darss on the gamasid fauna (Arachnida) and Collembola

Annett Wegener & Gerd Alberti
Zoological Institute & Museum, University of Greifswald, J.-S.-Bachstr. 11/12, D-17489 Greifswald, Germany.
E-mail: AnnettWegener@gmx.net

In December 1999 a winter storm largely destroyed pine-spruce stands on the peninsula Darss. After the decision to leave the windthrows in the protected zone to natural succession, a research project was started in 2000 to investigate responses of selected endo- and epigeic arthropod groups to this process. The soil fauna was explored at two sites within the windthrow area and at one site right in front of an uprooted spruce root plate. A pine stand and a spruce stand in the direct neighbourhood, both undisturbed, were chosen as references. The results show the development of the gamasid mite communities from 2000 to 2003. A total of 54 species were found. The structure of the predatory mite communities changed substantially over the period investigated, especially at the destroyed sites. In the central site within the windthrow area under study abundance and species number decreased significantly. Furthermore, the dominance structures differed in the course of time as well as in comparison with the undisturbed sites. In the site in front of the uprooted spruce root plate gamasid mites gradually colonized the highly disturbed and exposed soil substrate. The results indicate that the windthrow event has caused a considerable disturbance to the predatory mite communities in the affected sites, probably due to changed microclimatic conditions and to changes in food supply.

Key words: Gamasida, windthrow, natural succession, soil ecology

Windthrows arise as unpredictable natural events. They can break up biocenotical relations, which in turn lead to new developments in the affected forest ecosystems (Fischer, 1998). Since windthrow events can strongly influence the nutrient supply and species diversity, they are not only of economical importance for forestry, but also of general ecological interest.

The Darss peninsula in north-eastern Germany (Mecklenburg-Vorpommern) arose from marine sand sedimentation, starting about 7,000 years ago. Thus, there is a characteristic alternation of dune ranges (Reffs) and dune slacks (Riegen). Since 1990 the peninsula is part of the National Park "Vorpommersche Boddenlandschaft" (805 km²). The forest of Darss covers an area of 47 km². Under natural conditions it would be a mixed forest with beech (*Fagus sylvatica*), pine (*Pinus sylvestris*), oak (*Quercus robur*) and birch (*Betula pendula*) on the dune ranges and with alder (*Alnus glutinosa*) in the mostly moist dune slacks. However, a history of forest management led to the increase of coniferous stands composed of pine and spruce (*Picea abies*).

In December 1999 the pine-spruce stands of the forest were largely destroyed by a winter storm. Especially the shallow-rooted spruce trees could not withstand the storm. Altogether about 10,000 m² of wood were affected. After the decision to leave about 2,400 m² of the windthrows in the strictly protected zone to natural succession, a research project supported by the State Office of Forests and Protected Areas was started in 2000 to investigate responses of selected animal groups, e.g. soil-dwelling gamasid mites, to this process. Because of their abundance and species richness, their position at a high trophic level (being mainly zoophages), and their relatively high mobility, the gamasid mites are generally good indicators for changes in environmental conditions.

MATERIALS AND METHODS

Sites

The exploration of the soil fauna took place at two sites of a large-area pine-spruce windthrow (WT I and WT II). Here, the site WT I was located in the central part of the windthrow area, whereas WT II was situated closer to the border. Additionally, a site right in front of an uprooted spruce root plate (RP) was investigated. A pine stand (Pi) and a spruce stand (Sp) in the immediate vicinity of the windthrow area, yet not destroyed by the storm, were chosen as references. The herb layer in all sites was dominated by *Pteridium aquilinum*.

Sampling and processing of predatory mites and Collembola

The investigation was carried out from 2000 to 2003. Sampling was done three times a year, i.e. in spring, summer and autumn. Three samples were taken per site and date using a steel cylinder (diameter 6.4 cm and to a depth of 5 cm). In the laboratory the soil arthropods were extracted from the samples using a high gradient Macfadyen-type-apparatus. With keys of Karg (1989, 1993), the gamasid mites were identified to species level (except for the juvenile stages) from specimens permanently mounted on slide. The nomenclature is according to Błaszak et al. (1997). Specimens of Collembola were counted, but not identified.

Statistical analysis

To study the development of the predatory mite community in each sampling site in the four years after the windthrow event, the abundances (number of individuals/m²), the species numbers and the dominances of species were compared. For the Collembola only the abundances were evaluated. Differences between years in each site were analysed

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by Tukey's honestly significant difference test using SPSS 12.0. In addition, the Renkonen-Index was calculated to reveal dominance similarities between sampling sites.

RESULTS

After the windthrow event, in both windthrow sites under study abundances of the gamasid mites have decreased (Fig. 1). The decrease of abundance from 11,100 in 2000 to 4,300 ind./m² in 2003 in the windthrow site WT I was significant ($P<0.05$). However, in the windthrow site WT II the abundance returned in 2003 to the level of the first year. In the reference pine site the gamasid mites were found in their highest abundance of approx. 14,000 and 15,000 ind./m² in 2000 and 2001 respectively. But in 2002 and 2003 a decrease of the abundance was observed in this site, while the abundance in the reference spruce site barely differed between the years. The investigated site RP showed very low abundances. Over the investigated period they increased slightly from approx. 500 to 1,900 ind./m².

Abundance of the Collembola clearly decreased ($P<0.05$) from 2000 to 2002/2003 in WT I (Fig. 2). In WT II the abun-

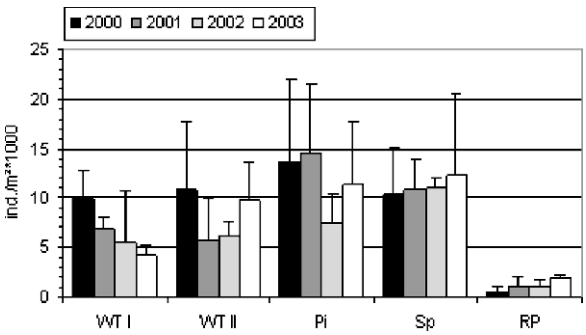

Figure 1 Abundances of the gamasid mites in the sites under study.

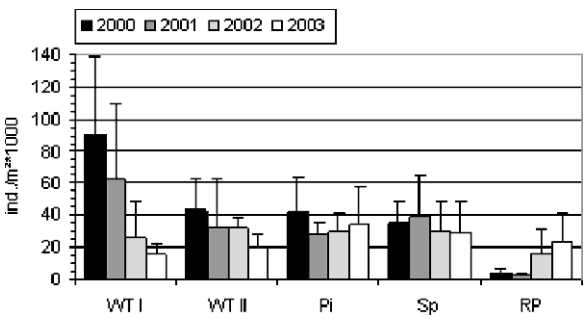

Figure 2 Abundances of the Collembola in the sites under study.

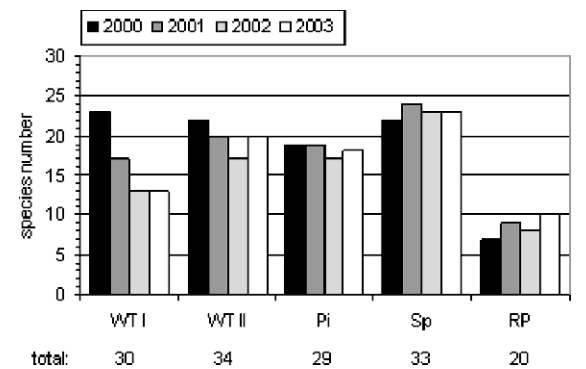

Figure 3 The observed species numbers of gamasid mites in the sites under study.

Table 1 Gamasid species identified at sites indicated within brackets (see Materials and Methods for explanation of the acronyms).

Antennophorina
- Celaenopsidae
  - *Celaenopsis badius* C.L. Koch, 1839 (WT I)

Sejina
- Sejidae
  - *Sejus togatus* C.L. Koch, 1836 (WT I, WT II)

Uropodina
- Polyaspidae
  - *Polyaspinus cylindricus* Berlese, 1916 (WT I, WT II, Pi, Sp, RP)
- Trachytidae
  - *Trachytes aegrota* (C.L. Koch, 1841) (WT I, WT II, Pi, Sp)
  - *T. pauperior* (Berlese, 1914) (WT II)
- Trematuridae
  - *Trichouropoda ovalis* (C.L. Koch, 1839) (WT I, WT II, Sp, RP)
- Urodinychidae
  - *Urodiaspis tecta* (Kramer, 1876) (WT I, WT II, Pi, Sp)
- Uropodidae
  - *Cilliba cassidea* (Hermann, 1804) (WT I, WT II, Pi, Sp)
  - *Uropoda minima* Kramer, 1882 (WT I, WT II, Pi, Sp, RP)

Gamasina
- Ascidae
  - *Arctoseius minutus* (Halbert, 1915) (RP)
  - *Zerconopsis michaeli* Evans et Hyatt, 1960 (WT II)
  - *Z. remiger* (Kramer, 1876) (Pi)
  - *Antennoseius avius* Karg, 1976 (WT II)
  - *Asca aphidioides* (Linné, 1758) (WT I, WT II, Pi, Sp)
  - *Cheiroseius borealis* (Berlese, 1904) (RP)
  - *Ch. bryophilus* Karg, 1969 (RP)
  - *Gamasellodes bicolor* (Berlese,1918) (WT I, WT II, Pi, Sp, RP)
  - *Lasioseius lawrencei* Evans, 1958 (Sp, RP)
  - *Proctolaelaps jueradeus* (Schweizer, 1949) (RP)
  - *P. pygmaeus* (Müller, 1860) (WT II, Sp)
- Digamasellidae
  - *Dendrolaelaps acornutosimilis* Hirschmann, 1960 (Sp)
  - *D. disetosimilis* Hirschmann, 1960 (Sp)
  - *Dendrolaelaps* sp. (Sp)
  - *Cornodendrolaelaps cornutulus* Hirschmann, 1960 (WT II)
- Epicriidae
  - *Epicrius* sp. (WT II, Sp)
- Eviphididae
  - *Eviphis ostrinus* (C.L. Koch, 1836) (WT I, WT II, Pi)
- Laelapidae
  - *Hypoaspis aculeifer* (Canestrini, 1883) (WT I, WT II, Pi, Sp)
  - *H. lubricoides* Karg, 1971 (Sp)
  - *H. vacua* (Michael, 1891) (Sp)
  - *Hypoaspis* sp. (Sp, RP)
  - *Ololaelaps placentula* (Berlese, 1887) (Pi)
- Macrochelidae
  - *Geholaspis longispinosus* (Kramer, 1876) (WT II)
  - *Macrocheles opacus* (C.L. Koch, 1839) (WT I, WT II)
- Pachylaelapidae
  - *Pachylaelaps longisetis* Halbert, 1915 (WT I, WT II, Pi)
- Phytoseiidae
  - *Amblyseius obtusus* (C.L. Koch, 1839) (WT I, Pi)
  - *Amblyseius* spp. (WT I, WT II, RP)
- Rhodacaridae
  - *Rhodacarus coronatus* Berlese, 1921 (WT I, Pi)
- Veigaiaidae
  - *Veigaia cerva* (Kramer, 1876) (WT I, WT II, Pi, Sp)
  - *V. kochi* (Trägardh, 1901) (WT I, Sp, RP)
  - *V. nemorensis* (C.L. Koch, 1839) (WT I, WT II, Pi, Sp)
- Parasitidae
  - *Vulgarogamasus kraepelini* (Berlese, 1905) (WT I, WT II, Pi, Sp)
  - *Holoparasitus calcaratus* (C.L. Koch, 1839) (WT I, WT II, Pi, Sp, RP)
  - *H. stramenti* Karg, 1971 (WT I, WT II, Sp)
  - *Paragamasus conus* (Karg, 1971) (WT I, WT II, Pi, Sp, RP)

P. lapponicus (Trägardh, 1910) (WT I, WT II, Pi, Sp)
P. robustus (Oudemans, 1902) (WT I, WT II, Pi, Sp)
P. runcatellus (Berlese, 1903) (WT I, WT II, Pi, Sp, RP)
P. vagabundus (Karg, 1968) (WT I, WT II, Pi, Sp, RP)
Pergamasus crassipes (Linné, 1758) (Pi)
P. mediocris (Berlese, 1904) (WT I, WT II, Pi, Sp, RP)
P. septentrionalis (Oudemans, 1902) (WT II, Pi, Sp, RP)
Zerconidae
Parazercon radiatus (Berlese, 1910) (Pi, RP)
Prozercon kochi Sellnick, 1943 (WT I, WT II, Pi, Sp, RP)
Zercon gurensis Mihelcic, 1962 (Pi)

dance decreased as well, but less rapidly than in WT I. The abundances of the two reference sites barely varied over the years. In the site RP the Collembola were found in very low abundances in the first two investigated years, but as of 2002 a significant increase could be observed ($P<0.05$).

In total, 54 species of gamasid mites from 35 genera and 19 families were identified (Table 1). Over all four years of this study the windthrow sites and the reference sites show similar species numbers, viz. circa 30 (Fig. 3). In the soil samples of site RP, 20 species were found. Species number decreased significantly ($P<0.05$) from 23 (2000) to 13 species (2002, 2003) at WT I, but it was relatively stable at WT II, at the two reference sites, and at the RP site. However, with regard to the species composition and dominance structures of the sites remarkable changes occurred (Fig. 4A-E). Only the dominance structure of the spruce site seemed to be relatively stable. The dominance diagrams of the windthrow sites, the pine site, and, above all, the RP site, indicate that some species disappeared one or two years after the windthrow event, while other species first appeared in 2001 or 2002. At the RP site the small gamasid species (<600 µm), which were caught exclusively in 2000, were somehow replaced in the years thereafter by larger species, e.g. of the genus *Pergamasus* (>1000 µm). Moreover, the dominance of *Uropoda minima* strongly increased at this site. At WT II and at the two reference sites this species could be observed in higher dominance in 2002, while at WT I its dominance was continuously decreasing over the years. At both windthrow sites the dominances of parasitid species, such as *Paragamasus runcatellus*, *P. Conus*, *P. Robustus*, and *Pergamasus septentrionalis* (WT II), decreased clearly in 2002 and 2003. Contrary to this, at WT I and WT II the common species *Veigaia nemorensis* appeared in higher dominance after one (WT I) or rather after two years (WT II). At WT II *Polyaspinus cylindricus,* the most abundant species in 2000, showed distinctly lower dominance in 2001 and 2002, but it became highly dominant at WT I over the years.

The dominance similarities show that the gamasid mite communities of the two windthrow sites differed very much from that of the reference sites during the first 2 years of this investigation (Fig. 5A,B). But in 2002 and 2003, species composition at the windthrow sites, especially WT II, became

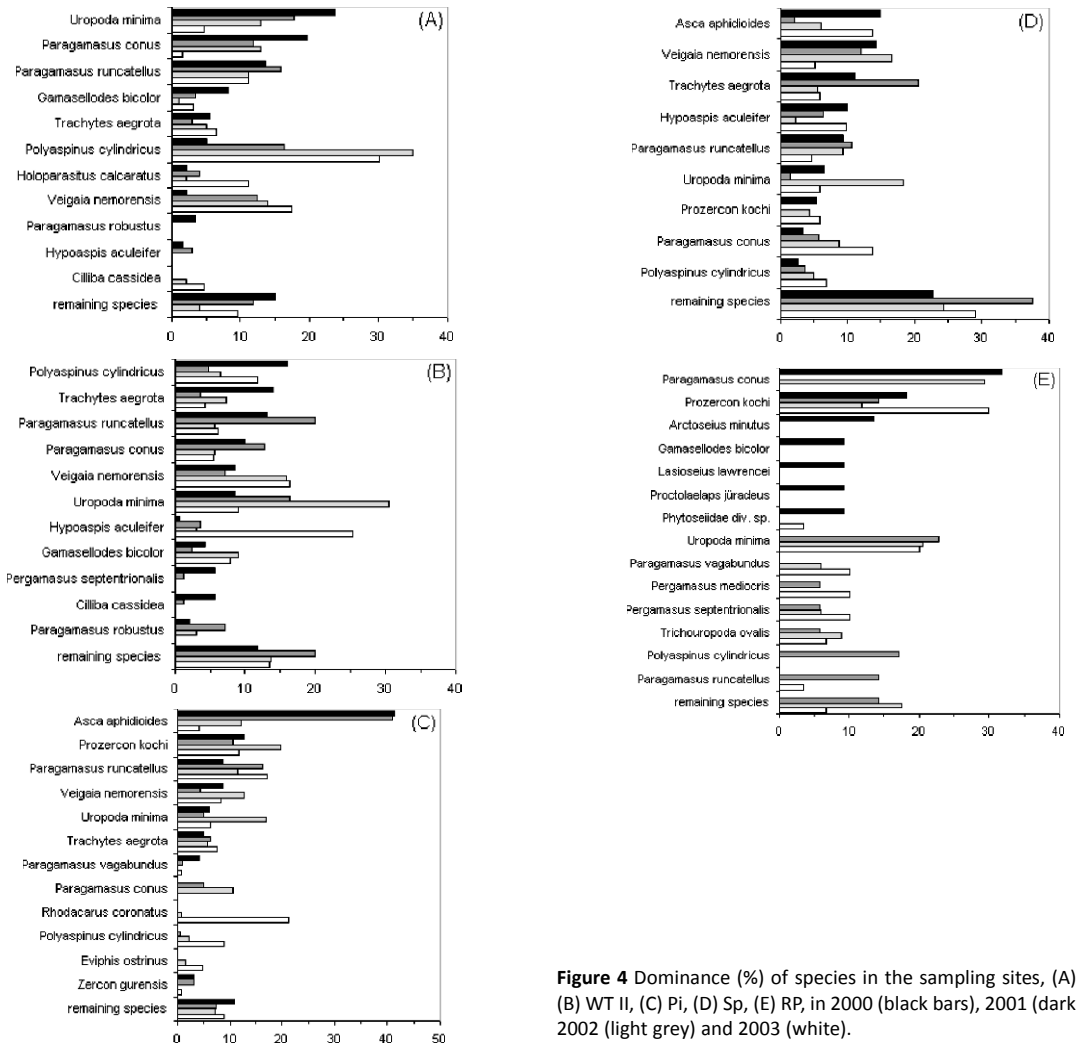

Figure 4 Dominance (%) of species in the sampling sites, (A) WT I, (B) WT II, (C) Pi, (D) Sp, (E) RP, in 2000 (black bars), 2001 (dark grey), 2002 (light grey) and 2003 (white).

gradually more similar to the reference sites (Fig. 5C,D). Species composition at the RP site was, except for 2001, very different from that at the other sites.

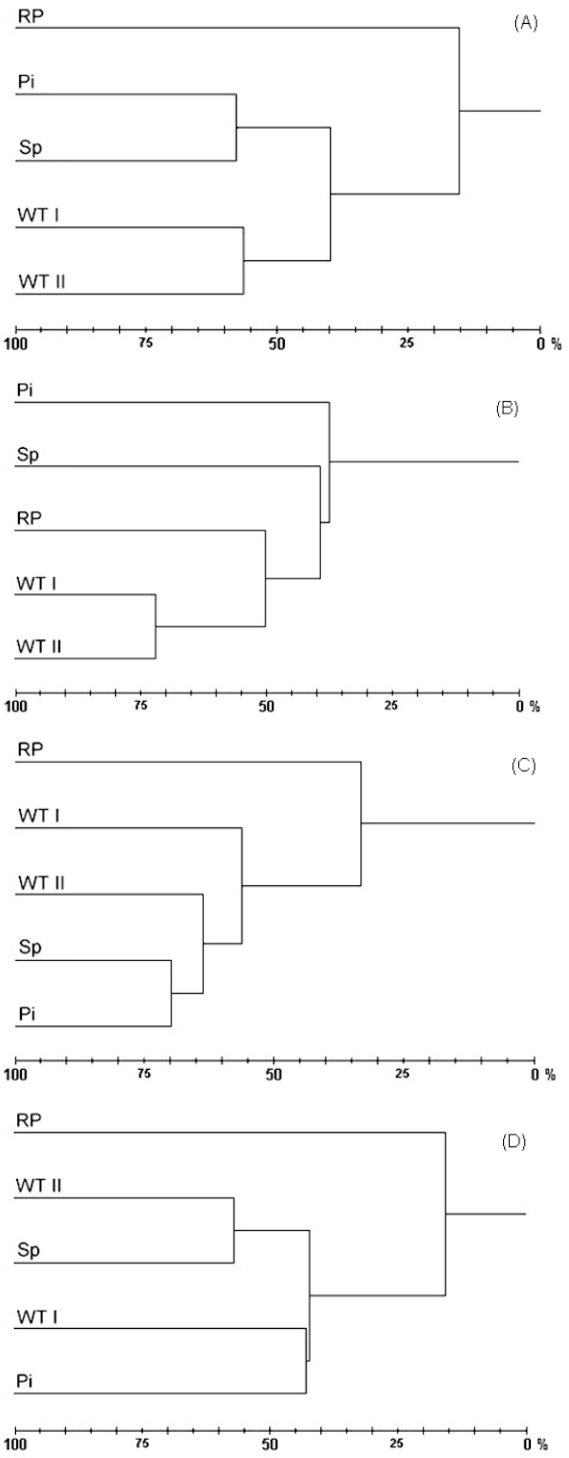

Figure 5 Dendrograms of the dominance similarities (Renkonen-Index) between the sampling sites in (A) 2000, (B) 2001, (C) 2002, (D) 2003.

DISCUSSION

The windthrow event in December 1999 caused considerable disturbance of the predatory mite communities. The structure of the gamasid mite community in the destroyed pine-spruce sites changed over time as well as in comparison with the undisturbed sites. However, the effects were most apparent in the central part of the windthrow area (WT I). The significant decrease of the abundance and of the species number from 2000 to 2003 in this site suggests that the conditions became worse for the soil dwelling gamasid mites. This could be due to a decrease of food resources in this destroyed site, as it was observed for the Collembola, the main prey for many gamasid species, especially for the Parasitidae. For example, this may explain the dominance decrease of *P. runcatellus* and *P. conus*. Strong correlations between the distribution of gamasid mites and Collembola in different forest type sites in north-eastern Germany were reported by Wegener (2006).

The significant abundance decrease of the Collembola in WT I over time, could result from microclimatic changes within the habitat after the windthrow event, since the crown layer, which normally absorbes a large part of solar radiation, has been partly removed (Bogenrieder et al., 1998). This will also have lead to changes in water balance and decomposition processes (Fischer et al., 1998), which are crucial parameters for the distribution of most collembolan species.

A smaller supply of food resources in WT I could have caused the decrease of *U. minima*. According to Hutu (1982) this species tends to avoid food scarcity. The general decrease in abundance of this dominant species and the putative reduction in food competition at this site of the windthrow area, seemed to have a positive influence on very common species like *V. nemorensis*, which is also often found in arable soils and meadows (Karg, 1993).

The most unstable and inhospitable conditions may be present in the soil in front of the uprooted spruce root plate. This is indicated by the very slow colonization of this habitat over the years, by its very uneven dominance structure, and by its dissimilarity from the other sites, as can be inferred from the Renkonen-indices. The early successional stage of this site shortly after removal of the spruce could also be inferred from the increased abundance of *U. minima*, an r-strategist according to Athias-Binche (1987). Alternatively, the increase of this species as well as that of the Parasitidae species as off 2001 could also reflect a rapid colonization of this habitat due to increased supply of potential prey such as Collembola.

The dominance development of *P. cylindricus* is noteworthy. In 2000 this species was most numerous at the border of the windthrow area, it showed a remarkable increase of dominance in the central part. According to Błoszyk & Athias-Binche (1998), this species feeds on fungal hyphae and probably other organic liquid substrates. Therefore, fungi that colonize dead wood could be an important food source for this species in the windthrow area. Accordingly, this species was also found abundantly in rotten tree trunks.

As can be seen from the Renkonen-indices, the shifts in dominance in the gamasid mite communities were most obvious in the first two years following the windthrow event. However, the dominance structures of the gamasid mites between the marginal windthrow site, WT II, and the reference sites became more similar, as of the third year. At the border of the windthrow area, close to the unaffected forest

stands, the effects seemed to wane. This must have contributed to the re-increase of the abundance of the gamasid mites in WT II in 2003.

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Succession of oribatid fauna (Acari, Oribatida) in fallen spruce trees: Deadwood promotes species and functional diversity

Piotr Skubala & Magdalena Maslak
University of Silesia, Department of Ecology, 40-007 Katowice, Bankowa 9, Poland. E-mail: piotr.skubala@us.edu.pl

The purpose of this study was to contribute to our understanding of oribatid mites inhabiting fallen logs, and to study successional patterns in Oribatida associated with deadwood. This research was carried out in the spruce forest in the Babia Góra National Park, Carpathian Mountains. Per season, five samples of decaying wood were taken from five downed spruce logs of similar size. Forty soil and litter samples were additionally collected around logs. Overall 55,723 specimens of oribatid mites belonging to 131 species were collected in 140 samples. Spruce deadwood is settled by a rich mite fauna. It becomes a more rewarding food resource for mites as they tend to increase with log age, although the maximum density was observed in log IV (i.e., the last but one decay class). It was both surprising and interesting to find that oribatid mite fauna in deadwood is not depauperated in comparison with forest soil. For oribatid mites logs are a separate habitat rather than simply an extension of the forest floor. Some mite species may specialize on deadwood, because 55 species (of 131 in total) were obligate members of the intra-log community. The structuring forces that influence the oribatid communities in their successional stages of decaying wood were variable and dependent on the stage of wood decay. Against our expectations wood feeders did not prefer deadwood over soil habitat, whereas eurytopic and parthenogenetic species did not constitute a major part of the oribatid community in pioneer stages of wood decay.

Key words: Deadwood, logs, oribatid mites, succession, forest

Deadwood is an important element of forest ecosystems. It influences the geomorphological processes (erosion, pit and mound dynamics), is a carbon and nutrient storage, and it is essential for a lot of deadwood-dwelling organisms (Maser & Trappe, 1984; Harmon et al., 1986). There are many kinds of decaying wood in forest, e.g., small twigs or logs, standing or lying tree trunks, and stumps. Although the importance of deadwood in forest ecosystems has been reiterated in the 1980s (Maser & Trappe, 1984; Bobiec et al., 2005), data on the organisms associated with deadwood accumulate far slower than data on organisms surviving cutting of old-growth trees and removal of coarse woody debris (CWD) from forests.

During the last century, deadwood has been removed from most of European forests. Nowadays, in Central European forests under conventional management, the average volume of deadwood is less than 3 m^3/ha. This is in clear contrast to the volume of deadwood found in similar natural forests, where it ranged from 50 to 120 m^3/ha (Bobiec et al., 2005). Removal of deadwood in traditionally managed forests poses serious challenges for many forest-dwelling species to survive, as reflected by the number of threatened species in these habitats. The most strongly affected organisms are saproxylic species, which during some part of their life cycle depend on deadwood. Among these species, beetles are comparatively well studied (Speight, 1989), whereas many groups of forest species that are not strictly dependent on decaying wood have remained practically unstudied (Martikainen et al., 2000). As regards mites, they were usually omitted from studies on decaying wood. Fager (1968), Johnston & Crossley (1993), Paviour-Smith & Elbourn (1993), Seastedt et al. (1989), and Travé (2003) are the only more detailed studies on Acari associated with deadwood. This is surprising, given the number of studies on forest floor mites, in which authors focussed mainly on soil and litter. Oribatid mites are typical inhabitants of forest soil and litter. Given the exceptional abundance and high diversity of oribatid mites it is safe to assume that it is difficult to find an ecological process in which they do not play a significant role.

Decomposition of downed logs (the main kind of CWD) takes many years, as a result of their low nutrient content (Hövemeyer & Schauerman, 2003). A spruce log decomposes in 80 years or more (Holeksa, 1998) and it changes during this process, thereby providing a variety of habitats for mites. This environment allows for the study of oribatid mite succession and for the estimation of its share in biodiversity of micro-arthropods in the forest. In particular, the following five questions were addressed: (1) What is – and what variables best explain – the Oribatida species diversity and abundance in downed logs of different age? (2) Is the decaying wood of fallen logs a richer habitat than soil and litter? (3) Are some oribatid species obligate members of the intra-log community? (4) How well are arboreal and xylophagous species represented in decaying logs? (5) Do eurytopic and parthenogenetic species compose the major part of oribatid communities in the youngest stages of decaying wood?

MATERIALS AND METHODS

Sampling site

The research was conducted in the spruce forest (*Plagiothecio-Piceetum tatricum typicum*) in the Babia Góra National Park, Carpathian Mountains, southern Poland, next to the Slovakian border. A 0.25-ha plot (50 × 50 m) was selected in the best preserved fragment of the old-growth spruce forest. The study area lies in the upper montane belt at an elevation of 1,180 m a.s.l. (49°40'N, 19°33'E), on the northern hillside (10° inclination). The stand is dominated by Norway spruce,

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Picea abies (L.) Karst. The amount of deadwood in the area is about 84 m^3/ha (Holeksa, 1998).

Samples and statistical analysis

Five samples of decaying wood were taken from five downed spruce logs of similar size (length ca. 12 m, diameter ca. 50 cm at the wider end). Logs were classified into five decay classes using a degree scale by Pyle & Brown (1998), ranking from freshly dead trees (log I) to completely soft logs overgrown by forest-floor mosses (log V). Logs were situated in the same characteristic patch of vegetation (with *Vaccinium vitis-idea* as the main species) and had the same exposition. Each sample contained bark, bast, sapwood, and hardwood. Woody material was collected from the top, middle, and near the bottom of each log. The average dry weight of the wood samples was about 18 g. Forty soil and litter samples (18 cm^2 surface, 7.5 cm deep) were additionally collected from the homogeneous site in the nearest surroundings of the logs. Deadwood and soil samples were taken seasonally, every 3 months, from August 2004 until summer 2005. Additionally, five samples of decaying wood from each log and five soil samples were collected per season for analyses. Mites were extracted for 7 days (until thoroughly dry) in a modified Tullgren extractor. Adults of oribatid mites were identified to morphotaxa. Overall 55,723 specimens of oribatid mites belonging to 131 species were collected in 140 samples, 71.7% of the animals originated from decaying wood.

Eight environmental descriptors have been taken into account: moisture, pH in H_2O, density, temperature fluctuations, bark cover, plant cover, number of plant species, and presence of earthworms. They were measured using standard methods (Odór & Standovár, 2003; Bednarek et al., 2004). We estimated the proportion of four ecological groups of oribatid mites (xylophagous, arboreal, eurytopic, and parthenogenetic) in communities according to Behan-Pelletier & Winchester (1998), Luxton (1972), Norton & Palmer (1991), Schatz (1983) and Weigmann (2006).

Descriptive and univariate statistics were performed using Microsoft® Excel 1997. The differences in abundance of oribatid mites between sites, between collection dates (seasons), and site*date interactions were tested by two-dimensional analysis of variance (ANOVA). Data were log-transformed to approximate assumptions of parametric statistics. When a significant effect ($P<0.05$) was noted, differing pairs were identified with the LSD post-hoc test. Non-parametric ANOVA was done on proportions of ecological groups in communities. Analyses were performed using STATISTICA 5.0. The index of faunistic originality (IFO) (Ejsmont-Karabin, 1995) was used to describe proportion of exclusive and rare species in oribatid communities: $IFO = [\Sigma 1/m_j]/s$, with m_j representing the number of samples with i species, and s the total number of species.

A canonical correspondence analysis (CCA) was employed in this study, using CANOCO 4.02 and MVSP 3.1. Rare species (occurring in less than five samples) were omitted because they do not improve the CCA analysis, as was confirmed in an initial analysis with all species. The numbers of individuals were log(x+1)-transformed. A Monte Carlo permutation test was used to test statistically whether the species composition was related to distinct environmental factors. Pearson's correlation was applied in the analyses of the relationships between distinct environmental descriptors and the abundance of each of the dominant species that were included in the CCA analysis.

RESULTS

The overall abundance of oribatid mites increased with the age of decaying wood. Peak values [2,633 individuals/100 g dw (dry matter)] occurred in decay class log IV (Table 1). The abundance of oribatids in log V was lower than in log IV (1,687 ind./100 g dw). Deadwood (log I) already initially contained quite high numbers of oribatids (468 ind./100 g dw). The number of oribatids from the top 7.5 cm of soil and litter of the adjacent forest floor (2,388 ind./100 g dw) was similar for logs II-IV (ANOVA: $F = 16.96$, $P>0.05$). The tendencies for adults and juveniles separately were similar, although the highest abundance of juveniles was noted in log II. Two-way ANOVA revealed also significant differences in oribatid abundance between seasons and within the interaction of sites and seasons at all studied sites (Table 1).

A general trend towards increased numbers of species from decay class I (56 spp) to decay class IV and V (85 spp) was evident (Table 2). In general, 126 oribatid species were recorded in decaying wood of downed logs, whereas the number of species found in soil and litter was lower (76 spp).

Table 1 Mean abundances of oribatid mites and other groups of mesofauna in logs (I-V) and soil + litter. Abundance of oribatids (± SE; n = 20) is tested by 2-way ANOVA. Mean abundances (no. individuals/100 g dw) are compared by the LSD post-hoc test to test for differences between sites. Mean abundance of other mircroarthropods was compared with 1-way ANOVA and LSD test.

	Log I	Log II	Log III	Log IV	Log V	Soil + litter	F-ratio	P
Abundance in							*2-way ANOVA*	
sites	468 ± 224a	2,253 ± 1,012c	2,243 ± 1,449c	2,633 ± 1,815c	1,687 ± 1,472b	2,388 ± 1,893c	16.96	<0.01
seasons							2.24	0.09
sites*seasons							3.08	<0.01
Abundance of								
adults	239 ± 109a	999 ± 701c	1,273 ± 987c,d	1,564 ± 1,122d	835 ± 925b	1,093 ± 843c,d	18.43	<0.01
seasons							3.84	0.01
sites*seasons							3.57	<0.01
Abundance of								
juveniles	228 ± 164a	1,254 ± 1,030b,c	969 ± 567b,c	1,069 ± 1,193b,c	852 ± 943b	1,294 ± 1,413c	9.42	<0.01
seasons							14.22	<0.01
sites*seasons							2.46	<0.01
Other microarthropods							*1-way ANOVA*	
Gamasida	35 ± 37a	281 ± 305c	78 ± 79b	34 ± 25a	31 ± 35a	121 ± 166c	13.72	<0.01
Actinedida	114 ± 37b	332 ± 228d	201 ± 267c	75 ± 55a	64 ± 51a	99 ± 125a,b	7.41	<0.01
Acaridida	4 ± 9a	15 ± 22a	25 ± 32a	81 ± 123b	22 ± 40a	136 ± 318c	34.01	<0.01
Collembola	954 ± 866b	2,692 ± 1,170c	703 ± 551a,b	552 ± 845a	433 ± 391a	473 ± 427a	12.90	<0.01

Means followed by a different letter within a row differ significantly (LSD test, $P<0.05$).

Table 2 Species richness of oribatid communities in deadwood and in soil + litter. IFO = index of faunistic originality (Ejsmont-Karabin, 1995; see Materials and Methods section).

	No. species in sample			
	Total	Mean	Exclusive spp.	IFO
Log I	56	14.6 ± 4.7a	4	0.37
Log II	64	19.2 ± 6.9b,c	3	0.51
Log III	74	17.9 ± 5.6a,b	4	0.45
Log IV	85	22.8 ± 9.5c	6	0.53
Log V	85	21.7 ± 10.7b,c	7	0.48
Soil + litter	76	20.5 ± 6.3b,c	5	0.45

Means followed by a different letter differ significantly (LSD test, P<0.05).

The mean number of species per sample was highest in log IV (22.8), but this was not significantly higher than in log II, V, or soil + litter (ANOVA: F = 3.25, P>0.05). The highest value of the index of faunistic originality was recorded in log IV (0.53), whereas the highest number of exclusive species was noted in log V (Table 2). Out of a total of 131 species, 55 species occurred exclusively in deadwood, whereas only five species were restricted to soil and litter.

The canonical correspondence analysis yielded eigenvalues of axes 1 and 2 of λ_1 = 0.301 and λ_2 = 0.095, respectively (Fig. 1). The Monte Carlo permutation test proved that axis 1 is statistically important (F = 4.72, P = 0.004). More than 71% of the variance was explained by the first two axes, so there is little need to bother about further axes. Overall, axis 1 appears to reflect a gradient from young (right) to older logs (left) (Fig. 1). More specifically, axis 1 represents a bark-cover, plant-cover, and earthworm-presence gradient (intraset correlation = 0.93, -0.96, and -0.83, respectively). However, correlations for temperature, density, and pH are only slightly weaker. Axis 2 represents a water content gradient (intraset correlation: 0.54).

The emerging patterns of individual species are: Quadrant I contains several species abundant in the youngest log (I), e.g., *Anachipteria deficiens* Grandjean (dominant, >5% of all individuals), *Caleremaeus monilipes* (Michael) (dominant), *Autogneta longilamellata* (Michael), *Cepheus grandis* Sitnikova, and *Carabodes femoralis* (Nicolet). They may be positively favoured by bark cover and high density of wood. The Pearson correlation coefficients for *A. deficiens* and bark cover and density were statistically significant (r = 0.951 and 0.815, respectively; P<0.05) (Fig. 1, Table 3). Similarly, correlation between *C. monilipes* abundance and bark cover was statistically important (r = 0.849, P<0.05).

Quadrant II contains the most abundant species on logs of intermediate stages of decomposition (II and III). *Carabodes tenuis* Forsslund (dominant), *Melanozetes meridianus* Sellnick (dominant), *Eupthiracarus monodactylus* (Willmann), and *Autogneta parva* Forsslund occurred abundantly in both logs. *Oppiella (O.) maritima* (Willmann) (dominant) and *Suctobelbata prelli* (Märkel & Meyer, 1958) were most abundant in log II. These species were mostly influenced by pH and temperature. The Pearson correlation coefficient for *M. meridianus* and pH was statistically significant (r = 0.905, P<0.05), whereas for *O. maritima* correlations between abundance and number of plant species (r = -0.879) and between abundance and temperature (r = 0.900) were significant (Fig. 1, Table 3).

Steganacarus (Atropacarus) striculus (C.L. Koch) (dominant), *Quadroppia quadricarinata* (Michael) (dominant), *Berniniella conjuncta* (Strenzke), *Oppiella (Moritzoppia) neerlandica* (Oudemans), and *Suctobelbella falcata* (Forsslund) (quadrant III) responded positively to older logs (IV and

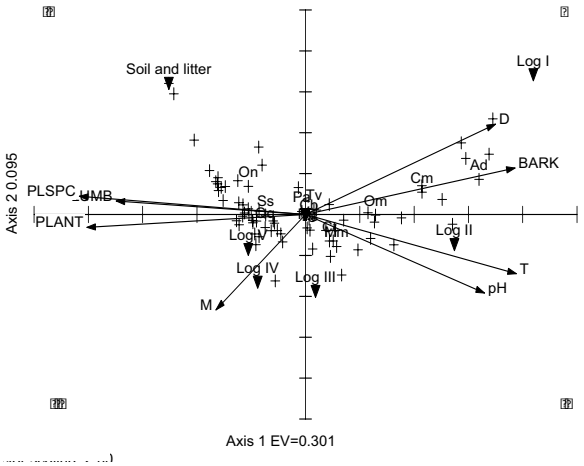

Figure 1 Canonical Correspondence Analysis (CCA) biplots showing species and site arrangements in relation to environmental factors. Triangles indicate the centroid position of the five logs and soil + litter. Scores were scaled by species (Hill's scaling). Codes: see Table 3 (codes are only marked for dominant species); EV = eigenvalues.

Table 3 Relationship between environmental factors and abundance of dominant oribatid species associated with decaying logs and soil. Pearson correlation coefficients are given; values statistically significant (P<0.05) are printed in bold.

	Moisture	pH	Density	Temp. fluctuations	Bark cover	Plant cover	No. plant species	Presence of earth worms
	[M]	[pH]	[D]	[T]	[BARK]	[PLANT]	[PLSPC]	[LUMB]
Anachipteria deficiens [Ad]	-0.608	-0.055	**0.815**	0.765	**0.951**	**-0.950**	**-0.864**	-0.758
Steganacarus (A.) striculus [Ss]	0.422	0.282	**-0.891**	**-0.896**	**-0.932**	**0.936**	**0.974**	0.783
Caleremaeus monilipes [Cm]	-0.233	-0.063	0.729	0.805	**0.849**	**-0.818**	**-0.853**	-0.571
Carabodes tenuis [Ct]	0.607	0.800	-0.442	0.344	-0.250	0.081	-0.107	-0.059
Chamobates borealis [Cb]	0.166	0.754	-0.709	-0.294	-0.603	0.420	0.459	0.137
Fuscozetes setosus [Fs]	0.372	**0.904**	-0.715	-0.057	-0.570	0.346	0.290	0.058
Hermannia gibba [Hg]	0.169	**0.823**	-0.599	-0.129	-0.403	0.244	0.279	0.034
Oppiella (O.) maritima [Om]	-0.129	0.171	0.531	**0.900**	0.712	-0.789	**-0.879**	-0.745
Melanozetes meridianus [Mm]	0.290	**0.905**	-0.319	0.454	-0.101	-0126	-0.229	-0.301
Oppiella(O.) nova [On]	-0.055	-0.216	-0.471	**-0.905**	-0.663	0.720	**0.858**	0.632
Phthiracarus anonymus [Pa]	0.068	0.480	-0.646	-0.686	-0.553	0.559	0.720	0.521
Quadroppia quadricarinata [Qq]	0.786	0.364	**-0.832**	-0.713	-0.744	**0.851**	0.797	0.405
Tectocepheus velatus velatus [Tv]	0.106	0.551	-0.724	0.342	-0.662	0.562	0.660	-0.564

In brackets are codes of species and environmental factors (see also Fig. 1).

V). The species may be favoured by high water content and high plant cover. Both dominant species (*S. striculus* and *Q. quadricarinata*) were strongly correlated with plant cover (r = 0.936 and 0.851, respectively) (Fig. 1, Table 3).

Quadrant IV represents species associated with soil and litter, that are absent or occurring in low numbers in deadwood. There were only few such species, e.g., *Oppiella (O.) nova* (Oudemans) (dominant), *Oppiella (Rhinoppia) fallax* (Paoli), and *Berniniella hauseri* (Mahunka). They may be favoured by low pH and low temperature (Fig. 1, Table 3).

Several dominant species, e.g., *Chamobates borealis* (Trägardh), *Fuscozetes setosus* (C.L. Koch), *Hermannia gibba* (C.L. Koch), *Phthiracarus anonymus* Grandjean, and *Tectocepheus velatus velatus* (Michael), were concentrated around the intersection point of the two axes. These species show no preference for any site (Fig. 1).

In the habitats studied, eleven species are regarded arboreal, a.o., *C. monilipes*, *C. labyrinthicus*, *Dometorina plantivaga* (Berlese), and *Licneremeus licnophorus* (Michael). The proportion of these species was highest in the youngest log (20% of total number), whereas in other logs and soil it was below 10%. The differences among communities were significant (Kruskal-Wallis analysis) (Fig. 2). In total 12 xylophagous species were recorded in the study [i.e., representatives of the Phthiracaridae and Cepheidae, *H. gibba*, *C. Monilipes*, and *Carabodes labyrinthicus* (Michael)]. The proportion of species that appear to feed on decaying wood was higher in soil and litter (32%) than in deadwood, where it ranged from 11 (log IV) to 28% (log I) (Fig. 2).

Twenty-four species recorded in the spruce forest are considered eurytopic. The highest proportion of species was noted in log V and soil (32 and 46% of total, respectively). Eurytopic species occurred in significantly lower number in the youngest logs (Fig. 3). Twenty-eight parthenogenetic species were noted in the study. Four dominant species (*S. striculus*, *O. nova*, *P. anonymus*, and *T. velatus*) are characterized by this mode of reproduction. The proportion of parthenogenetic species was highest in soil and litter (67% of total), in deadwood it varied from 13 (log I) to 35% (log V). The differences among the proportion of parthenogenetic species between sites were significant (Kruskal-Wallis analysis) (Fig. 3).

The micro-arthropod fauna of spruce decaying wood is dominated by oribatid mites. In deadwood samples they accounted for 75% (log I) to >93% (logs IV and V) of all mites. The proportion of oribatid mites was similar in soil and litter (87%). Other mites, e.g., Actinedida and Gamasida, were most numerous in earlier stages of decaying wood (peak values in log II) (Table 1). The differences in abundance of other groups of mites among logs and soil + litter were statistically significant. With regard to springtails (Collembola), the most abundant non-mite soil micro-arthropods, only in the youngest downed logs (logs I and II) they were more numerous than oribatid mites.

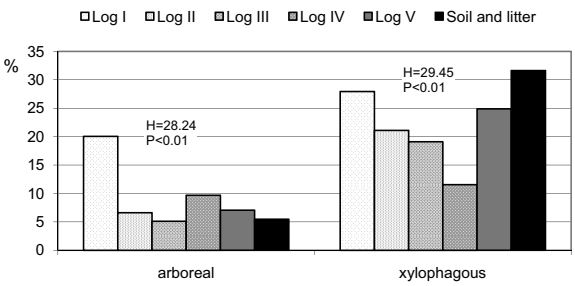

Figure 2 Proportion of arboreal and xylophagous species in oribatid mite communities in logs I-V and in soil + litter. The test results on the panels refer to Kruskal-Wallis ANOVA.

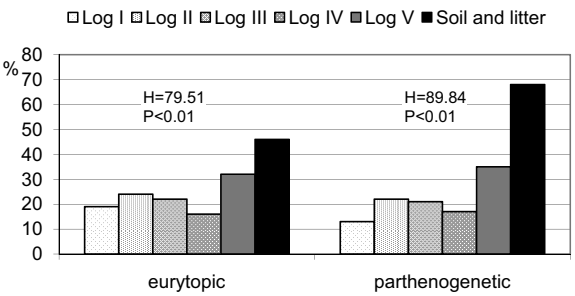

Figure 3 Proportion of eurytopic and parthenogenetic species in oribatid mite communities in logs I-V and in soil + litter. The test results on the panels refer to Kruskal-Wallis ANOVA.

DISCUSSION

Oribatids are among the most characteristic elements of soil fauna. They play a potentially important role in ground-related biotopes, e.g., in the decomposition of wood (Travé, 2003). Seastedt et al. (1989) remarked that, in conjunction with physical fragmentation, the effects of micro-arthropods on microbial processes may be most important in robust substrates such as wood.

Our present study revealed unexpected abundance and diversity of mites in spruce deadwood. Strong differentiation between oribatid communities was observed in logs of different decay stages. Spruce deadwood apparently becomes a more rewarding food resource for mites in the course of decomposition, because they tend to increase in numbers with log age. However, the difference in abundance between logs II, III, and IV was not significant. Also, the number of species appeared to increase throughout the course of succession, indicating that resource heterogeneity increased with log age. Many oribatids are fungivorous – the number of fungi increased during decomposition of wood and the highest diversity of fungi is observed in logs III and IV (Bader et al., 1995). Similarly, the maximum density of mites was observed in log IV in the current study. The number of species was also the highest in log IV, but the same as in log V. Such a trend might be expected because the remaining material from the oldest log (V) will not become more heterogeneous than from log IV. A general trend of increasing abundance with decaying wood age was previously observed by Hövemeyer & Schauermann (2003), Irmler et al. (1996), Braccia & Batzer (2001), and Abbott & Crossley (1982) for different groups of invertebrates. Maser & Trappe (1984) stressed that the mite fauna began to flourish as a fallen tree approached class IV. On the other hand, Seastedt et al. (1989) found peak values of micro-arthropods in decay class II logs, but values were not statistically different from older decay classes.

It was both surprising and interesting to find that oribatid mite fauna in deadwood is not depauperated in comparison with forest soil and litter. It was not simply a subset of the soil fauna. Deadwood was richer in species (126) than soil (76), whereas densities in logs II-IV were not statistically different from the density in soil and litter. Other authors described the mite fauna in wood as being depauperated in compari-

son with forest litter and soil. Seastedt et al. (1989) recorded 2-10 times lower densities of micro-arthropods in decaying wood than in an equivalent amount of litter and soil. Similarly, the number of species was twice as low in CWD as in litter; they recorded only 22 oribatid species inside decaying wood. Also Fager (1968) found 26 species of oribatids in decaying logs. However, Johnston & Crossley (1993) recorded 60 species in CWD. Paviour-Smith & Elbourn (1993) found 44 species in aerial deadwood on deciduous trees and Travé (2003) noted 68 species in decomposing wood. Nevertheless, mite species diversity in wood was lower than in litter (Johnston & Crossley 1993). Positive influence of the presence of downed logs in forests on the abundance of oribatid mites was observed by Evans et al. (2003). These authors associated this with the increased density of woody fragments which may contain a number of specialized fungi. This aspect warrants further detailed studies.

Succession and structural breakdown of wood is certainly influenced by many factors and a temporal and spatial differentiation of ecological niches is observed during decay of wood (Irmler et al., 1996). Bark cover and density of substrate appeared the most important environmental variables which influenced the formation of mite communities in downed logs of pioneer stages. Mite fauna settled in older spruce logs (IV and V) was strictly connected with high plant cover. *Anachipteria deficiens* and *C. monilipes* were noted in the colonisation phase (log I) as the dominant species. The frequent occurrence of *A. deficiens* is surprising as it is regarded as a hygrophilous mite, mainly occurring in peatbogs (Weigmann, 2006). It is a species of European distribution (Weigmann, 2006), recorded for the first time in Poland. As regards *C. monilipes*, the species is regarded as a forest, arboricol species (Weigmann, 2006), previously recorded only in two regions in Poland (Olszanowski et al., 1996). *Oppiella (O.) maritima*, *C. tenuis*, and *M. meridianus* were frequently recorded in logs of intermediate stages of decay (logs II and III). *Oppiella maritima* was noted mainly in coniferous forests, *C. tenuis* is known as a forest, montane species, whereas *M. meridianus* is described as a tyrphobiont and hygrophilous mite (Schatz, 1983; Weigmann, 2006). These three species were rarely recorded in Poland so far (Olszanowski et al., 1996). *Steganacarus (A.) striculus* and *Q. quadricarinata* were the most frequent dwellers of logs in the humification phase (logs IV and V). Both species are noted in different biotopes and are frequently noted in Poland. (Olszanowski et al., 1996; Weigmann, 2006).

Strong differences were observed between oribatid communities in deadwood vs. soil and litter in the current study, as was indicated by CCA analysis. It may be concluded that oribatid mites are using logs as a completely separate habitat rather than as an extension of the forest floor. Previously, authors pointed at the major importance of large branches and fallen logs in forest floor habitats as a refuge for mite species normally occurring in litter or soil (e.g., Johnston & Crossley, 1993). However, they also found some species use CWD exclusively. Seastedt et al. (1989) recorded members of the Oppiidae and Suctobelbidae as the most abundant in decaying wood and they were also the most abundant species found in litter and wood. Nearly all oribatid species obtained from deadwood were also found in the litter by these authors. Our results suggested that some mite species might specialize on deadwood, as 55 species (of 131 in total) were obligate members of the intra-log community. In our studies the dominant species in wood, e.g., *A. deficiens*, *C. monilipes*, *O. maritima*, and *M. meridianus*, were not generally the same species that dominate the fauna of litter and soil. It is noteworthy that 41 species are rare for the Polish fauna (36 were found in deadwood). Four species were new to the Polish fauna (*A. deficiens*, *Suctobelba regia* Moritz, *Mycobates carli* (Schweizer), and *Paratritia baloghi* Moritz). It just proved that this microhabitat was poorly studied so far.

Notwithstanding the publication of a few quantitative studies on micro-arthropods in deadwood, little information on ecological groups associated with decaying wood is available. Those species that appeared to directly ingest wood (12 species) were a minor component of the oribatid fauna in this substrate in our study. Against our expectations wood feeders did not prefer deadwood to soil habitat. Among 12 dominants in decaying logs only *C. monilipes*, *C. labyrinthicus*, *P. anonymus*, and *S. (A.) striculus* are regarded as wood-feeding mites (Luxton, 1972; Schatz, 1983). Surprisingly, the representatives of the phthiracarids, known to be common in deadwood (Seastedt et al., 1989), were more abundant in the soil than in deadwood. Johnston & Crossley (1993) noted a few wood feeders, such as *Atropacarus* sp. and *Carabodes* sp., among dominants in woody debris. This phenomenon was also observed by Seastedt et al. (1989). They found wood-feeding micro-arthropods to compose a small minority of the fauna in decaying boles. The authors concluded that oribatids were actually feeding on the microflora contained on the wood, instead of the wood itself. The arboreal species (11) were mostly occurring in the colonisation phase (log I).

New habitats are faster colonized by eurytopic species and these species are a characteristic element of pioneer mite communities (Ås et al., 1992; Skubala, 2004). Similarly parthenogenetic oribatid species dominate in newly formed habitats or disturbed environment (Norton & Palmer, 1991). Therefore, we expected that eurytopic and parthenogenetic species would compose a major part of oribatid communities in earlier stages of wood decay. However, the proportion of eurytopic and parthenogenetic oribatid species did not decrease with decay stage. This may mean that deadwood even in the initial phase of decay is still a natural microhabitat for mites.

Oribatida was the major component of the microarthropod fauna in spruce decaying wood throughout the year. Only in the youngest stages of wood decay, springtails slightly dominated oribatids. Previously, only the macroinvertebrates were considered to be important in early stages of wood decomposition (Harmon et al., 1986). Setäla et al. (1995) remarked on the important indirect contribution of Collembola to stump-decay. Oribatid mites may also play some role in decomposition of deadwood, even in the early stages of the decaying process. These mites do affect the structural integrity of the wood, and ultimately wood of decay class V is largely composed of faecal oribatid pellets.

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Effects of reforestation with conifers on the communities of mesostigmatic mites in northern Spain (Acari: Mesostigmata)

María Lourdes Moraza
Departamento de Zoología y Ecología, Facultad de Ciencias, Universidad de Navarra, C/ Irunlarrea 1, Pamplona 31008 (Navarra), España. E-mail: mlmoraza@unav.es

The effect of reforestation of *Pinus nigra* and *Pinus halepensis* in central and southern Navarra (northern Spain) on mesostigmatic mite communities inhabiting the soil was studied, and seasonal fluctuation of mite populations over 1 year of sampling. Reforestation causes a clear change in species richness of the community (71-72% drop) and a significant decrease of mite abundance (31-50% loss). There are no significant differences in overall Shannon's diversity (H) and evenness (J) when the Mediterranean shrub (H = 2.92, J = 0.66) is replaced by a pine forest (H = 2.73, J = 0.72). However, H and J significantly increased when a pine forest (H = 3.34, J = 0.84) substitutes the natural oak forest (H = 2.80, J = 0.63). Summer is the critical, unfavorable season for Mesostigmata of this region in terms of their abundance and community diversity.

Key words: Mesostigmata, reforestation, community analysis, species diversity, seasonal dynamics

Soil fauna as a bioindicator of soil productivity, health, and stability has been considered by several authors (Cassagnau, 1961; Bonnet et al., 1976, 1979; Ponge, 1983; Gers & Izarra, 1983; Arpin et al., 1984; Arbea & Jordana, 1985, 1988; Jordana et al., 1987; Lindel et al., 1994; van Straalen, 1997; Knoeppa et al., 2000). Oribatid mites have traditionally been the object of this type of study (Iturrondobeitia et al., 1997; Iturrondobeitia & Salona, 1985, 1992a,b; Arroyo et al., 2003). Attempts to link the impact of disturbance have been scarce (Maraun & Scheu, 2000; Migliorini et al., 2003; Huhta & Niemi; 2003), but Mesostigmata, as free-living acarine predators that depend on other soil fauna and on various soil parameters, have proven to be useful indicators of soil management (Siepel, 1996; Minor & Norton, 2004; Huhta & Räty, 2005). Knowledge of these mite populations in their natural habitat is essential to properly assess future changes.

The objective of this study was to sample the Mesostigmata community of the soil in natural environment and to assess fluctuations associated with seasonal changes and pine reforestation. Mites were sampled once per season (1982-1983) at various sites in Navarra, in the north of Spain.

MATERIAL AND METHODS

Sampling sites

The field study was carried out in two localities:
(1) Caparroso, Bardenas Reales (southern Navarra; UTM: 30TXM1184; altitude 300 m). Two sites were chosen for sampling: (i) an area of Mediterranean shrubs (Bs) in soil of gypsum rock (torriorthent typical), with arid vegetation belonging to the *Rosmarino-Ericion* alliance and *Rosmarineto-Linetum subfruticossi* association, an endemic *Gipsophilion* alliance on the gypsum outcropping (Ursua et al., 1985), and (ii) a pine forest (Bp) with *Pinus halepensis* Miller (planted 30 years ago), at 50 m from the previous site.
(2) Sansoain (central Navarra; UTM: 30XN1513; altitude 600 m). Natural vegetation of the area is oak forest (So) made up of *Quercus rotundifolia* Lam. of the *Quercetea-ilicis* class, *Quercion-ilicis* alliance, located on a north-facing slope. On the same slope, a substituted biotope of reforested *Pinus nigra* Arnold (Sp) was selected.

Sampling

Sampling was done once every season (spring, 20.04.1982; summer, 27.07.1982; autumn, 01.12.1982 and winter, 22.02.1983). Sample surface was 25 cm^2. Samples were taken at different depths depending on the layer of litter present. The samples were extracted layer by layer (leaf litter, humus, mineral soil). Results are presented per 1 kg dry sample weight.

When samples were collected, temperature was measured at every site at the soil surface and deep in the soil, as well as the pH in distilled water on the surface and in deep soil. Soil humidity (%) was calculated from the difference between fresh and dry sample weight (i.e., after drying at 20 °C and 40% r.h.) (Table 1).

Extraction

Modified Tullgren funnels were used (Jordana et al., 1987). Specimens were identified to species level using a light microscope, after clearing in Nesbitt's solution and mounting in Hoyer's medium. A list of all species found is presented in Table 2. The specimens are deposited in the acarology collection (Acarology Laboratory) of the Ohio State University (Columbus, OH, USA).

Data treatment and statistical analysis

Shannon's diversity index (H), relative diversity or evenness (J), community and species density, and species number were calculated for all samples using PAST software, developed by Hammer et al. (2001). Differences in the diversity of

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Table 1 Physico-chemical parameters of the soil samples throughout the year.

	Parameters	Spring	Summer	Autumn	Winter
Bardenas Reales	pH shrub surface / deep	7.59 / -	6.37 / -	6.35 / 6.94	7.22 / 7.23
	pH pine surface / deep	7.30 / 7.22	5.70 / 6.42	6.35 / 7.23	6.65 / 6.68
	% H_2O shrub	10.10	19.30	17.67	23.84
	% H_2O pine	15.95	36.76	22.91	57.51
	Tª shrub surface / deep	18.2 / 15.4	21.6 / 21.9	14.7 / 12.6	12.2 / 7.9
	Tª pine surface / deep	9.8 / 9	24.9 / 19.9	14.7 / 12.6	11.8 / 8.3
Sansoain	pH oak surface / deep	6.78 / 3.69	6.16 / 4.34	6.50 / 4	7.28 / 677
	pH pine surface / deep	7.50 / 7.03	6.71 / 7.39	7 / 7	7.28 / 7.57
	% H_2O oak	33.81	26.47	35.82	34.97
	% H_2O pine	28.64	2.92	2.71	28.26
	Tª oak surface / deep	13.0 / 8.6	17.6 / 17.6	13.0 / 10	9.1 / 3.2
	Tª pine surface / deep	13.8 / 9.6	25.6 / 18.5	13.0 / 2.71	12.3 / 3.4

mesostigmatic mite communities were analyzed by calculating diversity indices for the two samples, followed by comparison of the diversities using a permutation procedure [1,000 random matrices with two columns (samples) generated, each with the same row and column totals as in the original data matrix]. Similarity between habitats was calculated applying the Manhattan distance index (algorithm UPGMA: Unweighted pair-group average) with respect to all species found.

RESULTS AND DISCUSSION

Species composition and species density

Caparroso (Bardenas Reales)

In the shrub soil, a total of 22 species of Mesostigmata (535 specimens) were collected, 46% of which are rare (with abundances <1%) and only six species appear with densities >5% (Table 2, Fig. 1). The most abundant and frequent species was *Leitneria pugio* (190 specimens), classified as an expansive species (frequency of occurrence ≥75%). *Antennoseius bacatus*, *Rhodacarus mandibularis*, *R. coronatus*, *Ruehmnenteria nanca*, and *Gaeolaelaps* sp. 3 were species of low abundance, though very frequent (75%) in this habitat. Mites are usually found in soil litter throughout the year with only a very low percentage of the community inhabiting the thin humus layer (Fig. 1).

In pine forest soil, 13 species (90 specimens) were collected (Table 2). Four species have relative abundances >5% and one is rare. *Veigaia planicola* is the expansive species (high abundance and frequency of occurrence) and *Typhlodromus lituatus* (constant species), *Zercoseius spathuliger*, and *Gaeolaelaps* sp. 2 are all frequently found in this biotope, but at lower abundance. Except in the autumn, these mites generally inhabit the litter (Aoo) and humic soil layer (H) and they descend to the mineral layer (B) in spring (Fig. 2).

Sansoain

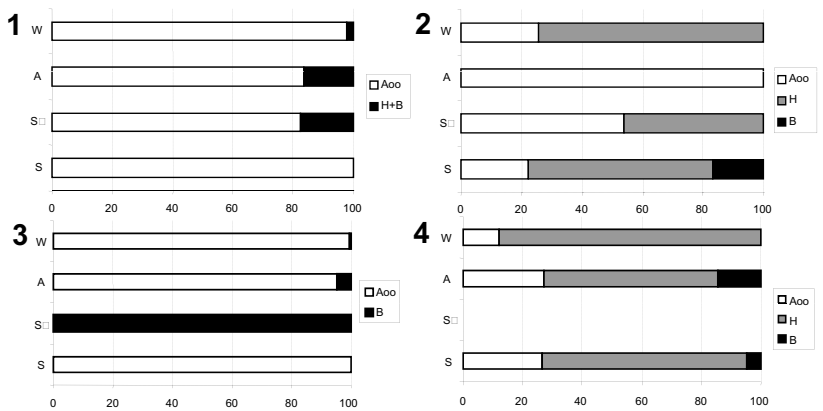
In the soil of the oak forest, a total of 21 species (543 specimens) were found, seven rare and three with relative abundances >5% (Table 2). Only *Zercon navarrensis* had a high relative abundance (52%) and frequency (75%). In this forest the community generally inhabits the surface litter, except in the summer when it migrates down to the humic layer (Fig. 3).

The pine forest soil is represented by 16 species (273 specimens), five rare and nine with relative abundances >5%. *Arctoseius* sp. is the most abundant species. Just as in the pine forests of the Bardenas Reales, the Mesostigmata prefer the humic soil layer and they colonize the mineral soil, though exhibiting low abundances (Fig. 4). The absence of Mesostigmata in the pine forest during the summer may either be due to its disappearance or to migration to deeper soil layers.

Effects of reforestation with conifer trees

The following trends in diversity indices could be noted (Table 3):

Decrease of abundance. The mesostigmatic mite community was more abundant in natural habitats throughout the year. A significant decrease of specimen abundance was observed, with losses of 31% (in Bardenas) and 50% (in Sansoain). Seasonal dynamics were similar in both reforested and natural biotopes, with the same minimum value of abundance in summer (unfavorable season).



Figures 1-4 Vertical distribution in soil of the Mesostigmata community in different seasons. (1) In Mediterranean shrub (Bs) in Bardenas. (2) In reforested pine forest (Bp) in Bardenas. (3) In oak forest (So) in Sansoain. (4) In reforested pine grove (Sp) in Sansoain. S, spring 1982; S´, summer 1982; A, autumn 1982; W, winter 1983; Aoo, litter layer; H, humic layer; B, mineral layer.

Table 2 Checklist of mesostigmatic mites, seasonal population density and overall relative abundance of Mesostigmata in the community (S, spring; S´, summer; A, autumn; W, winter).

	Caparroso (Bardenas Reales)								Sansoain								Overall relative abundance			
	Mediterranean shrub				Pine forest				Oak forest				Pine forest							
Mesostigmata	S	S´	A	W	S	S´	A	W	S	S´	A	W	S	S´	A	W	Bs	Bp	So	Sp
Amblyseius filixis Karg	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0				0.5
Amlyseius meridionalis Berl.	0	0	0	3	0	9	2	0	0	0	0	0	0	0	0	0	0.4	4.2		
Amblyseius obtusus (Koch)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0			0.2	
Amblyseius sp. 1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0.4			
Amblyseius sp. 2	0	0	12	0	0	0	0	0	0	0	5	0	0	0	0	0	1.5		0.4	
Antennoseius bacatus A.-H.	13	3	0	79	0	0	0	0	0	0	0	0	0	0	0	0	11.7			
Arctoseius minutus (Halb.)	0	0	0	0	0	0	0	0	3	0	0	5	0	0	0	0			0.6	
Arctoseius sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	27	0	19	105				23.2
Asca aphidioide (L)	0	0	0	0	0	0	0	0	47	0	9	5	0	0	0	0			4.7	
Asca nova Willmann	0	0	67	0	0	0	0	0	0	0	0	0	0	0	0	0	8.2			
Cilliba cassidea (Herm.)	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0.7			
Cosmolaelaps vacua (Mich.)	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2			
Dendrolaelaps reticulatus S.	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0		0.8		
Discourella cordieri (Berlese)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	25				4.0
Gamasellodes bicolor Berlese	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2			0.2	0.3
Gaeolaelaps aculeifer (Can.)	0	0	0	0	0	0	0	0	0	0	0	0	50	0	12	11				11.2
Gaeolaelaps sp. 1	0	3	0	0	0	0	0	5	0	0	0	3	0	0	0	0	0.4	1.9	0.2	
Gaeolaelaps sp. 2	0	34	9	0	4	9	11	0	3	4	2	11	0	0	3	0	5.3	9.3	1.5	0.5
Gaeolaelaps sp. 3	0	6	5	9	0	0	0	0	0	0	0	0	0	0	0	0	2.5			
Gaeolaelaps sp. 4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.2			
Laelaspis austriaca Sellnick	0	0	2	6	0	0	0	0	0	0	0	0	0	0	0	0	1.0			
Leitneria pugio (Karg)	244	2	0	121	0	0	0	0	0	0	0	0	1	0	0	0	45.0			0.2
Macrocheles montanus (Will.)	0	0	0	0	0	0	0	0	0	0	0	0	9	0	2	25				5.5
Pachylaelaps brachyperitrematus Koroleva	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	0	1.1			
Paragamasus navarrensis A.-H.	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0.4			
Paragamasus sp.	0	0	0	12	0	2	0	0	0	0	0	0	0	0	0	0	1.5	0.8		
Pergamasus robustus (Oud.)	0	0	0	0	0	0	0	0	15	0	10	19	0	0	0	0			3.4	
Polyaspinus cylindricus Berlese	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0			0.2	
Prozercon ornatus (Berlese)	0	0	0	0	0	0	0	0	3	0	0	24	0	0	0	0			2.4	
Prozercon sp. 1	0	0	0	0	0	0	0	0	12	0	19	0	0	0	0	0			1.9	
Pseudolaelaps doderoi Solari	0	0	0	0	0	0	0	0	12	0	0	13	0	0	0	0			2.1	
*Ruehmnenteria manca (*Berl.)	0	3	19	9	0	0	0	0	0	0	0	0	0	0	0	0	3.8			
Rhodacarus coronatus Berlese	0	13	26	3	1	0	0	5	9	11	17	16	0	0	0	0	5.2	2.3	4.1	
Rhodacarus mandibularis Berl.	0	36	30	3	0	0	2	5	0	0	0	0	16	0	4	21	8.5	2.7		6.3
Saprosecans baloghi Karg	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	64				10.3
Trachytes aegrota (CL Koch)	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0			0.5	
Trachytes eustructura H. & Z.	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0.4			
Trachytes pauperior Berlese	0	0	0	0	0	0	0	0	20	0	3	45	0	0	0	0			5.2	
Typhlodromus lituatus A.-H.	0	0	0	0	1	5	17	5	0	0	0	0	0	0	0	0		10.8		
Typhlodromus setubalis Dosse	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0			0.2	
Uropoda minima Kramer	0	0	0	0	5	0	0	5	38	0	2	66	64	0	10	25		3.9	8.2	15.2
Veigaia nemonensis (CL Koch)	0	0	0	3	0	0	0	0	6	0	36	21	31	0	3	0	0.4		4.8	5.2
Veigaia planicola (Berlese)	0	0	0	0	4	0	6	91	17	0	12	29	22	0	6	9		39.0	4.5	5.7
Zercon franzi Willmann	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0		1.9		
Zercon navarrensis Moraza	0	0	0	0	0	0	0	0	142	0	167	365	3	0	24	9			51.9	5.5
Zercon parivus Moraza	0	0	0	12	0	0	0	0	0	0	0	0	0	0	2	0	1.5			0.3
Zercon pinicola Halasková	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0		0.8		
Zercon pustulescens A.-H.	0	0	0	0	1	0	0	0	0	0	4	27	0	0	0	0		0.4	2.4	
Zercoseius spathuliger (Leo.)	0	0	0	0	6	0	20	29	6	0	0	0	21	0	7	11		21.2	0.5	6.0

Decrease of species richness. The fauna of Mesostigmata in reforested pine forests became poorer, with losses of approximately 35% of species.

Change in the species composition of the community. In Bardenas Reales, only five species with high ecological importance, of the 22 present in the Mediterranean shrub, were able to adapt to the new conditions, and nine (69%) of the 14 species present in the pine forest were not present in the natural habitat. In Sansoain, species substitution as a consequence of reforestation was significant and only six species (28%) could adapt to the pine forest soil.

Changes in Shannon's diversity and population balance. The substitution of Mediterranean shrub with pine forest in this area produces a moderate decrease of seasonal diversity in the community of Mesostigmata, at least during the favorable seasons (summer to winter), and an insignificant decrease ($P = 0.19$) in the overall Shannon's diversity, although the average value of this index is slightly higher in the reforested habitat. The eveness of the communities is higher in the reforested habitat ($J = 0.72$) and at its lowest in winter, but no significant differences are noted ($P = 0.092$). The seasonal fluctuations of the new community remain

Table 3 Seasonal and overall diversity indices of mesostigmatic mite communities in four sampled habitats from northern Spain. S, spring; S´, summer; A, autumn; W, winter; Bs, Mediterranean shrub from Bardenas Reales; Bp, pine forest from Bardenas Reales; So, oak forest from Sansoain; Sp, pine forest from Sansoain; H, Shannon's diversity index; J, eveness index.

		Bs	Bp	So	Sp
Abundance	S	259	22	345	251
	S´	102	27	15	2
	A	170	60	288	92
	W	284	150	649	307
Species richness	S	3	7	17	13
	S´	9	5	2	-
	A	8	7	13	11
	W	16	8	14	11
	Total	22	14	22	16
H	S	0.35	2.50	3.00	3.01
	S´	2.35	2.06	0.84	-
	A	2.46	2.32	2.25	3.01
	W	2.62	1.88	2.46	2.84
	Mean ± SD	1.95 ± 1.1	2.19 ± 0.3	2.14 ± 0.9	2.22 ± 0.1
	overall	2.92	2.73	2.80	3.34
J	S	0.22	0.89	0.73	0.81
	S´	0.74	0.89	0.84	-
	A	0.82	0.85	0.61	0.87
	W	0.65	0.63	0.63	0.82
	Mean ± SD	0.61 ± 0.3	0.82 ± 0.1	0.70 ± 0.1	0.83 ± 0.1
	overall	0.66	0.72	0.63	0.84

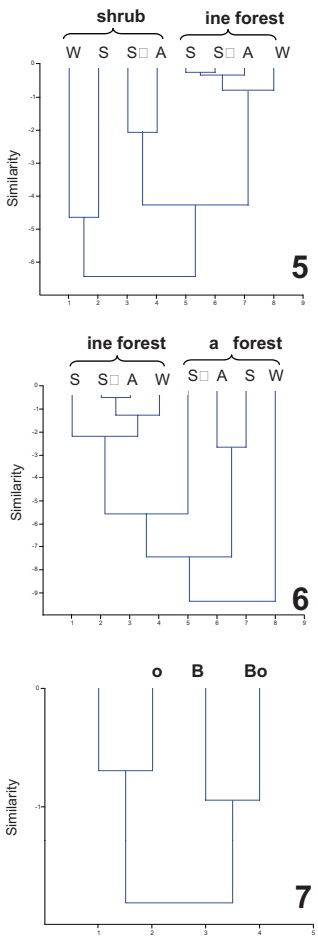


Figure 5-7 Cluster of similarity (Manhattan Index) between habitats. (5) Between seasonal samples of soil from shrub and pine forest in Caparroso (Bardenas). (6) Between seasonal samples of soil from oak and pine forest in Sansoain. (7) Between all four habitats. Bs, Mediterranean shrub in Bardenas; Bp, pine forest in Bardenas; So, oak forest in Sansoain; Sp, pine forest in Sansoain.

similar and, in fact, its stability even improves in spring and summer, possibly due to the shade provided by the pine trees which, at least in the superficial layers of the soil, results in more favourable temperatures (9-19 °C) for these mites than those in the shrub (15.4-21.9 °C).

However, when pine forest substitutes the natural oak forest (Sansoain), diversity and eveness increase. The seasonal and overall diversity and eveness are significantly higher (Perm value p = 0) in the pine forest (Table 3), except in the critical summer season. Clearly, reforestation in this habitat disrupts the stability of the community in the summer season (Fig. 4), whereas a slight increase in diversity was observed during the cooler months of autumn and winter.

The substitution of oak with pine forest (in Sansoain) led to different physico-chemical changes in the surface soil layers, compared to the substitution of shrub with pine (in Bardenas Reales). The change from oak to pine did not come with a decrease in temperature during the warmest months of spring and summer (Table 1), probably because pine and oak trees provide similar shading. Relative water availability was affected: the litter of oak retains more water than that of pine during spring and summer. Hence, in summer and autumn water stress is more pronounced in the pine forest substituting the oak forest, than in pine forest substituting the shrub vegetation.

When drawing similarities (calculations based on all Mesostigmata species found) in each of the regions (Figs. 5, 6), the communities in pine soil were clearly different from the corresponding natural forests. However, when comparing similarities among all studied habitats, the similarities between different soils of one geographic region are greater than between forest-type soils of different regions (Fig. 7).

The Mesostigmata communities of the two reforested pine forests have only five species in common, none exclusive for pine (Table 2). Apart from the summer, the seasonal and average diversity and species density in the *P. nigra* pine forest (Sansoain) are greater, whereas in terms of eveness both pine forests are similar (Table 3).

In conclusion, the Shannon's diversity estimates for the communities obtained in this study (2.73-3.34) are considered to be high values by other authors (Arroyo et. al.,, 2005). Mesostigmatic mites have population dynamics and a community structure comparable to those of other microarthropods (oribatids, collembolans) in the same biotopes (Jordana et al., 1987) and abundance of mesostigmatic mites is lowest in the summer in these habitats. The overall eveness values of ≥0.8 found in both pine forests indicate uniformity in species abundance (Magurran, 1988), and they suggest an optimal population balance (Daget, 1979). Hence, the community of Mesostigmata experience a notable 'improvement' with the pine reforestation, substituting natural habitats in this region.

Acknowledgements

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Actinedid mite community diversity in a succession gradient in continental sand-dune habitats of central Europe

David J. Russell & Gerd Alberti[1]

Senckenberg Museum of Natural History, Postfach 300 154, 02806 Görlitz, Germany. E-mail: David.Russell@senckenberg.de;
[1]Zoological Institute & Museum, University of Greifswald, Bachstr. 11-12, 17489 Greifswald, Germany

The actenidid mite communities were studied across a small-scale successional gradient in continental sandy habitats in southwest Germany. Nine sites – ranging from open sands to woodlands – were sampled between 1993 and 1996. Along the gradient, increasing vegetation corresponded significantly with increasing soil eutrophication. Species-poor communities with strong eudominances of single species occurred in the open sands. Parallel to increasing successional status, actenidid densities and species richness increased and dominances shifted strongly, with balanced dominance structures in the most developed sites. Specialized, psammophilous species were mostly limited to the open sands and short-grass sites. Eurytopic species were often limited to and dominant in the more highly eutrophied sites. Many psammophilous taxa are distributed world-wide but extremely disjunctly, occurring only in nutrient-poor, abiotically extreme psammic habitats.

Key words: Actinedida, sandy habitats, psammophilous

Ever since the publications of Coineau and colleagues (Coineau & Massoud, 1977; Coineau et al., 1978; Coineau & Seely, 1983), soil zoologists have been interested in the microarthropods of psammic habitats. Studies in many sand habitats around the world have provided lists of microarthropods (i.e., Wallwork, 1972; Santos et al., 1978; Steinberger & Whitford, 1984, 1985; Kinnear, 1991; Cepada-Pizzaro et al., 1996; Noble et al., 1996a). Most of these studies have shown that Actinedida (in the sense of the paraphyletic group comprising Prostigmata and 'Endeostigmata'; OConnor, 1984; Norton et al., 1993) are the most abundant microarthropods occurring in such habitats. André et al. (1994) found more than 1.5 million individuals m^{-2} in coastal dunes in southern France, with a dominance of undescribed prostigmatic species, mostly Microtydeids. Many new species, as well as genera and families, have been discovered in psammic habitats (i.e., Coineau et al., 1967; Théron et al., 1970; Schubart, 1973; André, 1996), most of which have been found exclusively in sandy soils.

Most reports on psammic habitats concern either the general microarthropod composition of specific habitat structures (Edney et al., 1975; Franco et al., 1979; Cepeda-Pizzaro & Whitford, 1989a; Steinberger et al., 1990) or associated with certain ecosystem functions (Santos et al., 1978; Leetham & Milchunas, 1985; Cepeda-Pizzaro et al., 1996), or have reported only taxonomic specialties (Coineau et al., 1967; Coineau & Théron, 1983; André, 1996). Few of these, however, have studied the community composition at the species level or the β-diversity of acarine communities. Except for studies in southern France (Coineau et al., 1967; Coineau & Théron, 1983; André et al., 1994), which provided no quantitative data or information on community composition, the Acari of psammic habitats in Europe have remained largely uninvestigated.

The present paper reports on the communities of Actinedida found during investigations of the microarthropod fauna in continental sand dunes in southwest Germany. The major aim was to identify changes in community composition and structure along a successional gradient (from open sands to wooded areas) in an attempt to understand the connection between habitat conditions and psammophilous microarthropod communities.

MATERIALS AND METHODS

The study took place in two environmentally protected continental dune areas ('Pt' and 'PS') in southwest Germany (in Sandhausen near Heidelberg; 8°39' E, 49°20' N). The two areas were 2.3 ha (Pt) and 19.0 ha (PS) large and approximately 2 km apart. Within these areas, sampling quadrates (5 × 5 m) within botanically well-defined plots representing homogenous psammophilous floral-society units (see Breunig, 1994) were sampled for microarthropods between 1993 and 1996. Within each dune area, the average distance between quadrates was 30-100 m. Nine sites are presented here, representing a successional gradient from open sands, short- and tall-grass sand-meadows, to wooded areas. The open sands showed only sporadic vegetation. Vegetation of the short-grass sites was diverse, yet dominated by *Koeleria glauca* (Pt) or *Corynephorus canescens* (PS), whereas that of all tall-grass sites consisted mainly of *Festuca guestfalica*. Woodlands were dominated by *Pinus sylvestris*. The specific sites and the major abiotic soil parameters are given in Table 1. Soils were all deep (approximately 5 m) aeolic sands of Pleistocene and Holocene origin and were very similar in composition (3% rough sand and gravel [>0.63 mm particle size], 47% medium sand [0.2-0.63 mm], 48% fine sand [0.063-0.2 mm] and 2% silt and clay [<63 µm]). Along the successional gradient, soil-organic-matter and plant-nutrient

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Table 1 Names, location and abiotic soil parameters of the studied sites. Pt = Pferdstriebdüne; PS = Pflege Schönau. Average soil moistures and surface temperatures were measured during microarthropod sampling.

	Open sands		Short-grass sites		Long-grass sites					Wood-land
Location (dune)	Pt	Pt	PS	PS	Pt	Pt	Pt	Pt	Pt	PS
Site	Sand (93)	Sand (96)	Sand	*Coryne-phorus*	*Koeleria* I	*Koeleria* II	*Festuca* I	*Festuca* II	*Festuca* III	*Pinus*
C_{org} [%]	0.31	0.31	0.24	0.99	0.55	1.19	1.95	2.40	2.64	7.17
N_{tot} [%]	0.028	0.027	0.014	0.071	0.030	0.085	0.136	0.190	0.206	0.359
Na^{+} [mg/kg]	0.4	0.7	0.9	1.5	0.8	2.0	0.8	1.8	2.1	4.3
Ca^{++} [mg/kg]	675	738	603	774	714	912	1193	1408	1712	2205
pH	7.57	7.46	7.62	7.07	7.38	7.11	7.04	6.42	6.15	6.09
Soil moisture [%]	3.1	19.4	2.7	4.5	5.0	23.8	7.1	25.3	13.2	14.7
Soil Temp. [°C]	18.1	15.9	20.2	19.6	17.7	19.6	18.3	14.2	15.1	16.5

content increased significantly and pH decreased (P<0.05-0.001; Table 1); thus, the spatial chronosequence also represents a gradient of increasing soil (sand) eutrophication. With increasing successional status, average soil moistures increased and surface temperatures decreased. Daytime surface temperatures in the open sands and short-grass sites often reached >50 °C (max. >70 °C) during summer.

Due to the close spatial vicinity and the absolutely protected legal status of the sampling areas, microarthropod sampling was restricted. Most quadrates presented here were only sampled in one year; the data from the open-sand site from one dune area (Pt), however, are presented for two sampling years to show successional tendencies. The quadrants were sampled three times per year, with 3-5 samples each (6.4 cm diameter, 5 cm depth). Since quantitative data were desired, the microarthropods were extracted in a high-gradient extraction device as opposed to flotation methods. The animals thus obtained were sorted under the dissecting microscope at up to 50× magnification into major arthropod groups. Actinedida were mounted in gum-chloral hydrate media ('Hoyer's fixative') and determined to the generic and, where possible, species level under a phase-contrast microscope at maximally 1,000× magnification.

Standard faunistical analyses were carried out on the data: total microarthropod densities (in individuals m^{-2}), microarthropod group composition (in % relative abundance), total Actinedida densities, species richness, and relative abundances of each taxon were assessed, all calculated as yearly averages per sampling site. Due to the non-normal distribution of soil microarthropods, differences in densities and species richness (measured as species per sample) between sites were tested for significance using a non-parametric one-way ANOVA for multiple observations (= samples) per cell (= site and sampling date) (modified Friedman test; Zar, 1999). This ANOVA is based on ranked data per sample and on the χ^2 rather than the F distribution. A post-hoc Tukey-like multiple comparison procedure for this non-parametric ANOVA (Zar, 1999) was used to test for significant differences between individual sites. The species abundances were correlated with each other and with abiotic soil parameters using a non-parametric Spearman correlation in SPSS v.10. A canonical correspondence analysis (CCA) was carried out on the environmental and individual species' abundance data using CANOCO v.4.5. Only the 60 most abundant taxa were submitted to the correlation analyses, which were carried out without data transformation and (CCA) centered on species.

RESULTS

Approximately 34,000 microarthropod individuals were recorded in the present sites. Of these, over 21,000 were actinedid mites, one third of which were 'Endeostigmata'. In the open sands and *Corynephorus* site, actinedid mites (mostly 'Endeostigmata') represented over 90% of the microarthropods (Fig. 1). In the remaining short-grass and all long-grass sites, prostigmatic taxa strongly dominated the microarthropod communities. In the woodland site, the communities were fairly evenly distributed among the major microarthropod groups (Fig. 1).

Along the successional gradient, the densities of endeostigmatic mites decreased significantly (χ^2 = 23.228, P<0.001; Fig. 2), often reaching more than 100,000 individuals m^{-2} in the open sands, while being minimal in the long-grass sites. Contrarily, prostigmatic mites increased significantly along the

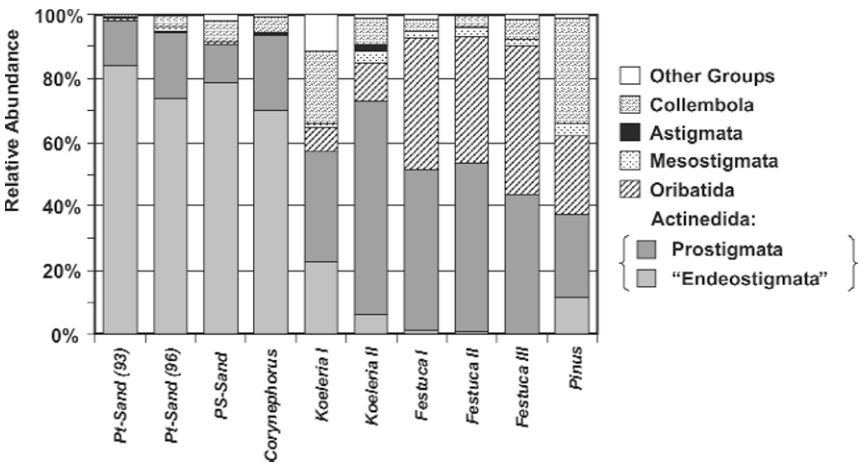

Figure 1 Group composition of the microarthropod communities found in the different sites.

gradient (χ^2 = 32.349, P<0.001; Fig. 2), with sometimes more than 120,000 individuals m^{-2} in the long-grass sites.

A total of 73 different taxa were registered (see Appendix). Taxon richness increased along the successional gradient, being largest in the long-grass sites (Fig. 3). Due to large per-sample variability, the increases were not quite significant across all sites (χ^2 = 9.714, P = 0.084), but were significant between the Pt-sites (χ^2 = 6.786, P = 0.034).

The communities in the open sands were species-poor and dominated by single taxa, mostly 'Endeostigmata' such as *Neonanorchestes ammolitoreus*, *Nanorchestes* sp. or *Micropsammus littoralis* (see Appendix). These species accounted for 70-90% of all individuals. *Linotetranus cylindricus* was also highly dominant in 1994 (data not shown). The assemblages in the short-grass sites were more species-rich, but often showed similarly strong dominances of single species, i.e., *N. ammolitoreus* and *M. littoralis*. Besides these species, microtydeid species were abundant in these sites. The second *Koeleria* site, however, showed relatively balanced actinedid assemblages, where the number of dominant species was larger: *M. littoralis*, *Paralorryia* sp., *Microtydeus* sp. and *Alycosmesis* n.sp., none of which accounted for more than 15% of the individuals. The long-grass sites harboured the most taxon-rich communities, which were dominated by microtydeid, eupodid and stigmaeid species as well as *L. cylindricus*. The woodland site, finally, was only somewhat species-poorer than the long-grass sites, but highly dominated by *Nanorchestes* spp. (Appendix). Otherwise individual-rich populations of mostly eupodid and tydeid taxa were found here.

In the correlation analyses, C_{org} and N_{tot} correlated almost perfectly, as did C_{org} and pH albeit inversely (rho = -0.973, P<0.0001). Especially *M. littoralis* and *Neonanorchestes* correlated significantly with low C_{org}, N_{tot} and higher pH (= nutrient poor sands) (Table 2). Other psammophilous species (see below) did not correlate significantly with nutrient status due to low individual densities. Other, more eurytopic species correlated significantly positive with eutrophication (Table 2).

Table 2 Spearman correlation coefficients (Spearman's rho) for species correlating significantly with soil abiotic parameters. Only correlations with [C_{org}] are shown. *P<0.05; **P<0.01; ***P<0.001.

Species	rho
Neonanorchestes ammolotoreus	-0.729 **
Micropsammus littoralis	-0.520 *
Microtydeus beltrani	0.509 *
Tydides ulter	0.544 *
Eustigmaeus cf. *arcuatus*	0.572 *
Alycus sp.	0.572 *
Cocceupodes paradoxus	0.620 *
Bakerdonia sp.	0.657 **
Cocceupodes sheppardi	0.669 **
Eupodes ereynetoides	0.718 **
Alicorhagia sp.	0.758 **
Lorryia sp.	0.807 ***

In the CCA analysis, 77% of the data variance was explained by the two selected axes (axis 1: eigenvalue 0.566, 43.8% of variance; axis 2: eigenvalue = 0.422, 33.2% of variance; Fig. 4). Especially axis 1 associated strongly and positively with C_{org} and N_{tot}. Three strong groups of species were delineated in the bi-plot diagram. One group, associating

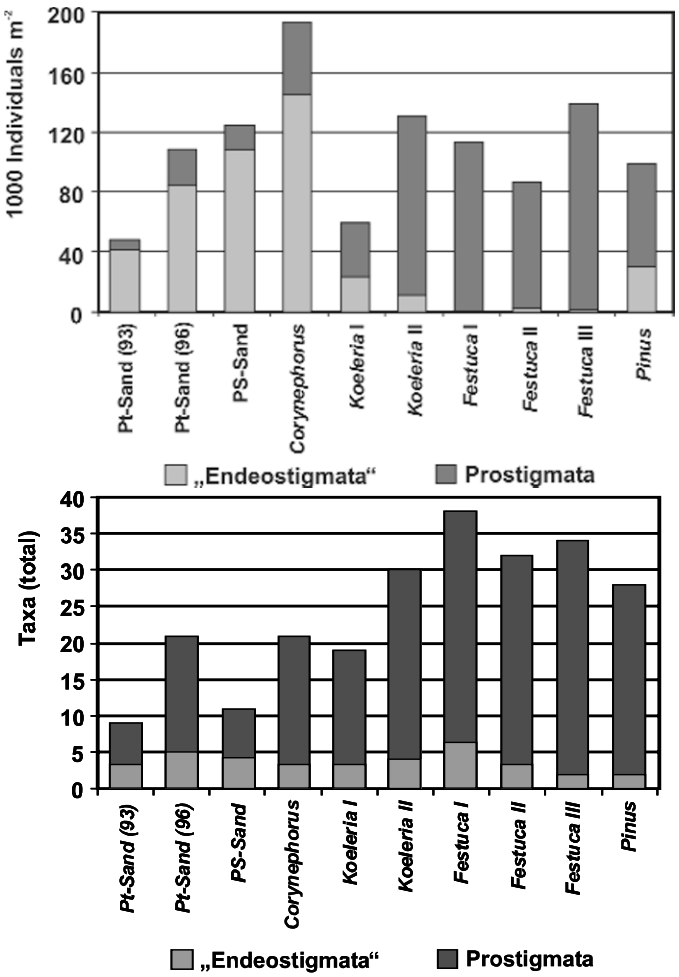

Figure 2 Registered densities of Actindedid mites, differentiated between 'Endeostigmata' and Prostigmata.

Figure 3 Number of Actinedid taxa found in the different sites.

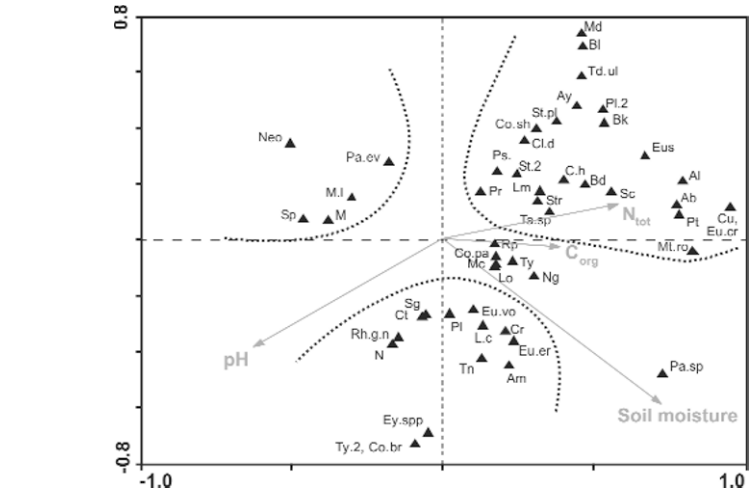

Figure 4 Bi-plot diagram of the CCA analysis (non-transformed data; species-centred; singletons, doubletons, and undetermined larvae omitted). For abbreviations of taxa, see Appendix. Dotted lines delineate species groups.

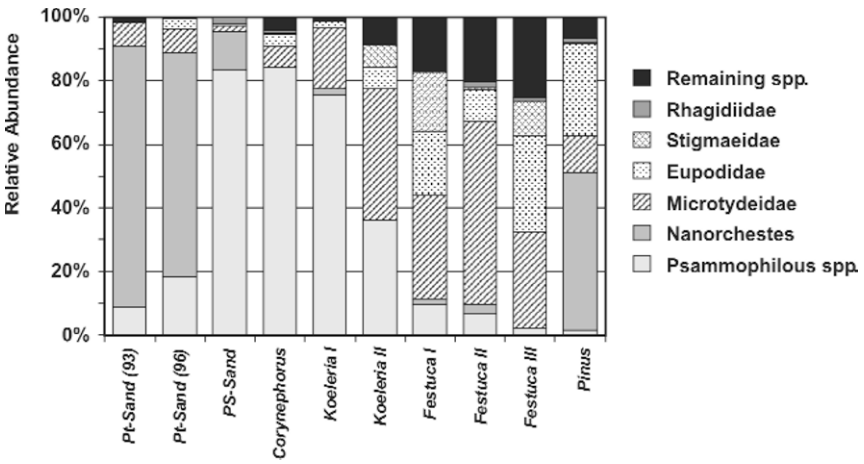

Figure 5 Composition of the Actinedid mite communities from the different sites, based on psammophilous species (see text and Appendix) and selected families.

negatively with axis 1 and positively with axis 2 (left and above in Fig. 4), were found almost exclusively in the open sands and short-grass sites. Associating negatively to axis 2 but neutrally to axis 1 (below in Fig. 4) was a group of species that occurred predominately but not exclusively in the open sands and short-grass sites. A large group of species associated positively to both axes (right and above in Fig. 4) and were found predominately or exclusively in the long-grass, highly eutrophied sites.

Based on this analysis as well as cluster analyses of the correlation matrices of the species' abundances (data not shown) and literature distribution data, certain species could be classified as being 'psammophilous' in the present study (see Appendix). Left out of the classification was *Nanorchestes*, since multiple species were most likely hidden in this taxon. Based on this classification, site specificity in the distribution of certain taxa became apparent. Psammophilous as well as *Nanorchestes* species were present in all sites, but were highly dominant in the open sands and short-grass sites, decreasing dramatically in the long-grass and (except *Nanorchestes*) woodland sites (Fig. 5). On the other hand, microtydeid species, which were present in all sites, as well as eupodid and stigmaeid species increased dramatically in the more eutrophied sites, where they often became the dominant taxa.

DISCUSSION

Few European soil-fauna studies of psammic habitats have yielded quantitative data at a community level. The present results can thus only be compared with geographically different sites, which – at first sight – appear comparable to the present sites (i.e., soil type and abiotic parameters, psammic vegetation, etc.). The studied sand dunes are locally xerotherm, but located within the temperate climates, with 400-700 mm precipitation per year, and are spatially limited, surrounded by more mesic woodland, agrarian and other habitats.

These habitat similarities and differences to comparable sites were reflected in the soil fauna. For instance, average microarthropod as well as actinedid mite densities were well above those of other xerotherm psammic habitats (Wood, 1971; Edney et al., 1975; Franco et al., 1979; Steinberger, 1990; Steinberger et al., 1990; Kinnear, 1991; Nobel et al., 1996a) and were similar to or even higher than those of temperate short-grass prairies and grasslands (Lussenhop, 1972; Peterson & Luxton, 1982; Leetham & Milchunas, 1985; Curry, 1994). The actinedid taxa were also more numerous (70) than those in similar habitats (10-50 species: Franco et al., 1979; Estrada et al., 1988; Cepeda-Pizarro & Whitford, 1989b; Kinnear, 1991; Cepedo-Pizarro et al., 1996; Noble et al., 1996a), despite the somewhat limited taxonomic resolution here. Thus, general zoocoenotic parameters in the present sites were comparatively high, probably reflecting the regional geography and climate rather than the local xerothermic psammic conditions.

On the other hand, community composition was very similar to that found in other xerotherm and/or psammic sites. That is, the most abundant microarthropods were mostly small, soft-bodied Actinedida (Loots & Ryke, 1967;

Wood, 1971; Wallwork, 1972; Santos et al., 1978; Steinberger & Whitford, 1984; Walter, 1987; Cepada-Pizarro & Whitford, 1989a; Steinberger, 1990; Steinberger et al., 1990; Kinnear, 1991; Cepada-Pizarro et al., 1996; Noble et al., 1996a). At the species level, the sites correspond well with acarofaunal results from similar habitats. Many of the genera and species have, to date, been found abundantly or exclusively in psammic habitats. One of the most common families in Sandhausen, Nanorchestidae, is often dominant in sandy and/or xerotherm habitats, often with the genus *Speleorchestes* (Franco et al., 1979; Wallwork, 1972; Whitford & Santos, 1980; Santos & Whitford, 1983; Cepeda-Pizarro & Whitford, 1989a, b; Steinberger, 1990; Steinberger et al., 1990; Kinnear, 1991; Cepeda-Pizzaro et al., 1996; Noble et al., 1996a). Although *Speleorchestes* was rarely abundant in Sandhausen, *Neonanorchestes ammolotoreus* accounted for up to 70% of all Actinedida. This genus has been found only in littoral sand habitats in southern North America and Mexican desert areas (McDanial & Bolen, 1981; Sanchez-Rocha & Palacios-Vargas, 1996), as well as Norwegian shoreline foredunes (unpubl.). *Micropsammus littoralis* also occurred abundantly in the open and sparsely vegetated sites. To date, the genus has been found exclusively in xerothermous sand habitats, albeit very disjunctly (Coineau & Seely, 1983; Coineau & Théron, 1983; Estrada et al., 1988; Kethley, 1990; Cepeda-Pizarro et al., 1996; Noble et al., 1996b; Russell, 2000) and is probably distributed world-wide (J. Kethley†, pers. comm.).

Stigmalychus n.sp. occurred here in somewhat more vegetated sites and only single individuals were found per site. Other than the description of *S. veretrum* from southern Africa (Théron et al., 1970), no other records have been published for this genus. It is known, however, to occur in various sandy sites in North America, Hawaii as well as coastal dunes in Norway (unpubl. Data; J. Kethley†, pers. comm.; Russell, 2000). Another taxon occurring in Sandhausen is *Cunliffea* n.sp. (Nematalycidae). The genera of this family appear to occur world-wide, yet very disjunctly in sandy soils (Schubart, 1973; Haupt & Coineau, 1999; Norton & Kinnear, 1999; Russell, 2000) and show strong adaptations to mesopsammal life (Schubart, 1973; Coineau & Massoud, 1977; Coineau et al., 1978; Haupt & Coineau, 1999). The spatial separation of the second and third coxae as well as the elongation of the posterior hysterosoma are considered to be adaptations to the loose texture and small, unstable pore spaces of sandy soils and can also be seen in *Stigmalychus*, *Micropsammus*, and – partly – *Linotetranus* (see below). These taxa are all most likely psammobiontic and typical of actinedid sand communities.

Although perhaps not as extremely psammobiontic, other actinedid taxa found in the sites appear to be important members of the psammophilous actinedid community. For instance, the genus *Linotetranus* has been found almost exclusively in sandy biotopes (Athias-Henriot, 1961; Estrada et al., 1988; Arganaraz & Alzuet, 1991; Kinnear, 1991; André, 1996; Cepedo-Pizzaro et al., 1996; Noble et al., 1996a; Sanchez-Rocha & Palacios-Vargas, 1996). Although very little is known about the distribution and occurrence of *Neognathus*, which was also found abundantly in another sun-exposed, short-grass sandy site nearby (unpubl.), Kethley (†, unpubl. manuscript) lists it as occurring in 'sandy soils with grass'. *Coccotydeolus*, a tydeid genus for which little is known about its distribution, has often been found in xerothermous sand communities (Estrada et al., 1988; Cepado-Pizzaro & Whitford, 1989a; Sanchez-Rocha & Palacios-Vargas, 1996). Two further, undescribed taxa partly occurred in large densities and conspicuously sympatric with the other psammophilous Actinedida: *Alycosmesis* n.sp., other species of which have only been found in sands (Théron, 1975). An undescribed Rhagidiidae genus, which was also found in sand dunes near Bornholm, Denmark (unpubl.), also appears to belong to the actinedid psammophilous synusia. All these taxa parallel many reports of Actinedida from sand habitats around the world, partly even at a species level. Important is the sympatric, often very abundant occurrence of these taxa, thus confirming the occurrence of specific actinedid sand communities in xerothermous, psammic habitats. It remains to be determined whether the world-wide disjunct distribution of these psammophilous, often basally derivative species represent relict habitats or long-distance dispersal (i.e., 'air-plankton') of stenoeceous species (cf. Noble et al., 1996a).

These psammophilous and -biontous taxa occurred mostly in the successionally less-developed sites within species-poor communities. Their populations decreased dramatically along the successional gradient, whereby actinedid densities and taxa number increased, extreme dominances became reduced and community structures became balanced. Most significant, however, was the shift in species composition within the communities along this gradient. Most psammobiontous taxa were restricted to open sands and short-grass sites. In the more developed grass sites, prostigmatic species (i.e., from the families Tydeidae, Stigmaeidae, and Eupodidae) became dominant, genera and species which have not been reported to occur abundantly in psammic habitats, but are abundant in grassland habitats (Lussenhop, 1972; Price, 1973; Seastedt, 1984; Kethley, 1990). These taxa correlated well with increasing organic C and pH. Thus, the nutrient content of the sands (eutrophication) appeared to be a determinant of species composition, with most of the psammophilous taxa limited to nutrient-poor sands. Unknown remains whether the occurrence of the psammophilous species is limited directly by abiotic parameters or indirectly through pedobiological maturity of the soils, i.e., competition by taxa only occurring in nutrient-richer sites.

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Appendix

List of registered taxa, their abbreviations and relative abundances (in %) in the various sampling sites. Undetermined juveniles are omitted. Psammophilous taxa (see text) in bold.

Taxon	Abbr.	PS-Sand	Pt-Sand (1993)	Pt-Sand (1996)	*Coryneph-orus*	*Koeleria* I	*Koeleria* II	*Festuca* I	*Festuca* II	*Festuca* III	*Pinus*
'Endeostigmata'											
Alicorhagiidae											
Alicorhagia sp.	Al								1.5	0.5	0.2
***Stigmalychus* sp.n.**	Sg						0.1	0.3			
Bimichaeliidae											
Alycus sp.	Ay							1.7	0.3	0.6	
Micropsammidae											
***Micropsammus littoralis* Coineau & Théron, 1983**	M.l	11.5	1.0	3.8	19.0	11.4	15.6				
Nanorchestidae											
Nanorchestes spp.	N	11.9	82.2	70.8		1.8		1.7	2.5		49.7
***Neonanorchestes ammolotoreus* McDaniel & Bolen, 1981**	Neo	68.5	1.9	7.5	64.0	53.3		0.3			
***Speleorchestes* sp.**	Sp	0.3			0.2	2.0		0.3			
Nematalycidae											
***Cunliffia* sp.n.**	Cu			0.1				0.3			
Terpnacaridae											
***Alycosmesis* sp.n.**	Am			0.8			10.1				
Parasitengona											
Erythraeidae											
Abrolophus sp.	Ab								0.8	0.1	
Balaustium sp.	Bl			0.1			0.3	0.3		3.2	
Curteria sp.						0.2				0.1	
Eupodina											
Bdellidae											
Bdella sp.	Bd							1.4	0.4		
Biscirus (cf.) sp.							0.1				
Cyta sp.					0.2						
Cunaxidae											
Armascirus sp.										0.3	
Cunaxa sp.									2.3		
Pseudobonzia sp.											1.1
Cunaxidae sp.				0.1							
Ereynetidae											
gen. sp.							0.1				0.8
Eupodidae											
Claveupodes delicatus Strandtmann & Prasse, 1977	Cl.d			3.3	0.8	0.2	4.3	0.8	0.8	26.6	1.1
Claveupodes sp. II					0.2						
Cocceupodes breweri Strandtmann, 1971	Co.br										1.3
C. cf. *stellatus* Strandtmann & Prasse, 1977	Co.st							0.6			
C. mollicellus (Koch, 1838)	Co.mo							0.3			1.1
C. paradoxus Weis-Fogh, 1948	Co.pa							1.7	0.1		0.6
C. sheppardi Strandtmann, 1971	Co.sh							0.6		0.4	0.2
C. trisetatus Strandtmann & Prasse, 1977	Co.tr								0.3		0.8
Cocceupodes sp. (Lv)					0.1			2.3	0.7	1.4	12.5
Eupodes ereynetoides Strandtmann & Prasse, 1977	Eu.er					0.2	0.6		1.9	0.5	6.3
Eupodes [near *crozetensis* Strandtmann & Davies, 1972]	Eu.cr								1.2		
E. voxencollinus Sig Thor, 1934	Eo.vo			0.1			0.3	0.3	0.8		0.2
Eupodes sp. (Lv)		0.3		0.1	2.3	0.7	0.5	3.7	3.9	0.2	2.1

Appendix Continued

Taxon	Abbr.	PS-Sand	Pt-Sand (1993)	Pt-Sand (1996)	*Coryneph-orus*	*Koel-eria* I	*Koel-eria* II	*Fest-uca* I	*Fest-uca* II	*Fest-uca* III	*Pinus*
[Eupodina]											
[Eupodidae]											
Protereunetes sp.	Pr	0.3				0.2	0.4	5.9		0.2	
Linopodes sp.											0.2
Penthaleidae											
Penthaleus sp.					0.1						
Rhagidiidae											
Coccorhagidia clavifrons (Canestrini, 1886)									0.8		
C. pittardi Strandtmann, 1971	Co.pi									0.2	0.8
Foveacheles sg. *Usitorhagidia* sp.									0.6		
Parallelorhagia evansi Strandtmann & Prasse, 1977	Pa.ev	2.1								0.5	0.2
***Poecilophysis arena* Zacharda, 1980**											0.2
Shibaia longisensilla (Shiba, 1969)									0.7		
gen.n., sp.n.	Rh.g.n	0.3		0.9	0.1		0.3	0.3	0.1		0.8
Tydeidae											
***Coccotydaeolus bakeri* (Wood, 1965)**	Ct	2.8	5.3	2.7	0.8	7.6	0.9	1.4	6.6	0.8	0.2
Lorryia sp.	Lo							1.1	0.1	0.5	1.9
Metatydaeolus robustus (Kuznetzov, 1979)	Mt.ro						0.3	0.3	2.8		
Microtydeus beltrani Baker, 1944	Mc	1.0	1.9	1.4	2.7	6.5	14.1	6.2	10.5	4.3	4.2
Paralorryia sp.	Pl	0.7	4.8	0.3		4.9	14.8	3.7	0.4	1.5	2.7
Paralorryia sp. II	Pl.2						0.1			0.4	
Paratydaeolus lukoschusi André, 1980	Pt		1.0	1.5	0.7	1.1	1.4	0.6	40.6	2.1	0.2
Tydaeolus sphaeroclaviger Kuznetzov, 1972	Ty			2.1	2.7	5.8	5.6	0.3	0.7	5.3	0.8
T. tenuiclaviger (Thor, 1931)	Ty.2										2.5
Tydeus sp.										0.1	
Tydides ulter Kuznetzov, 1975	Td.ul					0.2		20.3	2.1	16.4	
Triophtydeus sp.								0.3			0.4
[near *Tyndareus*] sp.	Tn			0.3		0.2	4.0				0.2
Eleutherengona											
Caligonellidae											
Caligonella humilis (Koch, 1838)	C.h						0.5			0.5	
***Neognathus* sp.**	Ng			0.1	0.1		1.6			0.6	
Cryptognathidae											
Cryptognathus sp.	Cr						0.6	0.3			
Linotetranidae											
***Linotetranus cylindricus* Berlese, 1910**	L.c		0.5	2.2		1.1	7.5	6.8	0.1	0.8	
Tetranychidae											
Byobia sp.										1.7	
Eotetranychus sp.										0.1	
Raphignathidae											
Raphignathus sp.	Rp					0.2	0.3	0.6	0.1		
Stigmaeidae											
Ledermulleriopsis sp.n.	Lm							1.4		0.1	
Pseudostigmaeus sp.	Ps							2.3			
Eustigmaeus c.f. *arcuatus* (Chaudhri, 1965)	Eus							0.3	0.3	0.1	
Stigmaeus planus Kuznetzov, 1976	St.pl				0.2		4.3	3.1		10.0	0.2
Stigmaeus sp. II [sect. I-D]	St.II							3.1	0.1		
Storchia robusta (Berlese, 1885)	Str						1.5	3.9		1.2	
gen. 7 sp.							0.1	0.3	0.1		
Heterostigmata											
Microdispidae											
gen. sp.	Md			0.1	0.1					7.9	0.2
Pygmephoridae											
Bakerdonia sp.	Bk				0.6		0.2	2.8	3.7	2.7	
Mahunkania sp.	M		1.4	0.1							
Scutacaridae											
Scutacaridae sp.	Sc				0.2		0.6		3.0	1.9	
Tarsonemidae											
gen. sp.	Ta			0.1	3.4		5.8	8.5	7.6	5.1	

Communities of Oribatida associated with litter input in western red cedar tree crowns: Are moss mats 'magic carpets' for oribatid mite dispersal?

Zoë Lindo

Department of Biology, University of Victoria, PO Box 3020, Victoria, British Columbia, V8W 3N5, Canada. Present address: Department of Biology, McGill University, 1205 Docteur Penfield, Montreal, Quebec, H3A 1B1, Canada.
E-mail: zoe.lindo@mcgill.ca

Oribatid mite abundance, species richness, and community composition in annual litter fall were compared between the high canopy of an ancient temperate rainforest and the forest floor to evaluate whether litterfall, including moss debris, is a dispersal vector for these organisms. Oribatid mites were extracted from litterfall collected from canopy (30 m) and ground (1 m) litter traps associated with six western red cedar trees in the Walbran Valley on the southwest coast of Vancouver Island, Canada, over 3, 6, and 12 months. Total annual litter input was not significantly different between canopy and ground traps, as high amounts of litter were associated with both habitats. Litter composition differed between the two habitats and cumulative input over larger spatial scales may prove to be appreciably different. Fifty-seven species of oribatid mites were associated with total litterfall collected in canopy and ground traps over 12 months. Species richness over the entire sampling period was similar between canopy and ground habitats, but oribatid mite species composition differed significantly, and is most likely related to litter composition and the initial source of litter. Oribatid mite abundance (number of individuals per gram dry weight) associated with litterfall was low compared to suspended soil habitats, and not significantly different between litter accumulation in ground and canopy traps. Nevertheless, a general trend of high litter input and high species richness associated with litterfall in canopy habitats, combined with high disperser survivorship, suggests dispersal vectors such as moss mats are important for maintaining arboreal oribatid mite communities.

Key words: Canopy, oribatid mites, litterfall, dispersal vector

In ancient western red cedar trees (*Thuja plicata* D. Don) (Cupressaceae) of North American temperate rainforests, suspended soils form high above the forest floor (ca. 35 m) and occur as discrete patches of habitat ranging in surface area from 100 to 20,000 cm^2 (Lindo & Winchester, 2006). Suspended soils are habitat islands of accumulated organic matter and epiphytes separated from one another within a tree crown by a barren bark matrix and between trees by the atmosphere (Moffet, 2000). These habitats contain an abundant and species-rich community of arboreal micro-arthropods dominated by oribatid mites (Oribatida), many species of which are undescribed and not found on the forest floor (Lindo & Winchester, 2006). Arboreal specificity in micro-arthropod communities and in particular oribatid mites has been well documented (see Behan-Pelletier & Walter, 2000) and canopy/ground comparison studies show that the number of oribatid mite species in common between the two habitats is typically less than 40% (Wunderle, 1992a; Behan-Pelletier et al., 1993; Winchester et al., 1999; Lindo & Winchester, 2006). Among the factors affecting the diversity and abundance in arboreal oribatid mite communities are tree species, elevation (Fagan et al., 2005), and the availability of habitat (Lindo & Winchester, 2007a). Dispersal events, colonization history, local and regional scale structural complexity, and stability of suspended soil environments are also hypothesised as major determinants of arboreal oribatid mite species richness (Southwood, 1996).

Dispersal is a dynamic biological process that is a driver of many ecological theories such as island biogeography (MacArthur & Wilson, 1967), metapopulation (Hanski, 1999) and metacommunity dynamics (Wilson, 1992), and neutral models (Bell, 2000; Hubbell, 2001; Chave et al., 2002). Under low rates of dispersal, species are more aggregated, and rare, local, or endemic species can survive in high abundance because of lack of competitors and adequate ecological time to increase in abundance (Hubbell, 2001). In fragmented, isolated, or patchily distributed habitats like suspended soils, I would expect dispersal to be especially limiting, and may explain the arboreal specificity in forest oribatid mite communities.

The mechanism of oribatid mite colonization of canopy habitats is unknown. Colonisation of canopy habitats by ground-dwelling oribatid mites is unlikely based on ground/canopy comparison studies, and recently, the trunk has been dismissed as a potential dispersal corridor (Proctor et al., 2002; Beaulieu et al., 2006; Lindo & Winchester, 2007b). A more parsimonious explanation is that canopy oribatid mites colonize new canopy habitats from other canopy sources. The proposed mechanisms of canopy-to-canopy dispersal include cursorial transport (Beaulieu et al., 2006), active (Norton, 1980) and accidental (Krivolutsky & Lebedeva, 2004a, b) phoresy, and aerial plankton (Karasawa et al., 2005).

Another possibility for aerial transport is via dispersal vectors, such as moss and lichen propagules, leaf litter, branch tips, and twigs. The importance of a particular vector depends on the rate at which dispersers are carried to a recipient habitat (propagule load) and on the survivorship during transport. Dispersal vectors could have high propagule loadings and high survivorship of dispersers due to lesser changes in environmental conditions during transport. There is limited evidence for such a passive dispersal mechanism in arboreal oribatid mites (Wunderle, 1992b; Karasawa et al., 2005), but horizontal movement within canopy systems is probable, as is transport to lower canopy habitats or the forest floor by litterfall.

The degree to which dispersal and dispersal limitations contribute to the dynamics and structure of communities advocates the need to elucidate the mechanisms of dispersal, to quantify dispersal rates, as well as to quantify the spa-

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Table 1 Results of repeated measures ANOVA for oribatid mite species richness (average number species per trap), and abundance of micro-arthropods (total number individuals per g dwt litter; mean ± SD) collected in litter traps at 30 m and 1 m above the forest floor associated with six western red cedar trees after 12 months. Given are P-values for different sources of variation with degrees of freedom in parentheses.

	Canopy (30 m)	Ground (1 m)	Habitat (1,10)	Time (2,20)	Time × Habitat (2,20)
Oribatida species richness	19.17 ± 2.8	13.50 ± 5.1	0.007	<0.001	<0.001
Oribatida abundance	0.542 ± 0.32	0.506 ± 0.20	0.123	0.482	0.038
Prostigmata abundance	0.249 ± 0.09	0.224 ± 0.32	0.701	0.001	0.923
Mesostigmata abundance	0.082 ± 0.05	0.095 ± 0.17	0.686	0.260	0.125
Collembola abundance	10.407 ± 11.51	1.562 ± 0.60	0.096	0.039	0.069
Other micro-arthropod abundance	0.195 ± 0.28	0.139 ± 0.04	0.435	0.038	0.297
Total micro-arthropod abundance	11.475 ± 11.38	2.527 ± 0.86	0.234	0.044	0.152

tial scale in which dispersal occurs. The objectives of this study were: (1) measure and compare annual amounts of litter input into canopy and ground habitats, and (2) observe oribatid mite species and abundances associated with annual litter input in these two habitats to evaluate whether litterfall is a dispersal vector for these organisms.

MATERIALS AND METHODS

The study site was located in the temperate rainforest of the Walbran Valley on the southwest coast of Vancouver Island, British Columbia, Canada (48°39'N, 124°35'W). The valley lies entirely within the CWH biogeoclimatic zone (Meidinger & Pojar, 1991) where the climate is characterized by wet, humid, cool summers and mild winters, and where a mean annual precipitation of 2,990 mm is typical for this area (Environment Canada, 2006). Conifers are dominant in this rainforest and include western hemlock (*Tsuga heterophylla* (Rafn.) Sarg.), Sitka spruce (*Picea sitchensis* (Bong) Carr.), Amabilis fir (*Abies amabilis* (Dougl.) Forb.), and western red cedar (*Thuja plicata* D. Don). The six western red cedar trees used in this study were approximately 50 m tall, with the diameter of the trunks at breast height ranging from 2.13 to 3.65 m (mean ± SD = 2.71 ± 0.52 m). These trees are estimated to be 800-1,200 years old, and well developed suspended soils ranging in size from 0.1 to 2.0 m² are abundant within tree crowns at heights greater than 20 m where trunk reiterations and major limb junctions take place.

Single rope climbing techniques were used to access tree crowns. Litter traps constructed of grain feed bags sewn to 12-gauge wire hoops (surface area = 0.25 m², depth = 30 cm) (Hughes et al., 1987) were secured in the canopy at 30 m. Litter traps were secured in freestanding space by three attachment points to neighbouring limbs or trunks within each western red cedar tree crown. Litter traps were also placed 1 m above the forest floor, 1.5 m from the base of each tree on the north side. Ground litter traps were supported at three attachment points to the wire hoop using PVC legs (2 cm in diameter, 110 cm long) inserted 10 cm into the forest floor. Litter traps were installed in June 2005 and litterfall was collected after 3, 6, and 12 months.

Micro-arthropods were extracted from total litter accumulations after each collection date into 70% EtOH using modified Berlese funnels over 48 h (Norton & Kethley, 1988). Abundance data is calculated as number of individuals per gram dry weight and is used for comparisons with indigenous suspended soil-dwelling oribatid mite communities. Extracted micro-arthropods were sorted into Acari, Collembola, and others. Acari were further identified to suborder (Mesostigmata, Prostigmata, Oribatida), and all adult oribatid mites were identified to species.

I used repeated measures ANOVA to test for the effect of time and habitat on litter input in canopy and ground litter traps, abundance of major micro-arthropod groups, and oribatid mite species richness. These analyses were performed using Statistica 7.0 (StatSoft Inc., 2004) with a significance level of $\alpha = 0.05$. Community composition of adult oribatid mites in canopy- and ground-collected litterfall was evaluated using non-metric multidimensional scaling (NMDS) (Clarke, 1993) which arranged the samples (traps) with respect to the rank order of similarity in community composition based on Bray-Curtis similarity of √x-transformed oribatid mite species abundance data. The final ordination of a priori trap placement was assessed for significance of random occurrence based on analysis of similarities (ANOSIM) with 10,000 randomized permutations using habitat (30 m canopy, 1 m ground) and collection time (3, 6, 12 months) as factors (Primer, 2001).

RESULTS

Total amount of litterfall over 12 months in canopy vs. ground traps was not statistically different ($F_{1,10} = 3.079$, $P = 0.110$), although litterfall was consistently higher in canopy litter traps (Fig. 1). There was a significant effect of time on amount of litterfall collected, with the most litterfall collected between 3 and 6 months ($F_{2,20} = 14.212$, $P<0.001$). Litterfall primarily consisted of moss, cedar bark, litter and twigs, hemlock cones and needles, and herbaceous plant litter. Differences in litterfall composition were observed between canopy and ground with litter accumulation in canopy traps predominated by moss and cedar derivatives, whereas ground traps accumulated litter primarily from hemlock sources.

Average oribatid mite, other Acari, and total micro-arthropod abundance was low, and the total number of indi-

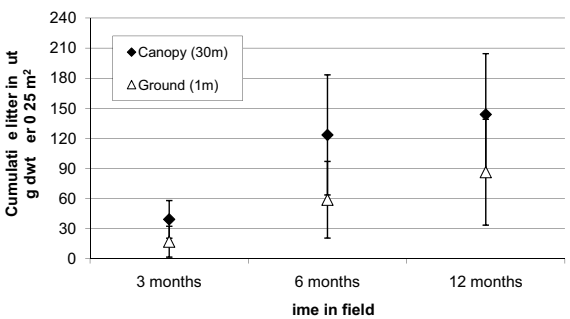

Figure 1 Cumulative litter input (mean ± SD) in canopy (30 m) and ground (1 m) litter traps (surface area = 0.25 m²) over 3, 6, and 12 months associated with western red cedar trees in temperate rainforest in the Walbran Valley on Vancouver Island, BC, Canada.

Table 2 Oribatid mite species (Acari: Oribatida) abundances from litterfall in canopy (30 m) and ground (1 m) litter traps associated with western red cedar trees in the Walbran Valley, Vancouver Island, BC, Canada.

Family	Species	Canopy	Ground
Brachychthoniidae	*Liochthonius* sp. 1	0	2
	Liochthonius sp. 2	3	1
	Liochthonius sp. 4*	0	1
Phthiracaridae	*Archiphthiracarus* sp.	28	4
Oribotritiidae	*Maerkelotritia* sp. nr. *alaskensis* Hammer 1967	25	2
	Mesotritia nuda (Berlese 1887)	9	0
Euphthiracaridae	*Euphthiracarus cernuus* Walker 1965	3	0
	Euphthiracarus monyx Walker 1965	1	0
Epilohmanniidae	*Epilohmannia* sp.	7	2
Camisiidae	*Camisia segnis* (Hermann 1804)	1	11
	Platynothrus sp. nr. *peltifer* (C.L. Koch 1839)	0	4
Trhypochthoniidae	*Trhypochthonius tectorum* (Berlese 1896)	10	0
Hermaniellidae	*Hermanniella* sp. nr. *occidentalis* Ewing 1918	0	1
Neoliodidae	*Platyliodes macroprionus* Woolley & Higgins 1969	1	5
Gymnodamaeidae	*Gymnodamaeus* sp.	1	0
Damaeidae	*Belba* (*Belba*) sp.	4	0
	Epidamaeus sp. nr. *floccosus* Behan-Pelletier & Norton 1985	7	0
Cepheidae	*Cepheus corae* Jacot 1928	0	2
	Eupterotegaeus rhamphosus Higgins & Woolley 1963	8	1
	Eupterotegaeus sp. nr. *rostratus* Higgins & Woolley 1963	30	5
	Ommatocepheus sp.	0	6
Caleremaeidae	*Caleremaeus* sp.	2	1
Eremaeidae	*Eueremaeus acostulatus* Behan-Pelletier 1993	1	0
	Eueremaeus chiatous (Higgins 1979)	7	2
	Eueremaeus marshalli Behan-Pelletier 1993	20	0
Megeremaeidae	*Megeremaeus montanus* Higgins & Woolley 1965	0	1
Liacaridae	*Dorycranosus* sp.	1	1
	Liacarus sp. nr. *bidentatus* Ewing 1918	0	9
	Liacarus sp. nr. *robustus* (Gervais 1844)	1	0
Peloppiidae	*Ceratoppia* sp. 1	0	3
	Ceratoppia sp. 2	7	11
	Ceratoppia sp. 3	2	3
	Dendrozetes sp.	2	14
	Metrioppia sp.	0	1
	Parapyroppia lamellata (Ewing 1909)	0	1
Kodiakellidae	*Kodiakella lutea* Hammer 1967	5	1
Tectocepheidae	*Tectocepheus velatus* (Michael 1880)	4	1
Oppiidae	*Oppiella* sp. 2*	0	2
Quadroppiidae	*Quadroppia quadricarinata* (Michael 1885)	4	1
Suctobelbidae	*Suctobelbella* sp. nr. *longicuspis* Jacot 1937	0	1
	Suctobelbella sp. 7*	1	0
Autognetidae	*Autogneta* sp. nr. *longilamellata* (Michael 1885)	0	1
Cymbaeremaeidae	*Scapheremaeus palustris* (Sellnick 1924)	6	12
Achipteriidae	*Achipteria* sp. nr. *curta* Aoki 1970	0	1
	Anachipteria acuta (Ewing 1918)	10	1
	Dentachipteria sp.	2	1
Scheloribatidae	*Parapirnodus coniferinus* Behan-Pelletier et al. 2002	2	0
	Parapirnodus hexaporosus Behan-Pelletier et al. 2002	4	2
	Scheloribates (*Scheloribates*) sp.	20	12
	Scheloribates (*Hemileius*) sp.	1	0
Oribatulidae	*Phauloppia* sp.	24	30
Oribatellidae	*Oribatella* sp.	0	1
Ceratozetidae	*Ceratozetes pacificus* Behan-Pelletier 1984	1	0
	Sphaerozetes winchesteri Behan-Pelletier 2000	14	0
	Trichoribates sp.	0	2
Mycobatidae	*Mycobates corticeus* Behan-Pelletier 2001	31	1
Galumnidae	*Pilogalumna* sp.	8	1
Total abundance		**318**	**165**

*species are numbered to be consistent with Lindo & Winchester (2006).
Numbers for each species are numbers of specimens collected within each habitat.

viduals collected over the 12 months was not significantly different between trap heights when standardized on a number/g dwt basis (Table 1). A total of 57 oribatid mite species were identified from 483 adult specimens (318 canopy, 165 ground) collected from all traps over 12 months (Table 2). Total oribatid mite species richness recorded over the entire sampling period was similar for canopy (40 species) and ground (42 species) habitats. The average number of species collected per trap was significantly greater in the canopy compared to the ground traps (Table 1). The composition of

oribatid mite species collected in 30 m and 1 m traps over the 12 months, differed significantly based on Bray-Curtis percent similarity in NMDS ordination (ANOSIM: Global R = 0.577, P = 0.001) (Fig. 2). There was also a significant effect of collection date, with communities collected at 3, 6, and 12 months (September, January, and June, respectively) being significantly different from one another in pair wise tests (ANOSIM: Global R = 0.595, P = 0.001). The most abundant species found in canopy traps were (Table 2) *Mycobates corticeus*, *Eupterotegeus* sp. nr. *rostratus*, *Archphthiracarus* sp., *Maerkelotritia* sp. nr. *alaskensis*, and *Phauloppia* sp., which accounted for nearly 50% of individuals collected in litterfall at 30 m. In 1 m ground traps 50% of individuals collected were *Phauloppia* sp., *Dendrozetes* sp., *Scheloribates* (*Scheloribates*) sp., *Scapheremaeus palustris*, and *Camisia segnis*.

DISCUSSION

Canopy research has increased in the last few decades, and with this research has come a greater appreciation for the interaction of above- and below-ground processes (Wardle, 2002; DeDeyn & van der Putten, 2005). This study shows high amounts of litterfall are produced and circulate throughout the year within canopy habitats, with only a fraction of that litter entering forest floor systems. Values of annual litterfall in the ground traps are similar to other old-growth temperate rainforests in Washington State (USA) (Edmonds & Murray, 2002), and interior forests of British Columbia (Canada) (Thomas & Prescott, 2000; Welke et al., 2005). Although the difference between litter input to forest floor and canopy systems was not statistically significant on the small spatial scale observed, annual litter input scaled over larger areas suggests considerable differences (canopy = 5,754.8 kg/ha, ground = 3,452.4 kg/ha). I acknowledge that litter traps used in this study may overestimate the amount of litter actually retained within the canopy because of the conical shape of the traps used. It should be noted however, that tree crown architecture in ancient western red cedar trees at this study site provide similarly shaped catchment areas. Tree crown and overall canopy architectural complexity is important in capturing and retaining litter within tree crowns, and most likely increases the capture and retention of micro-arthropods in the canopy (Sillett & Bailey, 2003).

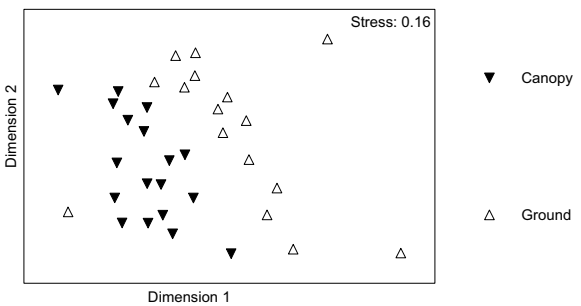

Figure 2 Non-metric multidimensional scaling (NMDS) plot of oribatid mite communities associated with total litterfall in three collection times over 12 months from canopy (30 m) and ground (1 m) litter traps associated with western red cedar trees on Vancouver Island, Canada. Ordination produced from Bray-Curtis similarity matrix of mean standardized (number individuals per 100 g dwt litterfall) species abundances. Stress value = 0.16 indicates data conform well within defined dimensions.

The distinction between active and passive dispersal is important as the two processes have different consequences for colonisation events, population dynamics, and community structure, particularly in a patchy habitat (Bowman et al., 2002). Beaulieu et al. (2006) found cursorial mesostigmatic mite dispersal across corticolous habitats within the canopy and between canopy habitat patches in arboreal mesostigmatic mite communities. Generally oribatid mite abundance is low in corticolous habitats, however, while bark is a more extreme environment for oribatid mites than either soil or suspended soil habitats, compensatory redistribution among microhabitats on exposed bark may enable mites to maintain viable moisture and temperature requirements during cursorial transport (Prinzing, 2005). However, rates of active cursorial transport are low for most species of oribatid mites (Berthet, 1964; Wunderle, 1992b; Ojala & Huhta, 2001). Oribatid mites, unlike their prostigmatic and mesostigmatic relations, are typically not known to participate actively in phoresy (with exceptions, e.g., *Mesoplophora* sp.; see Norton, 1980), but 'accidental' phoresy may occur as there is evidence for transport on birds (Krivolutsky & Lebedeva, 2004a, b). Transport by wind as aerial plankton (anemochory) has long been suggested though documentation of this is scant (Karasawa et al., 2005). Long-distance dispersal on air currents is possible and probable due to the small size of mites, and therefore individuals might also be carried considerable distances (Jung & Croft, 2001; Griffin et al., 2002), although there is often a low probability of dispersal success due to desiccation (Mitchell, 1970).

Colonisation history, structural complexity, and environmental stability are all major determinants of species richness (Southwood, 1996; Fagan et al., 2005). There was high species richness of oribatid mites recorded from litterfall in canopy and ground litter traps although relatively few individuals were collected. Abundance of individuals was generally low compared to abundances present in forest floor or suspended soils (Lindo & Winchester, 2006) but are comparable to corticolous cedar habitats (Lindo & Winchester, 2007b), and greater than the 17 species of oribatid mites collected from litterfall in beech forests of Germany (Wunderle, 1992b). The high species diversity associated with the large amount of litterfall into canopy habitats suggests dispersal vectors may be important in contributing to rescue effects in patchily distributed habitats like suspended soils (Brown & Kodric-Brown, 1977). Oribatid mite dispersal via dispersal vectors such as moss mats is probably more likely to supplement existing canopy populations than phoresy or aerial plankton mechanisms (Wunderle, 1992b). Coulson et al. (2002) suggest dispersal vectors may even enhance survival of trans-oceanic dispersal of terrestrial soil-dwelling arthropods. Aerial transport within organic matter substrate could protect against desiccation and ensure organisms arrive at a new destination alive. The method of active extraction to collect oribatid mites from the litterfall demonstrates that organisms were alive in collected litterfall, and suggests they are able to survive transport within dispersal vectors to a new habitat patch.

Canopy and ground litter-associated species richness over the entire collection period was similar, though the composition of the two communities was not. The number of species shared between canopy and ground traps (23 out of 57 species total) is the same percentage typically found in canopy/ground comparison studies (40%) (Wunderle, 1992a; Behan-Pelletier et al., 1993; Winchester et al., 1999;

Lindo & Winchester, 2006). However, while ground traps were composed of different species compared to canopy traps, the ground traps did not consist of ground species, but rather canopy species associated with other tree species. The influence of other tree species was apparent when comparing litter composition and the species associated with the litterfall in canopy vs. ground traps. The higher incidence of hemlock needles, cones, and branches in ground traps corresponded with species composition of oribatid mites known from previous hemlock canopy studies. *Dendrozetes*, *Scapheremaeus*, and *Camisia* are genera observed from canopy studies where hemlock is the focal tree (Winchester et al., 2008), and not found in suspended soils of western red cedar (Lindo & Winchester, 2006).

Horizontal movement of oribatid mites within canopy systems by wind via dispersal vectors such as moss mats appears to be an important mode of arboreal habitat colonisation for oribatid mites (Karasawa et al., 2005). Many individuals of canopy specific oribatid mite species additionally fall to ground habitats with litterfall, but these species are rarely encountered in samples of forest floor communities, suggesting factors associated with differences between these two systems play a role in the establishment of viable populations following dispersal. These factors are likely related to differences in physical habitat quality and species interactions (competition/predation levels), and future studies should focus on niche differentiation of species in seeking to elucidate community compositional trends between canopy and forest floor systems.

Dispersal in patchy habitats has been shown to affect species aggregation patterns, range sizes, and rates of spread (Shigesada et al., 1995; Clark, 1998; Chave et al., 2002), species coexistence and regional persistence (Brown & Kodric-Brown, 1977; Holyoak & Lawler, 1996), genetic variation and structure (Okamura & Freeland, 2002), and local and regional species richness (Kneitel & Miller, 2003; Cadotte & Fukami, 2005). Understanding mechanisms, rates, and spatial scales of dispersal processes are key to understanding how communities are formed and maintained. Colonization history associated with the dispersal events of individuals connected with litter input, in conjunction with tree crown complexity and the availability of suitable habitat for colonisation, are all important contributing factors of canopy oribatid mite communities associated with canopy habitats (suspended soils) in western red cedar.

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Oribatid communities (Acari: Oribatida) associated with bird's nest ferns (*Asplenium nidus* complex) in a subtropical Japanese forest – a mini-review

Shigenori Karasawa* & Naoki Hijii

Laboratory of Forest Protection, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan; *Present address: Fukuoka University of Education, 1-1 Akamabunkyo-machi, Munakata City, Fukuoka 811-4192, Japan. E-mail: karashi@fukuoka-edu.ac.jp

We reviewed the community structure of oribatid mites associated with bird's nest ferns in a subtropical Japanese forest, and seven trends became clear: (1) most of the bird's nest ferns on live trees occurred on host-tree species that typically grew in high density and/or large basal area in the forest, and they preferred concave slopes; (2) the amount of accumulated litter in the bird's nest ferns was positively correlated only with fern size; (3) there was a significant difference between the communities of oribatid mites between the litter and root components of bird's nest ferns; (4) oribatid communities in the bird's nest ferns were relatively insensitive to the spatial distribution of the fern in the subtropical forest, however, the density of oribatid mites in the litter decreased significantly with increasing height of the ferns above the ground; (5) species diversity of oribatid communities in the ferns was significantly lower than in the bark of trunks or the forest-floor litter and soil; (6) the oribatid faunas in the litter and roots of the ferns were more similar to those in both the forest-floor litter and soil than to the faunas in the other arboreal habitats; (7) presence of bird's nest ferns can enhance species richness of oribatid mites in the arboreal environment, but presence of the ferns might not always raise species richness of oribatid mites at the whole-forest scale, including the forest-floor habitats, because the species composition of oribatid communities in the ferns was very similar to that in the forest-floor habitat.

Key words: *Asplenium nidus* complex, bird's nest ferns, Oribatida, species diversity, subtropical forest

The bird's nest fern (*Asplenium nidus* complex) has a basket-shaped rosette of long fronds (up to 2 m in diameter) and a root portion underneath consisting of live fern roots and humus. The ferns are widely distributed from tropical to temperate regions (Murakami et al., 1999) and were found on 62 host-tree species in a tropical evergreen forest (Annaselvam & Parthasarathy, 2001), and individual ferns can grow to a fresh weight of more than 200 kg (Ellwood et al., 2002). The basket-shaped rosettes of the ferns can trap substantial amounts of leaf litter from the canopy and contain abundant and diverse invertebrate communities in forests (e.g., Walter et al., 1998; Ellwood et al., 2002; Ellwood & Foster, 2004). Thus, bird's nest ferns may enhance the biomass and diversity of invertebrate communities in tropical and temperate forest ecosystems more than other epiphytes do.

Oribatid mites (Acari: Oribatida) are often the numerically dominant group of arthropods in soils, and are key contributors to the dispersal of decomposer microorganisms and the acceleration of microbial activity in the decomposition of plant detritus (Wallwork, 1983; Seastedt, 1984). In addition, oribatid mites are species-rich and numerically dominant in several types of forest canopies (e.g., Hijii, 1989; Watanabe, 1997). They have been also observed in many arboreal habitats, including leaves, branches, bark, of trunks (e.g., Travé, 1963; Behan-Pelletier & Walter, 2000; Karasawa & Hijii, 2004a,b), and they colonize epiphytes attached to various trees (e.g., André, 1984, 1985; Winchester & Behan-Pelletier, 2003). In *A. nidus* in a subtropical forest in Japan, for example, oribatid mites accounted for about 40% of all invertebrate individuals in summer (Karasawa et al., 2008). Thus, investigations of habitat use and stratification of oribatid mites can help us to understand the distribution of invertebrates at a large scale in forests and nutrient dynamics in canopies that include epiphytes (Behan-Pelletier & Walter, 2000; Prinzing & Woas, 2003; Fonte & Schowalter, 2004).

The epiphytic biomass in a subtropical broad-leaved forest is compared with those in temperate coniferous and tropical forests (Hsu et al., 2002); however, there is little information on soil-invertebrate groups in suspended soils associated with large epiphytes till lately, excluding collembolans and ants in subtropical forests (Rodgers & Kitching, 1998; Yang et al., 2001a,b). Recently, our researches have revealed the community structure of oribatid mites within bird's nest ferns in this region (Karasawa, 2006; Karasawa & Hijii, 2006a,b,c; Karasawa et al., 2008), and thus the purpose of this study was to review the community structure of oribatid mites in the suspended soils associated with bird's nest ferns in a subtropical Japanese forest.

Study site

The site (ca. 4 ha) was located in an old-growth, evergreen, broad-leaved forest in the Yona Experimental Forest at the University of the Ryukyus, on the northern part of Okinawa Island (26°49'N, 128°5'E; 250-330 m a.s.l.). The evergreen forest is dominated by *Castanopsis sieboldii* with a maximum height of less than 20 m, and there is no history of logging or other artificial disturbance in this area in the past 50 years (Shinzato et al., 1986; Enoki, 2003). The area is characterized by a subtropical climate and abundant rainfall throughout the year. The mean annual air and soil temperatures are 23.3 and 24.0 °C, respectively, and annual precipitation between 1995 and 2003 averaged 2,363 mm (at the Experimental Forest). The bedrock is composed of sandstone and slate, and the soil is classified as yellow (Y).

Distributional pattern of bird's nest ferns

Within the site we found 41 ferns on 27 live trees of 13 species, and 12 on five dead trees, and they were attached to the trunks of the trees at heights of 0.1 to 10.5 m above ground level. About 85% of the bird's nest ferns on live trees

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occurred on host-tree species that typically grew in high density and/or large basal area in the forest, and they preferred concave slopes. Although many ferns were found on trees taller than 10 m and/or with greater than 20 cm DBH (diameter at breast height), the number and size of the ferns did not increase with increasing host-tree size (height and DBH). Thus, it is likely that under favorable conditions, such as higher humidity on concave slopes, bird's nest ferns may have become established on the tree species that were dominant in the area and only on those trees that were large enough to support the weight of the ferns (Karasawa & Hijii, 2006c).

In this forest we found no ferns on the ground or in the canopy, not even in tall trees. Hsu et al. (2002) reported that bird's nest ferns grew on the trunk base, main trunk, and main branching point of the host trees in a moist subtropical forest in northeastern Taiwan. These findings suggest that the vertical distribution of the ferns on host trees is limited by the structure of the trees. This distributional pattern of ferns may be characteristic of subtropical forests in eastern Asia (Hsu et al., 2002).

Factors of litter accumulation in bird's nest ferns

The projection area of the rosette at the frond tips reached up to 3.3 m^2, and the projection area of the region on which the litter was accumulated in the fern ranged from 13.0 to 551.1 cm^2. Both areas were significantly correlated with the number of leaves on the fern. These significant positive correlations indicated that the number of leaves could be used as an indicator of the size of the fern, which has a basket-shaped rosette. The amount of litter accumulated in the ferns reached ca. 120 g dry weight, and it was correlated neither with the size (height and DBH) of the host tree, nor with the height and position of the fern. There was a positive correlation only between the amount of litter and the number of leaves on the fern. In other words, the litter accumulation in each fern was determined only by the size of the basket-shaped rosette of the fern (Karasawa & Hijii, 2006c).

Effect of bird's nest fern structure on the structure of oribatid communities in the ferns

The organic matter of the bird's nest fern could be divided into two types of substrate: fresh litter deposited on the growing point of the fern, and a root portion underneath consisting of live fern roots and humus (decomposed litter). The non-metric multi-dimensional scaling (NMS) ordination of the oribatid communities collected from the litter and roots indicated that there was a significant difference between the communities of oribatid mites in these two habitats (Karasawa & Hijii, 2006a). We separately characterized the litter and root components of each fern (Karasawa, 2006, Karasawa & Hijii, 2006a), and thus the difference in the physical structure between the root portion and the litter accumulated on the growing-point areas of the ferns appears to generate different habitats for oribatid mites. As a result, the presence of these different substrates appears likely to enhance the diversity of the oribatid communities in the ferns of the subtropical forest.

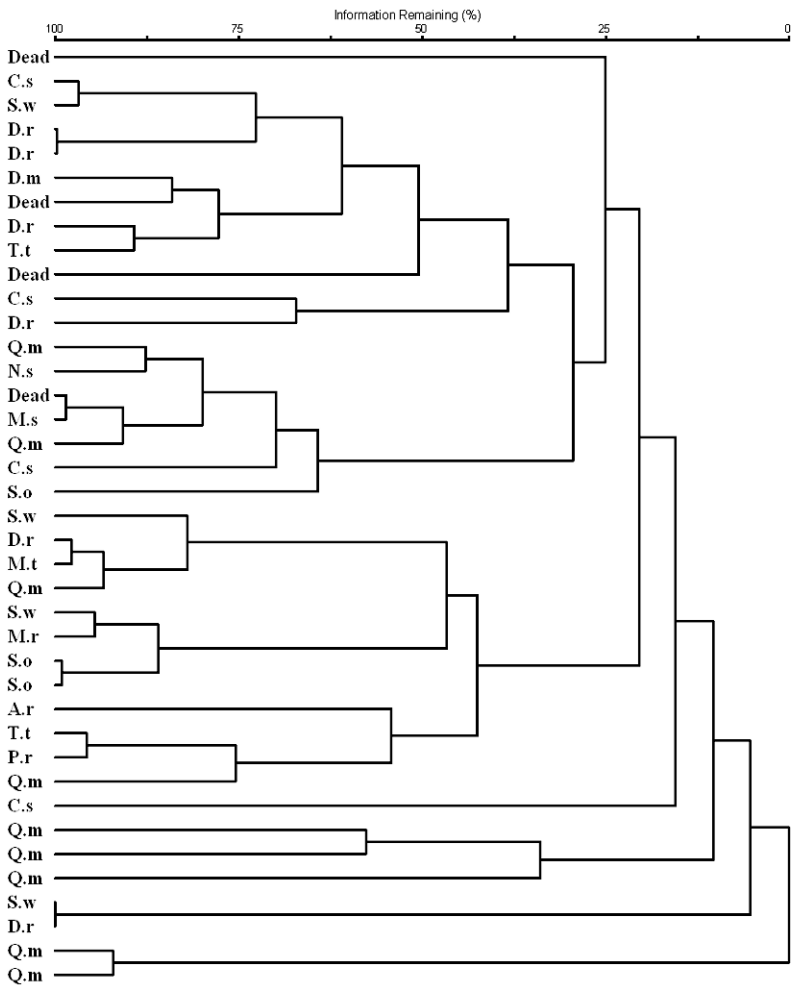

Figure 1 Similarity dendrogram (UPGMA) of oribatid communities in the litter of bird's nest ferns (Karasawa, 2006). A matrix of dissimilarities among samples was generated from Bray-Curtis distances. A.r: *Adinandra ryukyuensis*, C.s: *Castanopsis sieboldii*, D.m: *Diospyros morrisiana*, D.r: *Distylium racemosum*, M.r: *Meliosma rigida*, M.s: *Meliosma squamelata*, M.t: *Machilus thunbergii*, N.s: *Neolitsea sericea*, P.r: *Pileostegia riburnoides*, Q.m: *Quercus miyagii*, S.o: *Schefflera octophylla*, S.w: *Schima wallichii*, T.t *Trupinia ternate*, Dead: dead trees.

Effect of spatial distribution of bird's nest ferns on the structure of oribatid communities in the ferns

There were significant correlations between the numbers of individuals and species in the litter and fern size, but a total of 25 oribatid species was recorded from the litter of all small ferns (≤10 leaves), and 22 species were common to both large (>10 leaves) and small ferns. Fern size did not affect the density and diversity of species of the oribatid communities in the litter. There was also no significant effect of the height of the fern on species composition, numbers of individuals and species, and species diversity of the oribatid communities in the litter. However, the density of oribatid communities in the litter decreased significantly with increasing height of the ferns. There was no significant relationship between species composition, numbers of individuals and species, density and species diversity of oribatid communities in the roots, and the size or height of the ferns. Moreover, we found no significant correlations between the similarity of the oribatid communities and the horizontal distance between ferns (Karasawa & Hijii, 2006a).

The cluster analyses showed that species composition of oribatid communities in both litter (n = 39) and roots (n = 45) of the fern was not associated with host-tree species or condition (alive/dead), although there were two large groups recognized in each fern habitat (litter and root) (Figs 1 and 2). The results revealed that the oribatid communities in bird's nest ferns in this forest did not depend on qualities of host trees (species, alive/dead), because there was no relationship between species composition of the oribatid community in the fern and the varieties of host trees.

Our studies suggested that the oribatid communities in the bird's nest ferns were relatively insensitive to the spatial distribution of the fern in the subtropical forest. However, the density of oribatid mites in the litter component only decreased significantly with increasing height of the ferns above the ground. Arboreal environments are exposed to more insolation and wind, and undergo more frequent wetting and drying cycles, than the forest floor (Parker, 1995), and these environmental factors may thus decrease the oribatid densities in the ferns at high positions relative to those at low positions.

Species diversity of oribatid mites in bird's nest ferns

The species diversity (Simpson's 1-*D*) of oribatid communities in the ferns was similar to that in the branches, and significantly lower than those in the bark of trees and the for-

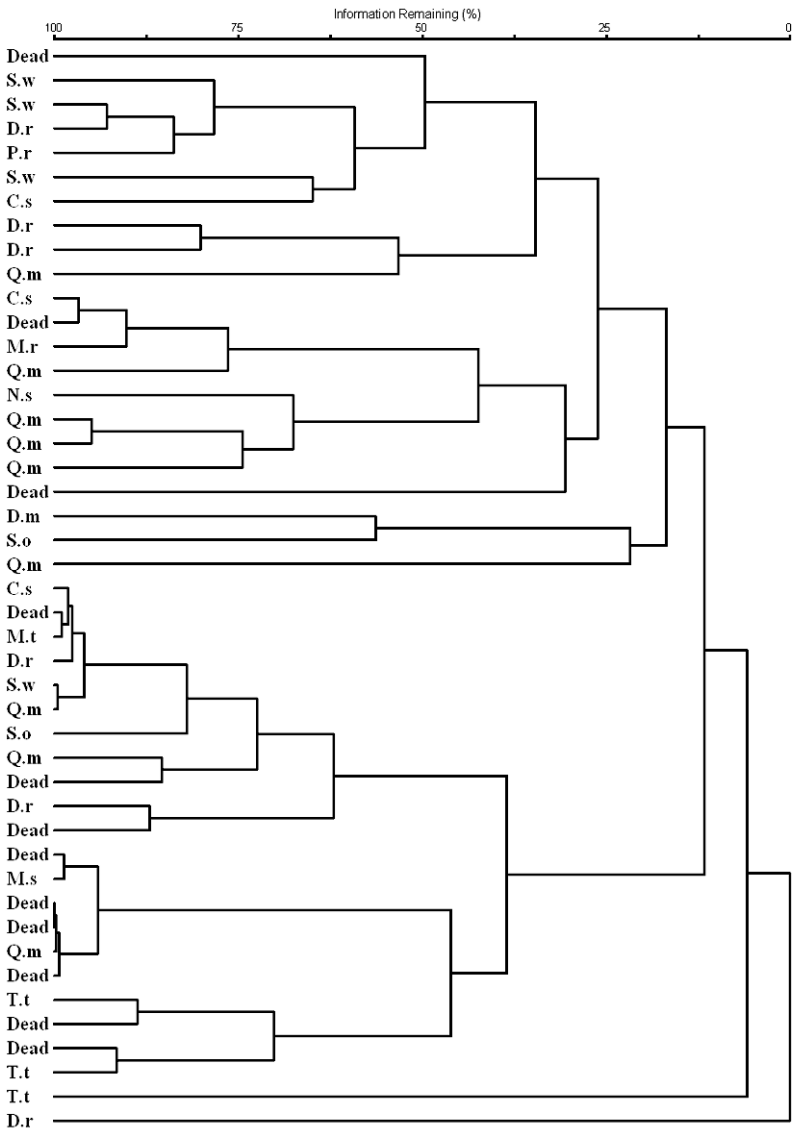

Figure 2 Similarity dendrogram (UPGMA) of oribatid communities in the roots of bird's nest ferns (Karasawa, 2006). A matrix of dissimilarities among samples was generated from Bray-Curtis distances. For abbreviations see Figure 1.

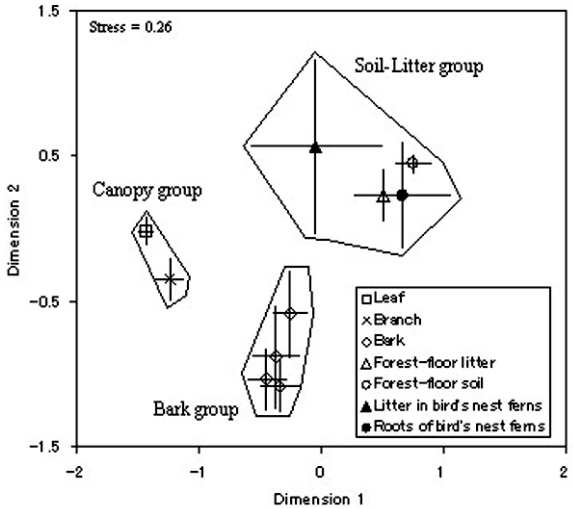

Figure 3 Non-metric multi-dimensional scaling (NMS) ordination of oribatid communities in samples from leaves, branches, bark of trunks, forest-floor litter, and roots, and in litter and roots of bird's nest ferns (Karasawa & Hijii, 2006b). Scores are expressed as mean values ± SD.

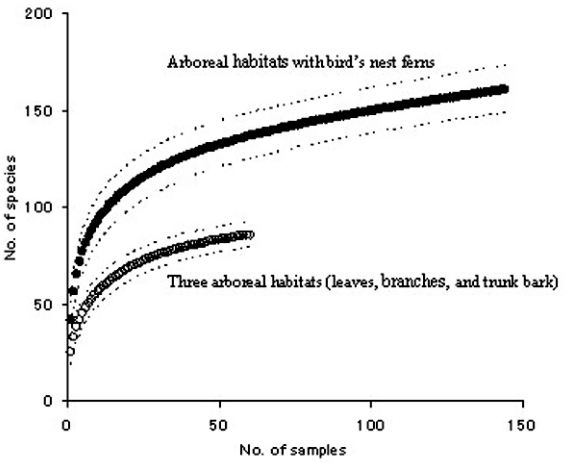

Figure 4 Species accumulation curves (dashed lines represent 95% confidence intervals) for samples from three arboreal habitats on *Castanopsis sieboldii* (leaves, branches, bark of trunks; n = 60) and all arboreal habitats (n = 144) with bird's nest ferns.

est-floor litter and soil, although we collected more than 130 oribatid species from bird's nest ferns (Karasawa & Hijii, 2006b). Most of the oribatid species that inhabit the trunk bark have some morphological modifications (e.g., claws and sensillus) to presumably adapt to aspects of plant architecture and/or microclimate (e.g., Aoki, 1973; Karasawa & Hijii, 2004b, 2008), and thus only a small number of oribatid species that inhabit the forest-floor habitats could move upward from the forest floor into the bird's nest ferns through the trunk bark or using wind currents (Karasawa et al., 2005). Therefore, harsh environments in arboreal habitats and low immigration from the species pools (forest floor) may have decreased the oribatid species diversity in the bird's nest ferns relative to that at the forest floor (Karasawa & Hijii, 2006b).

Species composition of oribatid faunas in bird's nest ferns

The NMS ordination classified the oribatid communities from the seven habitats into three clear groups (MultiResponse Permutation Procedure, $P<0.001$) on the basis of similarity of the species composition (Fig. 3). This result revealed that the oribatid faunas in the litter and the roots of the ferns were more similar to those in both the forest-floor litter and the forest-floor soil, than to the faunas in the other arboreal habitats, although the occurrence of some oribatid species was restricted to the litter in or the roots of the ferns (Karasawa & Hijii, 2006b). And then, the difference in the structure of the oribatid communities among the bird's nest fern, canopy, and bark of trunks suggests that oribatid communities should be examined separately in these components in future studies of arboreal systems.

Effect of the presence of bird's nest ferns on the species richness of oribatid communities in the forest

In the subtropical forest, we collected more than 130 oribatid species from bird's nest ferns, but species accumulation curves for the hypothetical forests of *C. sieboldii* showed no significant difference in the estimated number of oribatid species between the presence and absence of ferns (Karasawa & Hijii, 2006b). This result suggests that the presence of the fern may not greatly increase the number of oribatid species in the whole forest. Possible reasons for our result are that the component species of the oribatid communities from the two fern habitats are similar to those from the two forest-floor habitats, and that the species diversities of oribatid communities in both fern habitats are significantly lower than those in both forest-floor habitats (Karasawa & Hijii, 2006b).

Still, the presence of the ferns may approximately double the estimate of total oribatid species from all arboreal habitats (leaves, branches, and bark of trunks) other than the ferns (Fig. 4), because species compositions of oribatid communities within the ferns significantly differed from those within the other arboreal habitats (Fig. 3). Similar results of invertebrate biomass in a tropical rainforest were reported by Ellwood & Foster (2004).

The results suggested that the presence of bird's nest ferns can enhance the species richness of oribatid mites in the arboreal environment, but they also revealed that the species composition of oribatid communities in the ferns was very similar to that in the forest-floor habitat. This faunal similarity suggests that the presence of the ferns might not always raise the species richness of oribatid mites at the whole-forest scale, including the forest-floor habitats (Karasawa & Hijii, 2006b).

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Mites of the families Anystidae and Teneriffiidae from Baja California Sur, Mexico

Mercedes Guadalupe López-Campos & Ignacio Vázquez-Rojas
Facultad de Ciencias, UNAM, Laboratorio de Acarología, México, DF 04510. E-mail: lupycony@yahoo.com, mauro112003@yahoo.com.mx

The mite fauna of the state Baja California Sur is one of the least studied in Mexico. During an expedition to 10 locations in 1998, focusing on prostigmatic terrestrial mites, two families of the superfamily Anystoidea were identified: Anystidae and Teneriffiidae, the later being a new record for Baja California Sur. A phylogenetic analysis on Erythracarinae resulted in the diagnosis of two new genera, GN1 and GN2, both belonging to the family Anystidae. GN1 is monophyletic and includes three species, GN2 is monotypic. Other genera and species found in Baja California Sur include the anystids *Erythracarus nasutus* Otto, *Tarsotomus* sp., *Paratarsotomus* sp., *Chaussieria capensis* Meyer et Ryke, and the teneriffiid *Neoteneriffiola uta* Tibbetts.

Key words: Prostigmata, Anystidae, Teneriffiidae, Baja California Sur, Mexico, historical buildings

Baja California Sur is the second largest peninsula in the world. It shows a broad climatic variation, but is mostly dry, with a mean 200 mm annual rainfall. The northern part of the State is drier, with a mean annual temperature of 22 °C (annual low 5 °C, annual high 50 °C). Within this region there is a wide variety of ecosystems: deserts in the north west, southern deciduous rainforests, coniferous forests on the northern mountains, and oak-pine forest in the Sierra de la Laguna zone. A great part of the northern region has bands of volcanic ground interrupted by oases and diverse water bodies, such as streams, springs, lagoons, etc., with tropical vegetation. These areas contain a high biological diversity, serving as a shelter or permanent habitat for endemic relict flora and fauna. In 1998, the first author carried out a study on prostigmatic terrestrial mites in the Baja California Sur region. Three genera of the family Anystidae and various taxa of the Adamystidae, Erythraeidae, and Tetranychidae are being reported, together with mesostigmatic mites associated with historical buildings. The Adamystidae and Anystidae were most abundant, the family Teneriffiidae is reported for the first time for this region.

The phylogenetic relationships of the anystid subfamily Erythracarinae have been studied recently by Otto (2000). This author found 60 species of Erythracarinae within a sample of anystid mites, concluding that the study of semiarid and arid regions will show the existence of additional species. A total of 3,863 equally parsimonious cladograms of length 272 were found (strict consensus CI = 0.75, RI = 0.95). Phylogenetic analyses are tools to test for the monophyly of putative genera or to investigate the relationships of new species with other taxa. We used this phylogeny to test for the monophyly of the species in this study and for placing them within the subfamily of Erythracarinae.

MATERIALS AND METHODS

Collection techniques used along transects for arachnid collection comprised of pitfall traps with ethylenglycol-alcohol 1:1 and aspirators. Specimens were mounted on slides with Hoyer's medium (Krantz, 1978). Observations were made with clear field, interference, or phase contrast optics (Zeiss Axioskope 2 plus). Images were captured with an AxioCam MRc digital camera and processed using the Axiovision 4.4 program. The four new species were included in the anystid data matrix developed by Otto (2000) and coded for 151 characters. This data matrix with our species data was analyzed with Nona 2.0 (Goloboff, 1993) and the cladograms were mapped with Winclada 1.00.08 (Nixon, 2002). The character lines for the four species described here are presented in Appendix 1.

Localities and abbreviations

Collections were done at the following locations: Arroyo El Novillo (NOV), Laguna San Pedro (ASP), Presa Buena Mujer (BMJ), Arroyo 'El Chorro Santiago' (ACH), El Comitán (COM), Santa Rosalía (SRL), Loreto (LRT), Todos Santos (TST), La Paz (LPZ), and San José del Cabo (SJC) (Fig. 1). Genera abbreviations are: ERT, *Erythracarus*, CHS, *Chaussieria*, TRM, *Tarsotomus*, PTM, *Paratarsotomus*, NTF, *Neoteneriffiola*, GN1 = Genus nov. 1, and GN2 = Genus nov. 2

RESULTS

We studied 158 specimens from seven genera, six for Anystidae and one for Teneriffiidae. Two of the Anystidae genera are new, with four undescribed species. The formal description will be given elsewhere, here they are referred to as species a, b, c (GN1) and species d (GN2). Table 1 lists the genera found; it shows that GN1 was dominant, whereas only three specimens represented GN2.

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Table 1 Location data and relative abundance of each genus.

	GN1	ERY	CHS	TRM	GN2	PTM	NTF
LPZ	3	1	0	0	0	0	0
TST	9	1	0	0	0	0	0
SJC	7	0	0	0	0	1	0
LRT	11	0	0	0	0	0	0
SRL	13	0	0	1	0	0	0
COM	12	0	0	0	0	0	0
NOV	11	0	4	0	3	0	1
BMJ	44	0	4	0	0	0	0
ASP	0	0	0	0	0	0	31
ACH	0	0	0	0	0	0	1
Total	110	2	8	1	3	1	33
Relative abundance (%)	69.6	1.3	5.1	0.6	1.9	0.6	20.9

The addition of the four species to Otto's dataset matrix produced 33 trees, with 349 steps (CI = 65, RI = 91). The strict consensus of these cladograms led to the collapse of 20 nodes, increasing the length to 357 (CI = 63, RI = 90). All most parsimonious cladograms show that the new genera are monophyletic, but their relationships with other mites are unresolved; e.g., GN1 can be sister to *Lacteoscythis*, *Paratarsotomus*, or *Tarsotomus* (Fig. 2). These equivalent resolutions are a product of zero-length branches rather than data conflict. Zero-length branches are nodes without character state changes that support them; therefore, any taxon permutation involving these branches will not increase the tree length, when those nodes collapse at a consensus – see Coddington & Scharff (1994) and references therein for a discussion on different types of zero-length branches.

Below we describe the taxonomic features.

ANYSTIDAE

The family Anystidae Oudemans appears in literature by 1665, when Hooke describes these mites and their behaviour. Since then, various authors have described new species. Oudemans (1936) made a complete historical revision based on all known data. Grandjean (1943, 1952), Meyer & Ryke (1960), Barilo (1984a), Smith Meyer & Ueckermann (1987), Otto & Olomski (1994), and Otto (1999a,b,c, 2000) contributed to the current knowledge of Anystidae.

Both subfamilies of Anystidae are present in Mexico: the Anystinae are represented by *Anystis baccarum* L. and *A. wallacei* Otto, the Erythracarinae are represented by *Erythracarus* (= *Bechsteinia*), from Quintana Roo, Puebla, and Veracruz, and *Chaussieria* and *Tarsolarkus*, both from Veracruz. Hoffmann & López-Campos (2000) recorded several unidentified specimens from Nayarit, DF, Estado de México, Morelos, and Oaxaca.

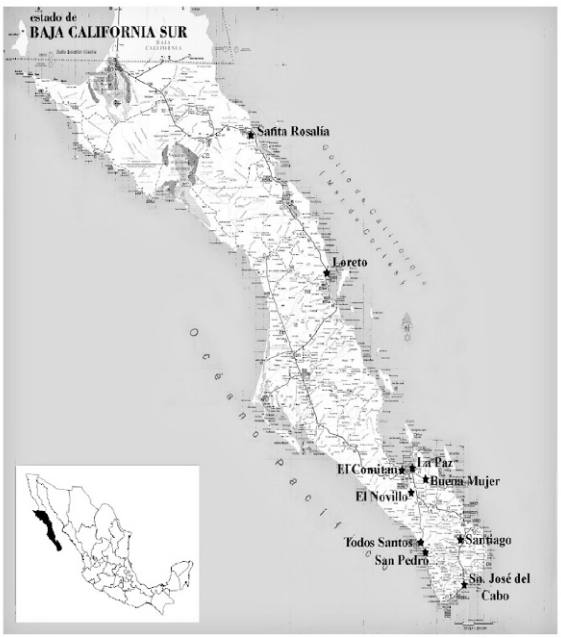

Figure 1 Map of locations in Baja California Sur State.

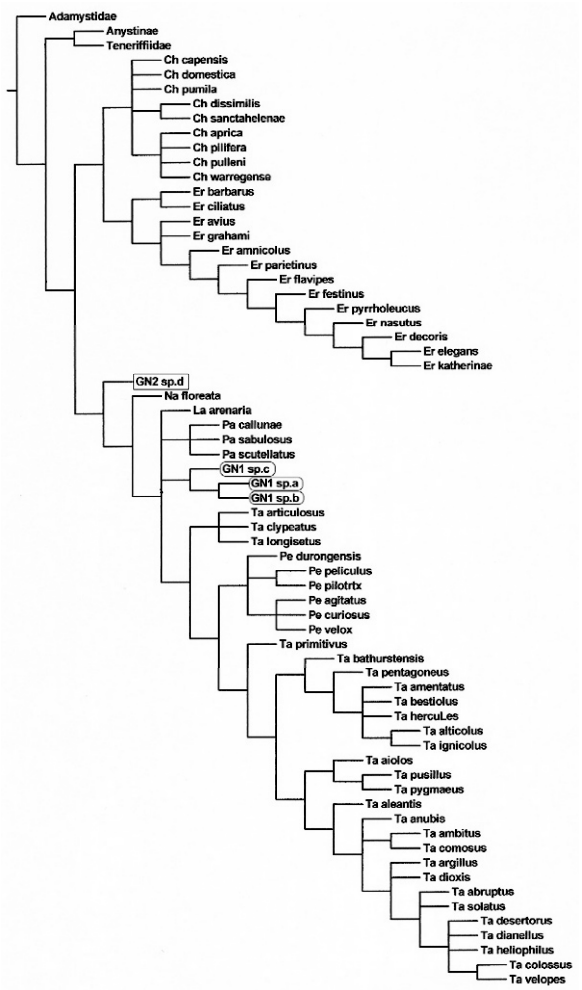

Figure 2 Strict consensus cladogram with new taxa added inside a box. See Otto (2000) for genera abbreviations.

Chaussieria capensis Meyer & Ryke

Chaussieria capensis Meyer & Ryke, 1960: 185.
Chaussieria venustissima (Berlese); Smith Meyer & Ueckermann, 1987: 20 (in part). Misidentification: Otto, 1999c: 264.

Diagnosis

Palp tibia with 4 setae (including claw-like seta); trichobothria *sci* approximately aligned with seta *sce*; tarsi I-III with two pseudosegments; male with a transverse row of 16 feathered setae on each side of the genital opening; trochanter IV with 1 seta, basifemur IV with 7 setae.

Material examined

Baja California Sur: NOV, 3 males, 1 protonymph (PN) 22-04-2004, soil, A. Corrales coll. (ACc); BMJ, 1 male, 2 females, 25-07-2004, soil, ACc; 1 female, 25-08-2004, soil, without collector name (wcn).

Tarsotomus Berlese

Tarsotomus Berlese, 1882: Oudemans, 1936: 438: Smith Meyer & Ueckermann, 1987: 24.

Diagnosis

Dorsal shield absent or present, prodorsal setae close to each other; peritreme reticulated or simple, 1 or 2 pairs of eyes, hypo- or hypertrichous; tarsi with articulations and ending in 2 dentated claws and an empodium; palp tibia with 2 claws; chelicera with 2 setae (Smith Meyer & Ueckermann, 1987). Without empodium on legs I and II, just paired claws.

Material examined

Baja California Sur: *Tarsotomus* sp., SRL, 1 male, 17-06-1997, soil, M. López coll. (MLc).

Erythracarus Berlese

Diagnosis

Palp tibia with 6 or 7 setae (including claw-like seta); setae *ve* further apart than setae *sce*.

Material examined

Baja California Sur: *Erythracarus* sp., LPZ, 1 larva (LV), 22-06-1997, wall, MLc.; *E. nasutus* Otto, TST, 1 female, 20-04-2005, soil, C. Palacios coll. (CPc)

Paratarsotomus Kuznetsov

Paratarsotomus Kuznetsov, 1983: 91. Type species: *Paratarsotomus scutellatus* Kuznetsov, 1983 by monotypy.

Diagnosis

Adults and tritonymphs with conspicuously swollen peritreme and with a prodorsal shield (Otto, 1999b).

Material examined

Baja California Sur: SJC, 1 deutonymph (DN), 21-06-1997, soil, MLc.

New genus GN1

Diagnosis

The following are some of the characters used by Otto (2000) for comparisons in the matrix; the same was done for GN2. Genus with different dorsal hysterosomal setation from any other Erythracarinae. Setae in series *c* have 2, 3, or 4 pairs, implying different species because they are constant, series *d*, *e*, and *f* are variable in number, in the same specimens; palp tibia with less than 6 setae; legs III longer than legs II; legs III with more setae on trochanter (>6), basifemur (>7), telofemur (>7), genu (>13), as well as in genu IV (>14); tibiae I and II longer than tarsi I and II; eupathidium (ζ) on fifth distal pseudosegment of tarsus IV.

Material examined

Baja California Sur: LPZ, 2 females, 1 male, 22-06-1997, wall, MLc; TST, 5 females, 3 males, 1 nymph (N), 22-06-1997, soil, MLc; SJC, 6 females, 1 N, 21-06-1997, soil, MLc; LRT, 11 females, 19-06-1997, soil, MLc; SRL, 7 females, 1 N, 17-06-1997, 5 females, 18-06-1997, soil, MLc; COM, 1 adult (AD), 23-06-1997, soil, MLc, 9 females, 1 N, 1 PN, 18-04-2005, soil, I. Vázquez coll. (IVc); NOV, 8 females, 1 male, 2 PN, 22-04-2004, soil, ACc; BMJ, 2 females, 2 males, 4 PN, 24-05-2004, soil, ACc, 9 females, 6 males, 2 PN, 1 N, 25-05-2004, soil, ACc, 4 females, 2 males, 24-05-2004, soil, ACc, 3 females, 6 males, 25-07-2004, soil, ACc, 2 females, 25-08-2004, soil, wcn.

New genus GN2

Diagnosis

Males and females with 5 setae on cheliceral base, 4 on the proximal half and 1 on distal half; with numerous (18) setae on palp genu; female: with 2 solenidia on tibia I, both in a deep cuticular depression; one tarsal lateral claw distinctly shorter than the other; trochanter II with 9 setae; pseudosegments of tarsi I and II scarcely separated; legs III with more setae than other genera; 11 setae on trochanter, >20 on genu, 8 on basifemur, and 12 on telofemur; more setae also on genu IV (>20); males with 8 pairs of setae on the external margin of genital valves and 16 pairs on internal margin, all setae are pilose-pectinate, no setae on posterior half of genital valves; females with 9 pairs of setae each on internal and external margin of genital valves.

Material examined

Baja California Sur: NOV, 2 females, 1 male, 22-04-2004, soil, ACc.

TENERIFFIIDAE

The family Teneriffiidae Thor is defined by McDaniel et al. (1976) on the basis of the following combination of characters: tarsal claws of leg I broadly bipectinate, claws of other legs various, and may be weakly bipectinate or not bipectinate; claw-like empodia either present or absent on legs III and IV; palps with reduced tarsus with setae arranged in a circular region, hypostome with acorn-shaped blunt spurs apically, and the chelicerae sickle-like.

This family is represented worldwide by 18 species in 5 genera (Eller & Strandtmann, 1963; Strandtmann, 1965; McDaniel et al., 1976; Judson, 1994, 1995; Ueckermann, 2005). At present only *Parateneriffia uta* (Tibbetts, 1958) from Nayarit, *Parateneriffia* sp. from Puebla, and *Teneriffia mexicana* McDaniel et al. (1976) from Sonora, have been reported from Mexico. Other data from unidentified specimens are known from Guanajuato, Morelos, Distrito Federal, Guerrero, and Hidalgo (Hoffmann & López-Campos, 2000).

Neoteneriffiola uta Tibbetts

Neoteneriffiola uta Tibbetts, 1958.
Syn. *Austroteneriffia hirsti* Baker, in Baker & Wharton, 1952: 223 (not *Austroteneriffia hirsti* Womersley), Eller & Strandtmann, 1963.
Parateneriffia uta (Tibbetts, 1958), in McDaniel et al., 1976: 532 (in part).
Neoteneriffiola Hirst, 1924, Judson, 1994: 116, revised status.

Diagnosis

Coxae with 4, 3, 4, 3, setae; venter of opisthosoma with 6 pairs of setae; tarsal claws III and IV weakly bipectinate, tarsi III and IV constricted in the proximal half, with a sensory seta; palpal genu with distal process, the palpal oncophysis of Judson (1994); legs shorter than body; 9 setae on palpal tarsus.

Comments

Eugenital setae of males are similar to those drawn by Judson (1994) for the genus *Neoteneriffiola*: 4 anterior setae (1-4), 2 pairs of median larger setae (5 and 6), 2 pairs of posterior setae (8 and 9) not seen in our specimens, besides k_1 k_2 k_3 setae associated with 3 genital papillae (Fig. 3). McDaniel et al. (1976) showed the setae (their Fig. 20) but didn't mention any number, nor named them, for *P. uta*.

Material examined

Baja California Sur: NOV, 1 female, 26-07-2004, pitfall trap, CPc; ASP, 25 males, 5 females, 1 DN, 20-04-2005, pitfall trap, CPc; Santiago, 1 male, 18-03-2005, pitfall trap, ACH.

DISCUSSION

The results of our survey showed that genus GN1 was abundantly present among specimens from all collection sites (Table 1). We used both the pitfall traps and the aspirator to collect the mites. No data on the distribution of anystids of Mexico are available, but ours suggest that some genera, like GN1, can be expected in other semi-desert environments in the country as well.

Erythracarus and *Chaussieria* were found in two locations, *Paratarsotomus*, *Tarsostomus*, and GN2 only in one. Other genera were very low in numbers, so they were ignored for the purpose of this study [project 'Los Arácnidos Asociados a los Oasis de Baja California Sur' del Centro de Investigaciones Biológicas del Noroeste (CIBNOR)]. However, the remaining genera still need to be revised to improve our knowledge of the erythracarine fauna of the Baja California Sur region.

Genus GN1 seems closely related to *Lacteoscythis* based on dorsal hypertrichosis, but it differs on several other characteristics, such as the number of hysterosomal setae: series *c*, *d*, *e*, and *f* are distinguishable in many specimens, some present 2 in *c*, whereas others have 3 or 4 pairs in *c*, and the number of setae in *d*, *e*, and *f* is variable. Number of setae in series *c* can be used, in combination with other characters, to separate species in the genus. Synapomorphies for GN1 are the number of setae on trochanter, femur, and genu of legs (see diagnosis) and the constant number of dorsal setae in series *c*.

Genus GN2 is a sister group of the group that includes genera *Namadia*, *Lacteoscythis*, *Paratarsotomus*, *Tarsotomus*, and *Pedidromus* (Fig. 2). The autapomorphies are 5 cheliceral setae and numerous setae (17) on palp genu.

Neoteneriffiola uta was abundant in three collection sites, namely Arroyo San Pedro, Arroyo 'El Chorro' Santiago, and El Novillo; the family Teneriffiidae is reported for the first time from Baja California Sur.

Spermatophores, spermatophore deposition, and eggs were not found for the new taxa, only a few immatures were found in the samples but they were not considered for the dataset matrix. We found that the characters listed by Otto (2000) are sufficient for interpretation of phylogenetic relationships among known erythracarine mites. For the new genera it is necessary to search for new characters that resolve the underlying relationships. The anystid fauna of Mexico is still poorly known and more surveys are needed to determine its diversity.

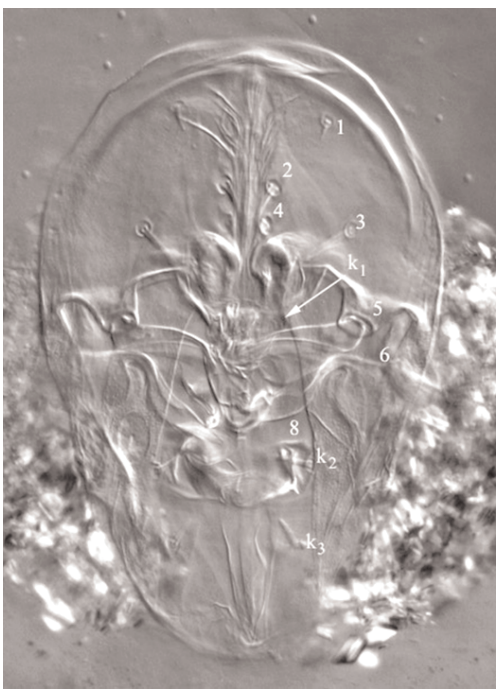

Figure 3 The *Neoteneriffiola uta* male eugenital setae. See text for numbers.

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Appendix

Data for the species added to Otto's data set matrix. See Otto, 2000 for the coding of characters. Numbers in bold are different character state found in this study.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
GN1 ♀ a	1	1	4	1	0	1	0	0	1	0	0	0	?	?	?	2	1	0	1	1	0	1
♀ b	1	1	5	1	0	1	0	0	1	0	0	0	?	?	1	2	1	0	1	1	0	?
♀ c	1	1	6	1	0	1	0	0	1	0	0	0	?	?	1	2	1	0	1	0	0	?
GN2 ♀ d	1	0	4	1	0	0	0	0	1	0	0	0	1	1	1	2	0	2	2	0	0	1

	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
GN1 ♀ a	0	1	1	1	1	2	0	0	3	1	1	1	0	1	0	1	0	0	0	0	1	0
♀ b	?	?	?	?	?	?	?	?	3	1	1	1	0	1	0	1	0	0	0	0	1	0
♀ c	?	?	?	?	?	?	?	?	3	1	1	1	0	1	0	1	0	0	0	0	1	1
GN2 ♀ d	0	1	1	1	1	3	0	0	1	1	0	0	0	1	0	?	1	0	2	0	0	1

	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
GN1 ♀ a	2	1	1	2	0	0	1	1	0	0	0	1	1	1	1	0	0	2	2	2	3	2
♀ b	2	1	1	2	0	0	1	1	0	0	0	1	1	1	1	0	0	2	2	2	3	2
♀ c	2	1	0	2	0	0	1	1	0	0	1	1	1	1	1	0	0	2	2	2	3	2
GN2 ♀ d	2	1	0	2	0	0	1	1	0	1	1	2	2	1	1	0	0	1	2	0	4	2

	67	68	69	70	71	72	72	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88
GN1 ♀ a	0	1	?	0	?	1	2	4	0	2	4	0	1	0	1	1	1	1	0	?	0	1
♀ b	0	1	?	0	?	0	2	4	0	2	4	0	1	0	1	1	1	1	0	?	0	1
♀ c	0	1	?	0	?	0	2	4	0	2	4	0	1	1	1	1	1	1	0	?	0	1
GN2 ♀ d	0	1	1	0	1	1	2	4	0	2	4	0	1	0	1	0	1	0	0	?	0	1

	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110
GN1 ♀ a	0	1	0	0	0	1	0	0	1	1	0	1	1	0	0	0	0	1	0	?	1	2
♀ b	0	1	0	0	0	1	0	0	1	1	0	1	1	0	0	0	0	1	1	?	1	2
♀ c	1	0	1	0	0	0	1	0	0	1	1	0	1	1	0	0	0	0	1	0	?	1
GN2 ♀ d	1	0	1	0	1	0	1	0	1	1	1	0	0	1	0	0	0	0	1	1	?	1

	111	112	113	114	115	116	117	118	119	120	121	122	123–151
GN1 ♀ a	2	1	0	1	1	1	0	0	2	1	0	0	? ?
♀ b	2	0	2	2	1	0	0	0	2	0	0	?	? ?
♀ c	2	1	0	1	2	1	0	0	2	1	0	?	? ?
GN2 ♀ d	1	1	1	1	0	1	0	0	0	1	0	0	? ?

Terrestrial species of the genus *Nanorchestes* (Endeostigmata: Nanorchestidae) in Europe

Matti Uusitalo
Zoological Museum, University of Turku, FIN-20014 Turku, Finland. E-mail: matti.uusitalo@koulut.tampere.fi

Five species in the genus *Nanorchestes* Topsent & Trouessart have been discovered from terrestrial habitats in Europe. The reasons are provided for naming them *N. arboriger* (Berlese), *N.* cf. *collinus* Hirst, *N. pulvinar* Grandjean, *N.* cf. *antarcticus* Strandtmann, and *N.* cf. *llanoi* Strandtmann. The species are keyed and the old descriptions are complemented by scanning electron micrographs of their prodorsa.

Key words: Nanorchestidae, *Nanorchestes*, taxonomy, soil environment, Europe

The genus *Nanorchestes* was originally described from crevices of rocks on the seashore, but these soft and sack-like species are also present in moss, litter, and humus material world-wide. The descriptions of *Nanorchestes arboriger*, *N. collinus*, and *N. pulvinar* refer to the species, living in the terrestrial environments in Europe. Their small size (200-300 µm in adult stage), heavy neotrichy, and tendency to die with the hairy appendages curled up under their venter, makes connecting of collected specimens with the existing descriptions sometimes difficult. Shortcomings in the existing diagnoses have also been noticed (Grandjean, 1942; Schuster, 1965; Niemi et al., 2002). Nevertheless, the species have been reported in the literature despite the fact that the often referred character of cheliceral seta (bifurcate or with one main branch only) can be considered insufficient to validate the use of the old species names. It is beyond the scope of this paper to revise any part of the world fauna. Instead it offers diagnoses and a key to the taxa recently collected from various European environments.

MATERIAL AND METHODS

A total of 197 fresh specimens from Finland and 124 specimens from Svalbard in the north, via Norway, Sweden, Russia, Poland, Austria, France, United Kingdom, Turkey, and Italy to Sicily in the south were examined by 3-dimensional scanning electron microscope (SEM; JEOL JSM-5200). The exact collection data will be published in a separate paper on the distribution and habitat selection of the taxa. The material is deposited in the Zoological Museum of the University of Turku.

Slides 23/1 and 23/2 of the syntype series at Berlese Acaroteca (Cascine del Riccio, Florence, Italy) and the fresh specimens collected from the Boboli Garden (in Florence), the environment of Florence, and in Sicily have been examined for *N. arboriger*. The fresh specimens collected in the backyard of Grandjean's house and the environment of Périgueux in France were compared with the description of *N. pulvinar*.

The prodorsal sensory area and the cheliceral seta on the gnathosoma are emphasized here because they are easily observable and different morphospecies are supposed to have differences between sensory structures. The prodorsa of the taxa encountered were micrographed and compared with all the descriptions published so far of the 33 nominal species of *Nanorchestes* and the three species of *Neonanorchestes*. The phrase 'cf.' in front of a species name indicates that for some reason the identification work is as yet uncompleted.

The setal nomenclature follows the universal terminology of Kethley (1990). The terms and their abbreviations adapted here are the following:

C anterior part of opisthosoma, corresponding C-segment;
Ch chelicera;
cha cheliceral seta;
Co collar or the joint area between chelicera and naso (gnathosoma and prodorsum);
Ep epistome (labrum of Kethley), approx. triangular area at the base of chelicera;
exp prodorsal seta located near posterior eyes (postocular bodies) [authors: *nr* or *xp*];
in prodorsal seta placed behind bases of sensilla *ve* [authors: *nm*];
Na naso for the skin structures between the two bases of *vi/ve*-complex;
sce prodorsal seta [authors: *ne* or *xa*];
sci posterior sensillus of prodorsum [authors: *nb* or *bo*];
vi prodorsal seta on base of which lies the tiny anterior sensillus *ve* [authors: *na* or *ro*].

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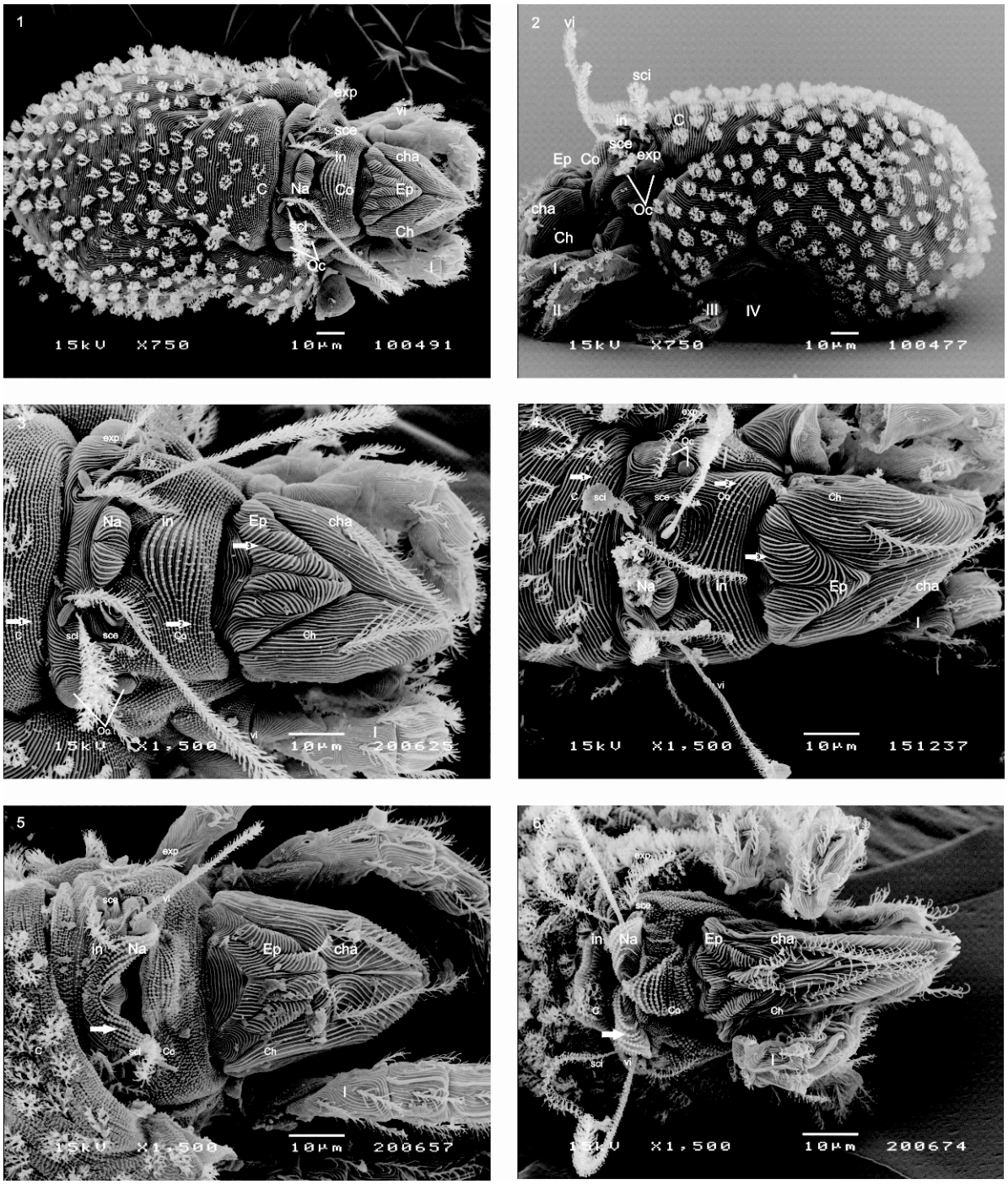


Figures 1-6 *Nanorchestes pulvinar* (1-4), and *N.* cf. *collinus* (5-6). (1) Dorsal habitus, variant 1, with deep grooves on epistome and lots of lamellae on collar and C-segment, see Fig. 3. (2) Lateral habitus, variant 2, with shallow grooves and small amount of lamellae, see Fig. 4. (3) Prodorsum, variant 1, dense lamellation (arrows 1-2), *cha* not furcate, with branchless cilia of various lengths, Ep with deep grooves (arrow 3), Na entire, *in* long, partly covering collar. (4) Prodorsum, variant 2, sparse lamellation (arrows 1-2), *cha*, Ep with shallow grooves (arrow 3), Na and *in* like in Fig. 3. (5) Prodorsum, *cha* bifurcate, with anterior arm twice as long as posterior and with branchless cilia, subequal in length, Ep grooved, Na divided into two wings, with lamellate ridges (arrow). (6) Prodorsum like in Fig. 5, *in* short, partly covering naso. [1-5 from Kangasala, Finland; 6 from Tärna, Sweden].

RESULTS AND DISCUSSION

The six major taxa which came out of the material are considered to represent five morphospecies. The taxa can be keyed or the main characters tabulated as below.

A key to the European taxa

1. Epistome grooveless (Figs. 8-9).
   1a. – Setae *in* long, lamellation sparse
   arboriger (Figs. 7-8).
   1b. – Setae *in* short, lamellation dense
   cf. *antarcticus* (Figs. 9-10).

– Epistome grooved 2.

2. Naso entire (Figs. 3-4).
   2a. – Grooves deep, lamellation dense
   pulvinar variant 1.
   2b. – Grooves shallow, lamellation sparse
   pulvinar variant 2.

– Naso divided (Figs. 5, 12).
   3a. – Wings lamellate, anterior arm of *cha* much longer, cilia simple
   cf. *collinus* (Figs. 5-6).
   3b. – Wings smooth, arms of *cha* subequal in length, cilia branched
   cf. *llanoi* (Figs. 11-12).

The following characters on the prodorsum were found to be without any significant differences and of minor importance when differentiating the European taxa – these were therefore not included in the diagnoses presented here:

– Prodorsal seta *vi* is stout and long, with short, branchless, and branched cilia, becoming progressively longer from base to apex.
– Prodorsal sensillus *ve* is extremely tiny and rarely seen.
– Prodorsal sensillus *sci* is slightly shorter than *vi*, slender, flexible, filiform, with branchless and branched cilia, becoming progressively longer from base to apex, club-like on larvae.
– Prodorsal seta *sce* is relatively short, delicate with branchless and branched cilia.
– Prodorsal seta *exp* is equal or shorter than *in*, with branchless and branched cilia.
– Dorsal setae are branched. The larval setae are bifurcate at base.

Nanorchestes pulvinar Grandjean, 1942

(Figs. 1-4)

Diagnosis

Cheliceral seta *cha* is not furcate and with branchless cilia of various lengths. Epistome's ridges are interrupted by grooves which are shallow or deep, and without lamellae. Naso is entire and without lamellae on ridges, ridges sometimes directed approx. transversely. Prodorsal seta *in* is longer than *exp*, reaching over collar, with branchless and branched cilia. Most of the opisthosomal ridges are without lamellae (Figs. 1-2). Number of lamellae on C-segment and collar varies from prominent to inconspicuous.

The two major variants with the above listed character states have differences in the deepness of the epistome grooves and in the number of transverse lamellae on the ridged skin (Fig. 3 vs. 4). The lamellae are not mentioned or drawn at all in the original description. Variant no. 1 (Fig. 3) has deep grooves on the epistome and the ridges of collar and anterior part of opisthosoma (C-segment, Fig. 2) are densely lamellate (compared to three other European species below, with dense lamellation on the whole body). I have collected only variant no. 1 from the backyard of Grandjean's house in Périgueux, France, but the epistome in Grandjean's Figure A rather resembles epistome of the variant no. 2. Variant no. 2 (Fig. 4) has shallow grooves and almost lacks lamellae. Both variants have been found in the same samples in Finland. There is also variation in the quality of cilia and ridges. The cilia of prodorsal setae by Grandjean are all branchless but prodorsal setae with branched cilia have been observed, and specimens with nasal ridges running transversely. At the moment I consider the two taxa as variants of *N. pulvinar*.

Nanorchestes cf. *collinus* Hirst, 1918

(Figs. 5-6)

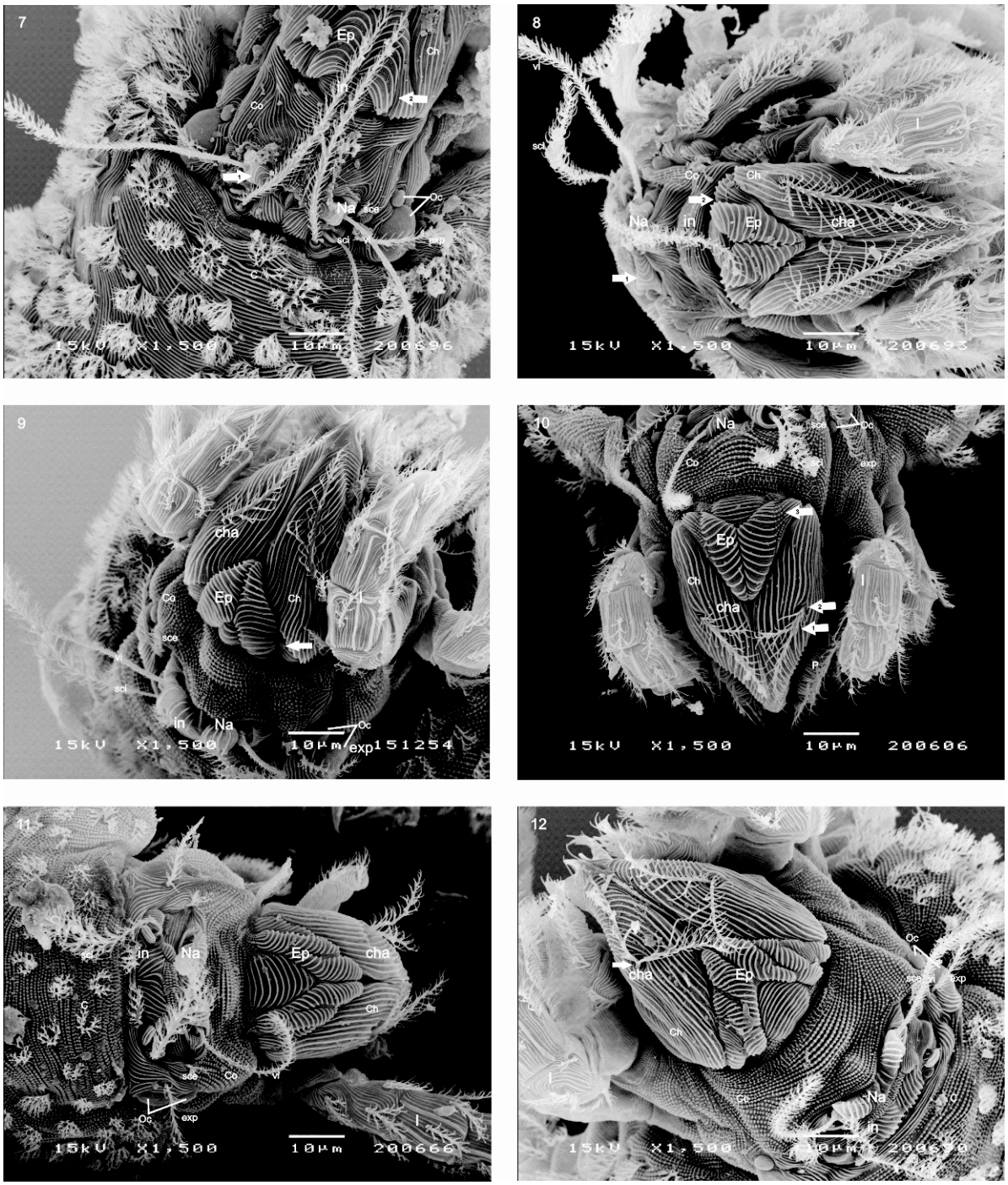
Diagnosis

Cheliceral seta *cha* is unequally bifurcate, anterior arm much longer than the posterior one, arms with branchless cilia of rather uniform lengths. Epistome is deeply grooved and without lamellae. Naso is medially divided into two wings with lamellate ridges. Prodorsal seta *in* is equal in length with *exp*, short, reaching over the nasal wings, and with branchless cilia.

According to Hirst (1918), the cheliceral seta of *N. collinus* from terrestrial inland habitat differs from the cheliceral seta of *N. amphibius* Topsent & Trouessart in three respects. The cheliceral seta of *N. collinus* is (1) slender and (2) divided close to the base into two plumose branches, (3) the outer one being considerably longer than the other. The type species of the genus *Nanorchestes* from France, *N. amphibius*, to which Hirst refers, was originally described without any illustration, but Hirst (1917) depicts a stout, non-furcate seta with branchless cilia for the chelicerae of the specimens he collected on the sea shore of the Isle of Wight. Accordingly, the specimens of this material with branchless cilia in their bifurcated cheliceral setae agree well with the original description of *N. collinus*. The holotype in alcohol is in the Museum of Natural History, London (Dr. A. Baker, in litt.), but I have not yet inspected it. Thor & Willmann (1941) refer to the original description but the specimens – examined, reported, and depicted as *N. collinus*

A table of the European taxa of the genus *Nanorchestes* Topsent & Trouessart, 1890

Species	epistosomal grooves	nasal wings	length of *in*	division of *cha*	opistosomal lamellation
pulvinar var. 1	deep	missing	up to collar	undivided	dense on C-area
pulvinar var. 2	shallow	missing	up to collar	undivided	sparse on C-area
cf. *collinus*	deep	lamellate	up to naso	unequal	dense all over
arboriger	missing	smooth	to epistome	undivided	sparse on C-area
cf. *antarcticus*	missing	smooth	up to naso	undivided	dense all over
cf. *llanoi*	deep	smooth	up to naso	subequal	dense all over



Figures 7-12 *Nanorchestes arboriger* (7-8), *N.* cf. *antarcticus* (9-10), and *N.* cf. *llanoi* (11-12). (7) Prodorsum, Na divided into two wings with smooth ridges (arrow 1), *in* very long, extending over epistome, Ep without grooves, depression not lamellate (arrow 2). (8) Prodorsum, *cha* not furcate, with branched cilia of various lengths, Ep, Na and *in* like in Fig. 7. (9) Prodorsum, *cha* not furcate, with asymmetrically elongated and branched cilia, Ep without grooves, depression lamellate (arrow), Na divided into two wings with smooth ridges, *in* short, partly covering naso. (10) Prodorsum, *cha* with one of the branched cilia elongated on basal half (arrows 1-2), Ep (lamellate depression, arrow 3), Na and prodorsal setae like in Fig. 9. (11) Prodorsum, *cha* branched, with branched cilia, Ep grooved, Na divided into two wings, with smooth ridges, *in* short, hardly reaching naso. (12) Prodorsum, *cha* bifurcate near its base (arrow), arms subequal in length, with branched cilia of various lengths, Ep, Na and *in* like in Fig. 11. [7-8 from Sicily, Italy; 9 from Tannu-Ola, Russia; 10 from Florence, Italy; 11 from Kangasala, Finland; 12 from Utsjoki, Finland].

by Willmann (1943, 1956), Womersley (1944), and Strandmann (1982) from Sweden, Germany, southern Australia, and northern Alaska (USA), respectively – have bifurcate cheliceral setae with forks of subequal length and branched cilia, and rather refer to a taxon like in Figs. 11-12.

Nanorchestes arboriger (Berlese, 1904)

(Figs. 7-8)

Diagnosis

Cheliceral seta *cha* is not furcate and with branched cilia of unequal lengths. Epistome is grooveless, i.e., the ridges are unbroken but with a depression at each basal corner. Naso is medially divided into two wings, ridges without lamellae. Prodorsal seta *in* is much longer in length than *exp*, stretching across naso, collar, and over the epistome, thick, especially towards the base, densely ciliate, branchless, and branched cilia longest along the middle.

The description of the dorsum by Berlese (1904: 14, Fig. 13) can be considered superficial and oversimplified by modern standards, but the pair of backward projecting setae in his description is stiff, stout, and even thicker than the anterior pair of setae *vi*. As a rule the sensilla *sci* are finer and more flexible compared to setae *vi*, like the setae which Berlese has drawn on the shoulders of his specimen. At closer inspection there are two character states of diagnostic value in his figure: (1) the long and tapering pair of setae, which obviously are the *interlamellars*, and (2) the cheliceral setae, which are not furcate. The slides of the type series are brown and specimens are poorly visible. Specimen 23/1 has the cheliceral seta unbranched but prodorsum cannot be seen properly, whereas on specimen 24/2 the pairs of *vi, sci*, *exp*, and *in* are well observable, and the seta *in* is long, tapering, and thicker than *vi*. The fresh specimens of the Boboli Garden and Sicily in figures 7-8, with a long, prominent, and tapering pair of *in* setae, stretching forward across the broad wings of naso and over the grooveless epistome, are equivalent to the *arboriger* described by Berlese. So far, I have found the species only in my Italian material. The earlier records of the species – e.g., by Womersley (1944), Haarløv (1957), Dindal & Norton (1979), Bååth et al. (1980), Purvis (1982), and Uusitalo & Huhta (1995) from Australia, Denmark, USA, Sweden, Ireland, and Finland, respectively – should be checked again.

Nanorchestes cf. *antarcticus* Strandtmann, 1963

(Figs. 9-10)

Diagnosis

Cheliceral seta has one main arm with branched cilia of various lengths. A branched cilium can be elongated only in one of the two cheliceral setae, i.e., the cilia may be asymmetrically branched (Fig. 9). In the most extreme case the seta may look divided, if one of the branched cilia is much longer than the others, but the exceptionally elongated and branched cilium is never the most basal one (Fig. 10). Epistome's ridges are grooveless and without lamellae except for the lamellation in the cuticular depression. The naso has coarse ridges without lamellae, divided into two wings, one over base of each *vi/ve* complex. Prodorsal seta *in* is shorter in length than *exp*, partly covering the naso.

The European specimens at hand suggest a large variation in lengths of the basal cilia of the cheliceral setae. Strandtmann (1967) reports similar variation on widely distributed and common *N. antarcticus* Strandtmann from various parts of Antarctica – therefore 'cf. *antarcticus*' as a work name. The quality of epistome, collar, naso, and *in* setae seems quite constant in the European material. Booth (1984) uses the number of setae of various leg segments to separate the Antarctic species of *Nanorchestes* but the differences of one or two setae in the key are based on counting of an unreported number of specimens, with unknown amount of pedal neotrichy. Intraspecific variation of the prodorsal characters should also be examined from fresh material of the type locality (Observation Hill, Site #1, Ross Island, Antarctica) and several localities around the world, rather by use of the SEM-technique and the type material of *N. antarcticus* (BISHOP 3427) should be re-examined by phase-contrast to confirm the species.

Nanorchestes cf. *llanoi* Strandtmann, 1982

(Figs. 11-12)

Diagnosis

Cheliceral seta *cha* is bifurcate at base, with subequal arms, both arms may be secondarily branched, and arms have branchless and branched cilia of uneven lengths. Epistome has prominent ridges without lamellae, with deep grooves. Naso with coarse ridges without lamellae is divided into two wings. Prodorsal seta *in* is shorter than *exp*, hardly reaching over the naso, cilia mostly branched.

Strandtmann (1982) described three species from Alaska and one of these has the posterior sensillum with a dense brush of long, branched cilia and the cheliceral seta has two arms arising from a single axis near its base, both arms having branched cilia of unequal lengths – therefore 'cf. *llanoi*' as a work name, but the Alaskan fauna should be re-examined.

Conclusion

The importance of sensory organs in taxonomy is well recognized, but inclusion of the elaborate skin pattern seemed to improve essentially the usefulness of the prodorsal sensory area. The detailed pictures of the prodorsa of the European nanorchestids could be used like passport photographs for the species. Even specimens with some injuries can be identified (e.g., Figs. 5 and 6). A database like this of the prodorsa of other mite taxa as well might have a positive effect on identification work and taxonomy in general. The data accessible and available for any student in the field might enable them to compare their specimens and to use similar symbolic names of the taxa at hand, regardless of the state and completeness of a revision work in a particular group of mites.

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Ptyctima (Acari, Oribatida) in various habitats in Finland

R. Penttinen & V. Huhta[1]

Zoological Museum, University of Turku, FIN-20014 Turku, Finland. E-mail: ritniemi@utu.fi; and [1]Department of Biological and Environmental Science, University of Jyväskylä, PO Box 35, 40014, Jyväskylä University, Finland

The oribatid species of the taxon Ptyctima were studied in eight habitat types (four forests, three bogs, and one shore). Altogether 19 species were found. The highest number of species (15) was found in marsh forests in southern Finland, and the lowest number (3) in eutrophic fens in northern Finland. *Atropacarus striculus* was the most common and abundant species in Finland. It was found in each habitat explored, especially abundant on shores, in marsh forests, and pine bogs. *Phthiracarus longulus*, *P. boresetosus*, and *Steganacarus carinatus* occurred in abundance in coniferous forests, especially in the southern and central parts of the country, whereas *Rhysotritia ardua* was found there only occasionally. *Hoploderma pavidus* favoured bog habitats in Finland. *Protoribotritia oligotricha* was new to Finland. It was found in low numbers in the north.

Key words: Bogs, Finland, forests, oribatids, Ptyctima, shore

The oribatid communities of various forests have been studied intensively in Finland (Karppinen, 1958a,b, 1972, 1977; Huhta et al., 1986; Huhta & Niemi, 2003, 2005). Therefore, the oribatid fauna is fairly well known. Nevertheless a country-wide, systematic study covering different habitats has been carried out only for the family Camisiidae (Karppinen, 1955). Here, we focus on the ptyctimous mites in eight habitats in Finland. This study is a part of a wider, ongoing investigation, entitled 'Occurrence and distribution of poorly known soil animal groups in Finland'.

MATERIALS AND METHODS

Eight habitat types were explored: four forests (dry coniferous, mesic coniferous, mesic deciduous, and marsh forest), three bogs (pine bog, open bog, and eutrophic fen), and one shore (lakeside). Nine sampling areas were chosen all over Finland (Fig. 1). In every sampling area, four replications of each habitat type were selected (three in northern Finland, occasionally on eutrophic fens), and in each three soil samples (area 25 cm^2) were taken.

Mites were extracted in the laboratory using a modified high-gradient apparatus (Macfadyen, 1961). Material was identified (Balogh & Mahunka, 1983; Niedbala, 1992, 2002) and counted. The areas sampled were taken together, grouped into three parts (southern, central, and northern Finland). Densities per area were not calculated. The original data are available upon request.

RESULTS

Numbers of specimens

In total 7,467specimens were collected. In southern Finland the number (3,582) was >3× higher than in the north (1,166), in central Finland the number (2,719) was intermediate. Most specimens were found in pine and open bogs, the fewest in eutrophic fens (Fig. 2). The numbers in marsh forests, especially in southern and central Finland were high, i.e., close to those in bogs.

Numbers of species

Altogether 19 species were found (Table 1). Most (15) were found in marsh forests in southern Finland, fewest (3) in eutrophic fens in northern Finland (Fig. 3). The number of species in northern Finland was lower in almost every habitat type, compared to southern and central Finland.

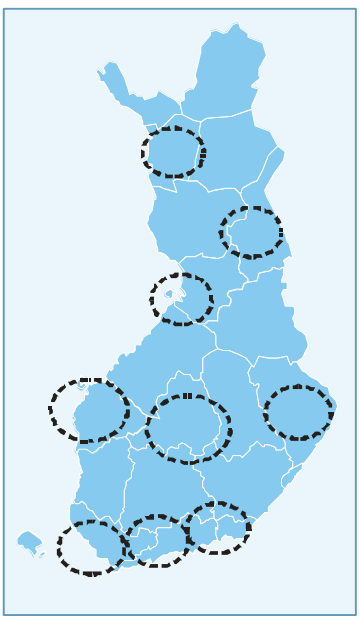

Figure 1 Sampling areas in Finland.

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Table 1 The oribatid species in three regions in Finland.

Species	South Finland	Central Finland	North Finland
Phthiracarus			
P. boresetosus Jacot	X	X	X
P. bryobius Jacot	X	X	X
P. globosus (CL Koch)	X	X	X
P. longulus (CL Koch)	X	X	X
P. nitens (Nicolet)	X	X	X
P. crinitus (CL Koch)	X	X	X
P. lentulus (CL Koch)	X	X	-
P. ferrugineus (CL Koch)	X	X	-
Atropacarus			
A. striculus (CL Koch)	X	X	X
Hoplophthiracaus			
H. pavidus (Berlese)	X	X	X
Steganacarus			
S. applicatus (Sellnick)	X	X	X
S. carinatus CL Koch	X	X	X
Mesotritia			
M. nuda (Berlese)	X	-	X
M. flagelliformis Ewing	X	X	X
Protoribotritia			
P. oligotricha Märkel	-	-	X
Euphthiracarus			
E. cribrarius (Berlese)	X	X	-
E. monodactylus (Willmann)	X	X	X
Microtritia			
M. minima (Berlese)	X	-	-
Rhysotritia			
R. ardua (CL Koch)	X	X	X

Communities of Ptyctima in habitat types

Dry coniferous forest (Fig. 4a)

Phthiracarus longulus, *Steganacarus carinatus*, and *Atropacarus striculus* occurred abundantly in coniferous forests. The first species was numerous in all three parts of Finland, *S. carinatus* was numerous in southern and central Finland, whereas *A. striculus* was common only in southern Finland.

Mesic coniferous forest (Fig. 4b)

The number of *S. carinatus* was highest in southern Finland, in central Finland it was as common as *P. boresetosus*, but in

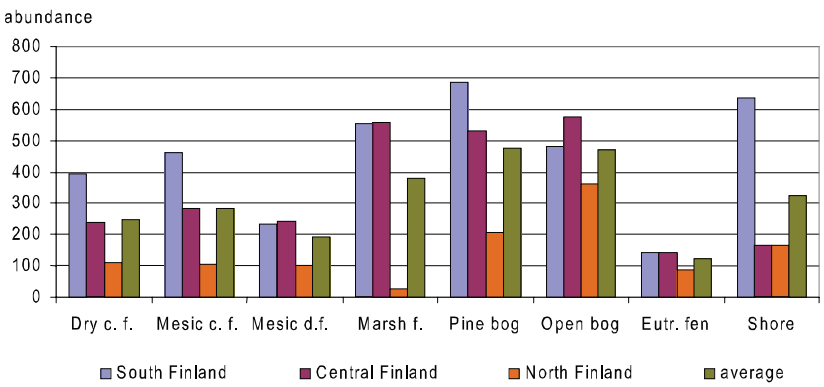

Figure 2 Oribatid specimens in eight habitats: dry and mesic coniferous forest, mesic deciduous forest, marsh forest, pine and open bog, eutrophic fen, and shore.

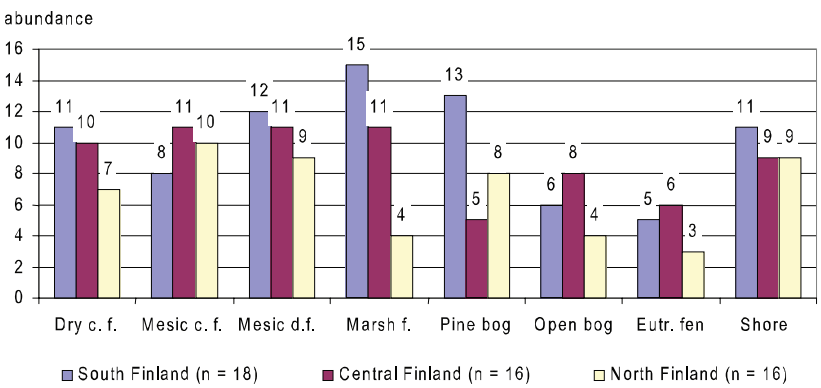

Figure 3 Oribatid species in eight habitats: dry and mesic coniferous forest, mesic deciduous forest, marsh forest, pine and open bog, eutrophic fen, and shore. n, total number of species.

northern Finland it was found only occasionally. *Steganacarus applicatus* occurred more than average in southern Finland.

Mesic deciduous forest (Fig. 4c)

Clearly, *A. striculus* dominated throughout the country. *Phthiracarus globosus* was found commonly, whereas it was rare in coniferous forests.

Marsh forest (Fig. 4d)

Atropacarus striculus dominated even more distinctly in marsh forests than in mesic deciduous forests. However, it is noteworthy that the numbers of almost all species were very low in northern Finland.

Pine bog (Fig. 5a)

Also in this habitat *A. striculus* dominated, especially in southern and central Finland. In southern Finland *S. carinatus* was abundant, but absent from other parts. *Hoplophthiracarus pavidus* was absent in forests, but clearly favoured pine bogs. In contrast to forests, the numbers of *Rhysotritia ardua* were high in pine bogs

Open bog (Fig. 5b)

More than other bog types, *H. pavidus* favoured the open bog, where it was 2-3× more abundant than in pine bogs.

Eutrophic fen (Fig. 5c)

Here *A. striculus and H. pavidus* dominated too. The number of *H. pavidus* was highest in southern Finland, *A. striculus* was more common than *H. pavidus* in central and northern Finland.

Shore (Fig. 5d)

Most dominant was *A. striculus*; it was more abundant here than in any other habitat.

Habitat preferences of the most common species (Fig. 6)

Atropacarus striculus was found in every habitat. It was the most common and numerous species in the whole country. It favoured shores, marsh forests and pine bogs. *Phthiracarus longulus* preferred dry coniferous forest to other habitats. Also *P. nitens* and *P. bryobius* favoured this habitat. *Steganacarus carinatus* as well as *S. applicatus*, *P. boresetosus*, and *Euphthiracarus monodactylus* preferred mesic coniferous forests to other forest types. Mesic deciduous forest was the most favourable habitat for *P. globosus*, marsh forest for *P. ferrugineus. Rhysotritia ardua* was mainly found in pine bogs. *Hoplophthiracarus pavidus* proved to be a true bog species. Open bog was the most suitable habitat for this species.

Species with exclusive occurrences

Protoribotritia oligotrichia – new to the fauna of Finland – was found only in northern Finland, whereas *Microtritia minima* was only found in southern Finland. *Mesotritia nuda*, *M. flagelliformis*, *Euphthiracarus cribrarius*, *P. lentulus*, and *P. crinitus* were found occasionally in habitats throughout Finland.

DISCUSSION

The oribatid group Ptyctima forms a minority in the overall oribatid community in Finland. Previous investigations in Finnish forests and bogs concerned whole communities of oribatids (Huhta et al., 1986; Huhta & Niemi, 2003, 2005; Karppinen, 1958a,b, 1972, 1977; Markkula, 1986) and species

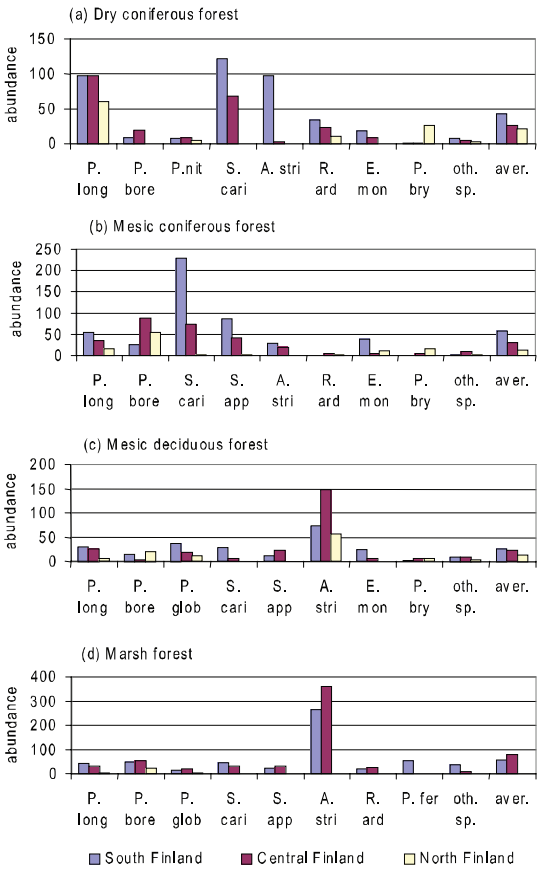

Figure 4 Oribatids in four forest types: dry coniferous forest (a), mesic coniferous forest (b), mesic deciduous forest (c), and marsh forest (d). See Table 1 for full species names.

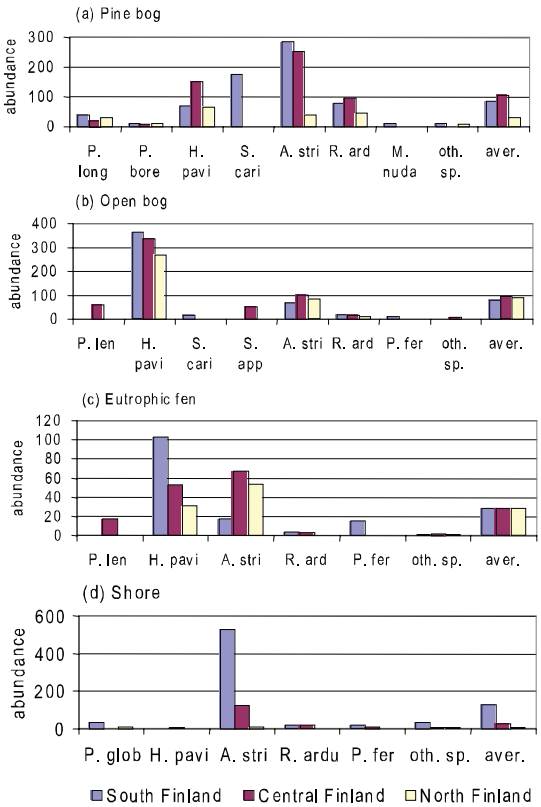

Figure 5 Oribatids in three bog types and one shore type: pine bog (a), open bog (b), eutrophic fen (c), and lakeshore (d). See Table 1 for full species names.

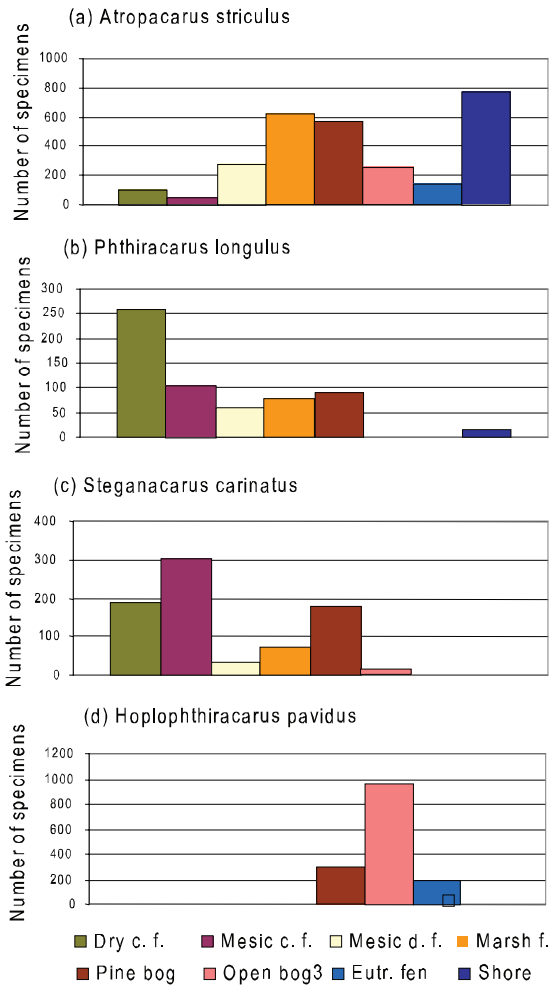

Figure 6 Habitat preference of four oribatid species: *Atropacarus striculus* (a), *Phthiracarus longulus* (b), *Steganacarus striculus* (c), and *Hoploderma pavidus* (d). Dry c.f. = dry coniferous forest, Mesic c.f. = mesic coniferous forest, Mesic d.f. = mesic deciduous forest, Marsh f. = marsh forest, Eutr. fen = eutrophic fen.

of Ptyctima received little attention. Our examination of this taxon only was suitable to reveal the relative numbers of these species in different habitats. Also, our study clearly demonstrated the habitat preferences of these species.

The numbers of specimens in eutrophic fens were very low, but this may be due to the small number of samples. Numbers in northern Finland were also low, which is partly explained by the smaller number of replicate habitats sampled (3). Seven species were found in low numbers only. This does not necessarily mean that these species are rare. They may live in other microhabitats than the ones sampled, e.g., decaying wood, stumps, or needles.

In conclusion, 19 species of Ptyctima oribatids were found. *Protoribotritia oligotrichia* was new to Finland. The most dominant species in three habitats – marsh forests, mesic deciduous forests, and lakeshores – was *A. striculus*, whereas *P. longulus* was the most abundant species in dry coniferous forests. *Steganacarus carinatus* was mostly found in mesic coniferous forests, *H. pavidus* occurred only in bog habitats. Almost all species were found throughout the whole country.

Acknowledgments

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Distribution of *Cosmochthonius* species (Oribatida: Cosmochthoniidae) in the eastern part of the Mediterranean, Ukraine and Tajikistan

R. Penttinen & E. Gordeeva[1]

Zoological Museum, University of Turku, FIN-20014 Turku, Finland. E-mail: ritniemi@utu.fi; and [1]Karadag Natural Reserve, Ukranian National Academy of Sciences, P.O Kurortnoe, Feodosia 98188, Crimea, Ukraine

Eleven species of the genus *Cosmochthonius* have been recorded in the eastern Mediterranean and in some parts of the former Soviet Union. Five of these species [*C. reticulatus* Grandjean, *C. foliatus* Subias, *C. lanatus* (Michael), *C. semifoveolatus* Subias, and *C. asiaticus* Gordeeva] have been found in the western Mediterranean as well, whereas the other six species (*C. ugamaensis* Gordeeva, *C. zanini* Niemi & Gordeeva, *C. macrosetosus* Ayyildiz & Luxton, *C. tenuisetosus* Gordeeva, *C. taurus* Niemi, Gordeeva & Ayyildiz, and *C. ponticus* Gordeeva) can be considered to be endemic. The most common species in the eastern Mediterranean was *C. reticulatus*. It was found on the Mediterranean islands (Crete, Cyprus, Rhodes, Symi-Rhodes, and Lesvos) and in the coastal zone of Turkey and the Ukraine. In contrast to this distribution, the species *C. foliatus* seemed to be absent from these islands and from the coastal zone of Turkey, but it regularly occurred in the southern and southeastern Crimea, Ukraine. The exact locations of the species found in the eastern region are documented here, and the habitus of eight species is presented in the form of SEM micrographs.

Key words: *Cosmochthonius*, oribatids, distribution, Mediterranean

The mites of the genus *Cosmochthonius* Berlese are found almost everywhere in the arid and dry subtropical regions of the world. The highest number of species in this genus has been recorded from the Mediterranean region (Subias, 2004). The species of the western Mediterranean region have been listed by Subias & Gil-Martin (1997), whereas those of the eastern Mediterranean were lacking. Here, we present the species both of the eastern Mediterranean region and of the southern territory of the former Soviet Union, based on published as well as unpublished material. The distribution of these species is described and the habitus of eight species is illustrated by means of SEM micrographs.

MATERIAL AND METHODS

The new material has been collected on the islands of Greece (Lesvos, 2006; Rhodes and Symi-Rhodes, 1997) and also from Russia, Tuva Republica (1995). The specimens have been studied by the aid of a scanning electron microscope (SEM, JEOL JSM-5200).

RESULTS

Cosmochthonius reticulatus Grandjean (Fig. 1a) is the most common species in the eastern Mediterranean. It has been found on the islands of Greece, Crete and Cyprus, and in the south-western coastal zone of Turkey and the Ukraine, Crimea (Penttinen & Gordeeva, 2006). In addition, it was now found in the material collected from the Greek islands Lesvos, Rhodes, and Symi-Rhodes.

Examined material

Greece – Rhodes, Petaloudes Kalopteras monastery, *Arbutus* sp. & *Quercus coccifera* litter 31.5.1996, Ritva Niemi leg., 26 exx. [ACA.ORI.PAL 0.061]ZMT; Rhodes, Stegna, *Pinus* sp. litter, 1.6.1996, Ritva Niemi leg., 8 exx. [ACA.ORI.PAL 0.063]ZMT; Rhodes, Atavyros, *Cupressus sempervirens* litter, 31.5.1996, Ritva Niemi leg., 4 exx. [ACA.ORI.PAL 0.066]ZMT; Rhodes, Stegna, *Pinus halepensis* litter, 1.6.1996, Ritva Niemi leg., 3 exx. [ACA.ORI.PAL 0.068]ZMT; Rhodes, Petaloudes, mixed litter, 31.5.1996, Ritva Niemi leg., 2 exx. [ACA.ORI.PAL 0.070]ZMT; Symi-Rhodes, Simi, *C. sempervirens* litter, 2.6.1996, Ritva Niemi leg., 73 exx. [ACA.ORI.PAL 0.067]ZMT; Lesvos, near Achladeri, pine forest, *Pinus* sp. litter, 39°08.989'N, 26°18.105'E, 22 m, 8.5.2006, R. Penttinen leg., 29 exx.; Lesvos, Vlachos, near Parakila, pine forest, *Arbutus* sp. litter, 39°10.918'N, 26°07.445'E, 135 m, 9.5.2006, R. Penttinen leg., 1 ex.; Lesvos, near Filia, *Juniperus macrocarpa* litter, oak woodland & meadow, 39°15.534'N, 26°09.225'E, 470 m, 9.5.2006 R. Penttinen leg., 103 exx.

Cosmochthonius lanatus (Michael) (Fig. 1b) is a rare species in the eastern Mediterranean. Only one record has been made (in the western Ukraine) under the name *C. novus* Sergienko, which has been confirmed to be the junior synonym of *C. lanatus* (Penttinen & Gordeeva, 2006).

Cosmochthonius foliatus Subias (Fig. 1c) is a common species in the western Mediterranean (Gil et al., 1991). Our studies also revealed that it regularly occurs in the coastal zone of the South Ukraine and Crimea (Penttinen & Gordeeva, 2006). However, it seemed to be absent from the Greek islands Crete, Cyprus, Rhodes, Symi-Rhodes, and Lesvos, and also from the southeastern coast of Turkey.

Cosmochthonius zanini Penttinen & Gordeeva (Fig. 1d) has been found in the southeastern Crimea, on the southwestern coast of Turkey, and on Rhodes (Penttinen & Gordeeva, 2003). In addition, the species was found in the new material collected from Lesvos.

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Examined material

Greece, Lesvos – Vlachos near Parakila, pine forest, *Cistus* sp. litter, 39°10.918'N, 26°07.445'E, 135 m, 9.5.2006, R. Penttinen leg., 7 exx.; Vlachos near Parakila, pine forest, *Pinus* sp. litter, 39°10.918'N, 26°07.445'E, 135 m, 9.5.2006, R. Penttinen leg., 9 exx.; Vlachos near Parakila, pine forest, *Arbutus* & *Erica* sp. litter, 39°10.918'N, 26°07.445'E, 135 m, 9.5.2006, R. Penttinen leg., 1 ex.; near Achladeri, pine forest, *Cistus* sp. litter, 39°09.198'N, 26°17.801'E, 20 m, 5.5.2006, R. Penttinen leg., 2 exx.; near Mesa Sanctuary, mixed forest, *Pistacia lentiscus* litter, 39°10.896'N, 26°17.928'E, 7 m, 5.5.2006, R. Penttinen leg., 1 ex.; Sykominia, woodland, *Quercus coccifera* & *Juniperus macropedia* litter, 39°21.320'N, 26°17.947'E, 520 m, 6.5.2006, R. Penttinen leg., 5 exx.; near Filia, oak woodland & meadow, *Quercus* sp. litter, 39°15.534'N, 26°09.225'E, 470 m, 7.5.2006, R. Penttinen leg., 8 exx.; near Parakila, Vlahos, *Rhododenron* litter, 39°11.158'N, 26°07.367'E, 200 m, 9.5.2006, R. Penttinen leg., 1 ex.; near Achladeri, sea shore, *Pistacia* litter, 39°10.276'N, 26°17.515'E, 20 m, 10.5.2006, R. Penttinen leg., 17 exx.

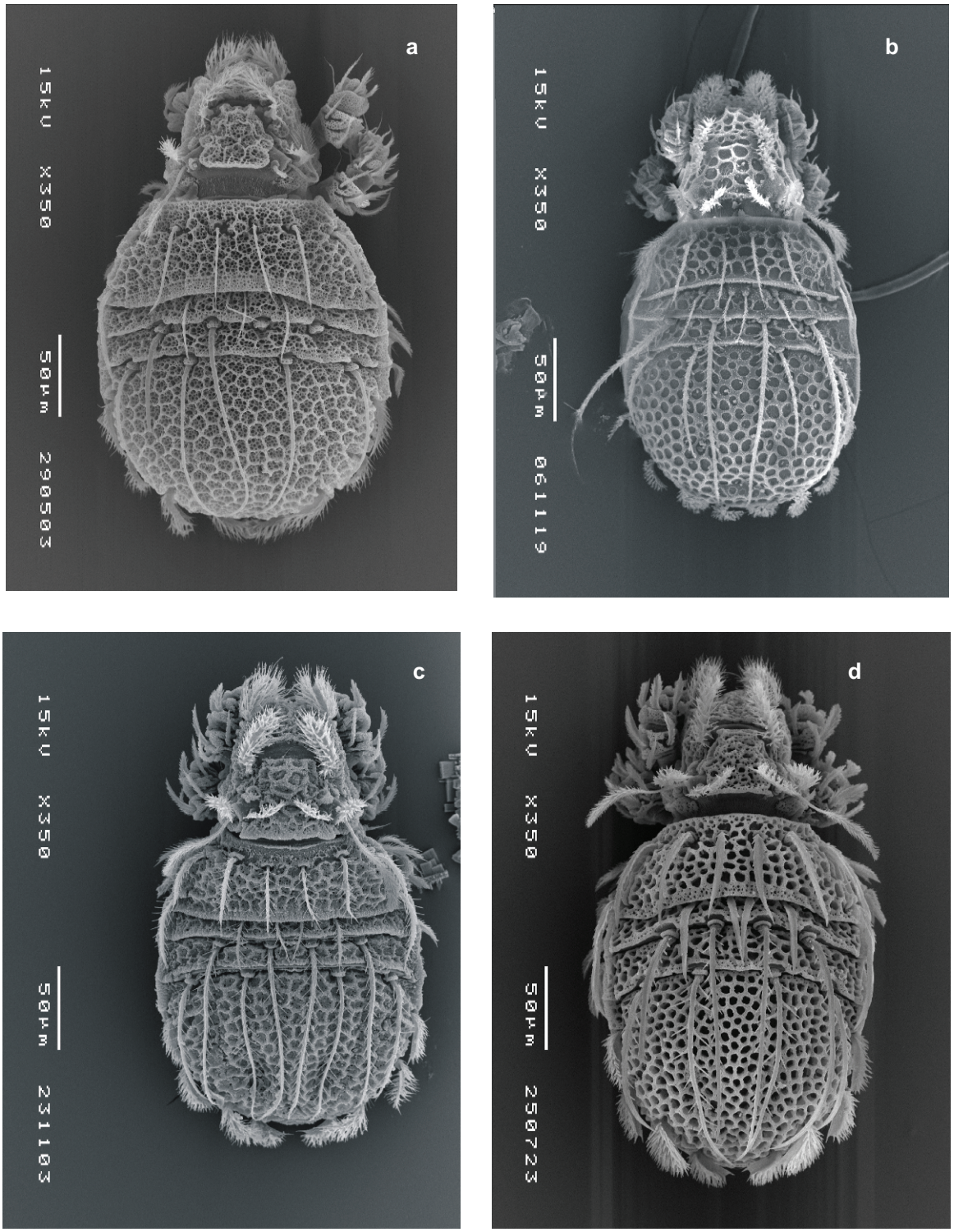

Figure 1 Habitus of (a) *Cosmochthonius reticulatus*, (b) *C. lanatus*, (c) *C. foliatus*, and (d) *C. zanini*.

Cosmochthonius semifoveolatus Subias (Fig. 2a). The specimens collected from the mountain side of the southeastern Crimea, Karadag, have earlier been identified as *C. ponticus* by Gordeeva (1980). However, our SEM study now revealed that the species is *C. semifoveolatus*.

Cosmochthonius ugamaensis Gordeeva (Fig. 2b) has been found earlier in Tajikistan (Gordeeva, 1980), and we also found it on the island of Lesvos.

Examined material

Greece, Lesvos – Sykominia, woodland, *Olea europea* litter, 39°21.320'N, 26°17.947'E, 520 m, 6.5.2006, R. Penttinen leg., 75 exx.; near Filia, oak woodland & meadow, *J. macrocarpa* litter, 39°15.534'N, 26°09.225'E, 470 m, 9.5.2006, R. Penttinen leg., 3 exx.

Cosmochthonius asiaticus Gordeeva (Fig. 2c) has been found in Tajikistan in the district of Dushanbe (Gordeeva, 1980), and a recent finding was made in the material collected from Russia, Tuva Republica.

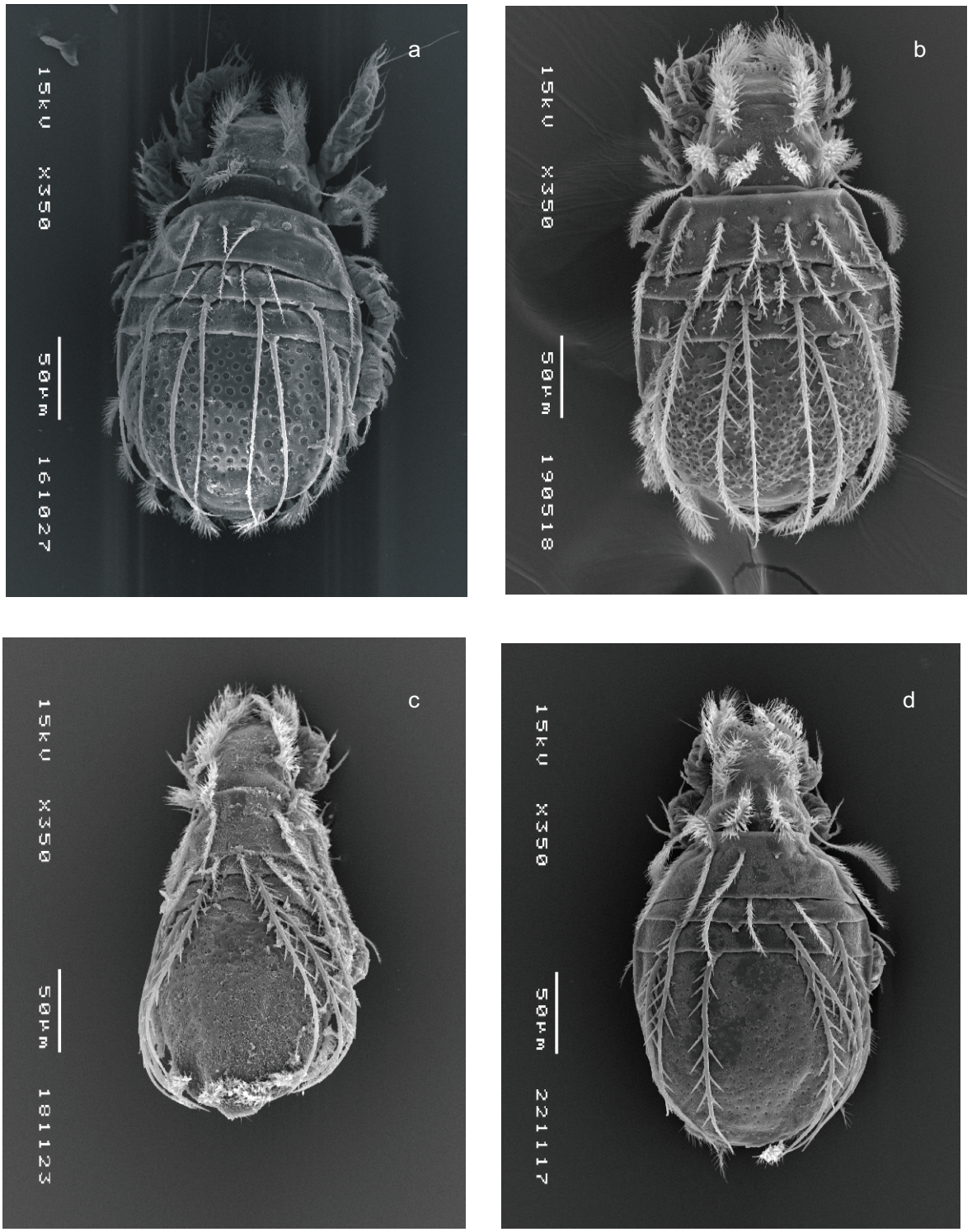

Figure 2 Habitus of (a) *Cosmochthonius semifoveolatus*, (b) *C. ugamensis*, (c) *C. asiaticus*, and (d) *C. tenuisetus*.

Examined material

Russia – Tuva Republica NE, in saline soil zone of salt lake, 15.6.1995, Matti Uusiatlo leg., 5 exx. [ACA.ORI.PAL 0.086]ZMT.

Cosmochthonius tenuisetus Gordeeva (Fig. 2d) has been found in the southeastern coastal zone of Crimea (Gordeeva, 1980). In the same article, the specimens – collected from the southern coastal zone of Crimea – were published under the name *C. plumatus*, but our recent SEM studies have revealed them to be *C. tenuisetus*.

Cosmochthonius taurus Niemi, Gordeeva & Ayyildiz, so far, has only been found in Turkey, in the mountains of Taurus.

Cosmochthonius macrosetosus Ayyildiz & Luxton has only been found in the eastern part of Turkey (Ayyildiz & Luxton, 1990).

Cosmochthonius ponticus Gordeeva has been found both in Ukraine in the district of Donetzk, and in the southern Crimea, close to Sevastopol (Gordeeva 1980). In both cases, the specimens had been collected in the steppe landscape.

DISCUSSION

In this study, we found 11 species from the genus *Cosmochthonius* in the eastern Mediterranean. Earlier the same number of species has been listed in the western Mediterranean (Subias & Gil-Martin, 1997). Five species (*C. reticulatus*, *C. foliatus*, *C. lanatus*, *C. semifoveolatus*, and *C. asiaticus*) have now been found in both regions, whereas six species (*C. macrosetosus*, *C. zanini*, *C. ugamaensis*, *C. taurus*, *C. tenuisetus*, and *C. ponticus*) were recorded only in the eastern part of the Mediterranean.

Six species (*C. foliatus*, *C. reticulatus*, *C. zanini*, *C. ugamaensis*, *C. tenuisetus*, and *C. ponticus*) were found on Crimea, which indicates a relatively high diversity compared to other areas, not too different in size. Karadag's mountain area is especially remarkable in this respect. It originated in the Jurassic period. Only a few species were found on the islands and in the coastal zone of Turkey. This low diversity may be partly due to insufficient sampling of different habitats in these areas.

Dependence between *Cosmochthonius* species and different species of plants cannot yet be proven conclusively. However, some features can be observed. For instance, the species *C. reticulatus* was found more frequently on plants of the family Cupressaceae; it was commonly found in the litter of *J. macrocarpa* in Lesvos, of *J. excelsa* in Ukraine, and of *C. sempervirens* in Rhodes. Most specimens of the species *C. foliatus* were found in the litter of *Q. pubescens* in the Ukraine. The species *C. ugamaensis* was found to be abundant in the litter of *O. europea* in the mountains of Lesvos.

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An inventory of oribatid mites, the main decomposers in bogs of Colchic Lowland (Caucasus, Georgia)

Maka Murvanidze & Eristo Kvavadze
Institute of Zoology, Chavchavadze av. 31, 0179 Tbilisi, Georgia. E-mail: maka.murvanidze@gmail.com

The bogs of Colchic Lowland are of great international importance, because they are a foraging site on the route for many migrating and hibernating birds. Productivity of the bogs is very high and they create a large mass of dead organic matter. We made an inventory of the oribatid mites in three types of bogs: peat, cereal-*Carex*, and *Juncus* bogs. A total of 47 species were identified, one of which, *Punctoribates manzanoensis* (Hammer), is new to the fauna of the Caucasus. Cluster analysis indicates that each bog type is characterized by a specific fauna, that shows low similarity to other bog types. In peat bogs the dominant species are *Platynothrus peltifer, Nothrus pratensis, Parachipteria punctata, Punctoribates punctum*, and *Scheloribates laevigatus*, in cereal-*Carex* bogs these are *Zetomimus furcatus, Nanhermannia nana*, and *P. manzanoensis*, and in *Juncus* bogs: *Steganacarus personatus, Chamobates kieviensis*, and *Oppia nitens.* The oribatid fauna also includes species known from bogs of northern regions, such as *Z. furcatus, O. nitens*, and *Oppiella neerlandica*. The density of oribatid mites varies from 3,250 to 23,500 individuals/m^2. In some sites mite density was high, whereas species diversity was low. Such high numbers per oribatid species indicate fast decomposition. Decomposition occurs in absence of many groups of other invertebrates and is mainly driven by oribatid mites.

Key words: Oribatida, Colchic Lowland, decomposition, bogs, similarity index

Bogs and flooded forests have long been common in the Colchic Lowlands, but in the 20th century most of them drained out and deteriorated. Only in some inaccessible places, such as Anaklia, Churia, Nabada, and Kobuleti (Ispani 2), the bogs have kept their natural form. Previous studies have show that the deep layers of these bogs are 5,500-6,500 years old (Tvalchrelidze et al., 2004; Connor et al., 2006).

The remaining bogs of Colchic Lowland are a refuge of rare, relict, and threatened species of flora and fauna of the Tertiary Period (65-1.8 Mya). Their international ecological importance is great. Together with other flooded ecosystems of Colchic Lowland and Black Sea Aquatorium they represent a foraging site on the route for migrating birds of African-Asian waters and bogs. Each year >300 bird species are nesting, resting, and hibernating in this region. Therefore this territory is included in the Colchic National Park and the Ramsar Site.

The bogs of Colchic Lowland are distinguished by high biological productivity. The main decomposers of dead organic matter, together with other groups of invertebrates, are oribatid mites. For this reason we made an inventory of the oribatids inhabiting these bogs.

MATERIALS AND METHODS

The oribatid mites of peat, cereal-*Carex*, and *Juncus* bogs, were studied. Material was taken in June, July, and October 2005. Ten plots were investigated. From each plot three soil samples were taken and arthropods were extracted by a Tullgren–apparatus. Only adult oribatid mites were identified and counted. For species determination we used mainly the keys of Ghilarov & Krivolutski (1975) and Weigmann (2006).

Jaccard's coefficient was calculated as index of faunal similarity between plots. Community similarity was expressed as Renkonen's coefficient. This coefficient is calculated by summing up the lesser of the dominance values of each species occurring in the two sites to be compared (Krebs, 1989).

The ecological characteristics of the plots under study are as follows:
(p1) Ispani 2. A bog with *Sphagnum*, *Juncus*, *Rhododendron luteum, Rh. ponticum, Osmunda regalis* (41°47.512'N, 41°51.890'E, 6 m a.s.l.).
(p2) Ispani 2, A bog with *Sphagnum*, cereals, and various grasses (41°51.825'N, 41°47.428'E, 4 m a.s.l.).
(p3) Ispani 2. A bog with *Sphagnum*, various grasses, and *Juncus* (41°51.828'N, 41°47.418'E, 3 m a.s.l.).
(c4) Dedabera. A bog with various grasses and *Carex* (42°05.162'N, 41°47.452'E).
(p5) Nabada Lake. A peat-bog (42°91.589'N, 041°41.095'E).
(c6) North-Eastern side of Parto Tskali Lake. A cereal and *Carex* bog.
(c7) Nabada. A cereal-*Carex* bog with alder trees.
(j8) Lower part of Churia River. A *Juncus* bog.
(j9) Anaklia. A *Juncus* bog.
(c10) Etseri. A bog with different grasses and cereals.

RESULTS

Forty-seven species of oribatid mites were recorded (Table 1). One of them, *Punctoribates manzanoensis* Hammer, is new to the fauna of the Caucasus. Most species were found in PartoTskali Lake (c6) – 16 species, and in Ispani 2 (p1) – 15 species. Fewest species (viz., 5) were found in Ipani 2 (p2) and Dedabera (c4). No species was found in all the sites. All three types of bogs harboured common species, such as *Steganacarus personatus, Punctoribates punctum*, and *Minunthosetes pseudofusiger* (Table 1).

High indexes of dominance were found for *S. personatus*

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Table 1 Dominance (%) of oribatid mites in Colchic Lowland bogs. For description of the codes (= bog types) see Materials and Methods section.

Species	P1	P2	P3	C4	P5	C6	C7	J8	J9	C10
Hypochthonius rufulus	6		8							
Hoplophthiracarus vanderhammeni										8
Mesoplophora pectinata	9									
Phthiracarus ferrugineus						1		6		
Ph. lentulus	6				8					
Ph. murphy						1				
Ph. nitens								3		
Ph. ligneus									7	
Steganacarus personatus					8	18		70		
St. serratus										38
St. spinosus	3									
St. striculus	21						15			
Microtritia minima	15									
Platynothrus peltifer	3	30			21		3			
Nanhermannia nana				14		18	3		14	8
Nothrus pratensis	6				21					
Nellacarus caucasicus		4								
Amerobelba decedens						1				
Eremobelba geographica						1				
Dissorhina ornata						1				
Oppia nitens									29	
Oppiella neerlandica	3									
O. nova						4				
O. tuberculata								15		
O. (R.) fallax				14		3				
Ramusella mihelcici	3									
Parachipteria georgica						2			21	15
P. punctata		26	8							
P. willmanni			15		8					
Eupelops acromios	33									
E. hygrophilus					21	1				
Pergalumna minor	12									
Ceratozetes gracilis								3		
Zetomimus furcatus						24	50			
Chamobates kieviensis									14	
Ch. voigtsi				29						
Globozetes microtus			15		4					8
Punctoribates manzanoensis				29		11				
P. punctum	6	19	23			13	26			8
Minunthozetes pseudofusiger			8	14		1				15
Protoribates capucinus						1				
Scheloribates laevigatus	3	22			4					
Sch. latipes	3				4				7	
Sch. quintus						2				
Sch. pallidulus							3			
Oribatula tibialis			23						7	
Total	15	5	7	5	10	16	6	6	7	7
Density (ind ×100/m^2)	56,4	135	32,5	35	39,8	235	170	165	35	65

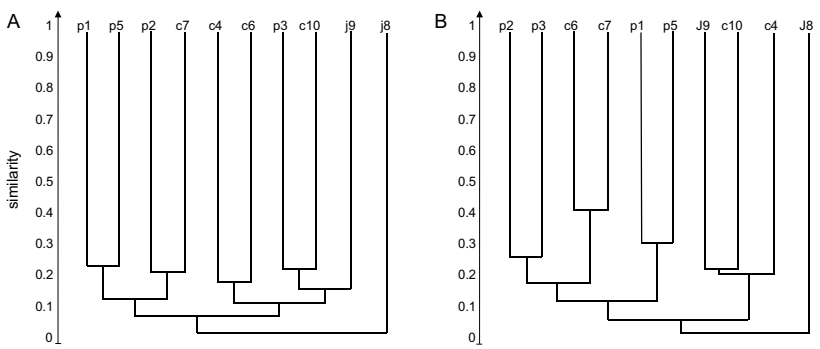

Figure 1 Cluster of (A) faunal similarity and (B) dominance identities of oribatid mites of Colchic Lowland.

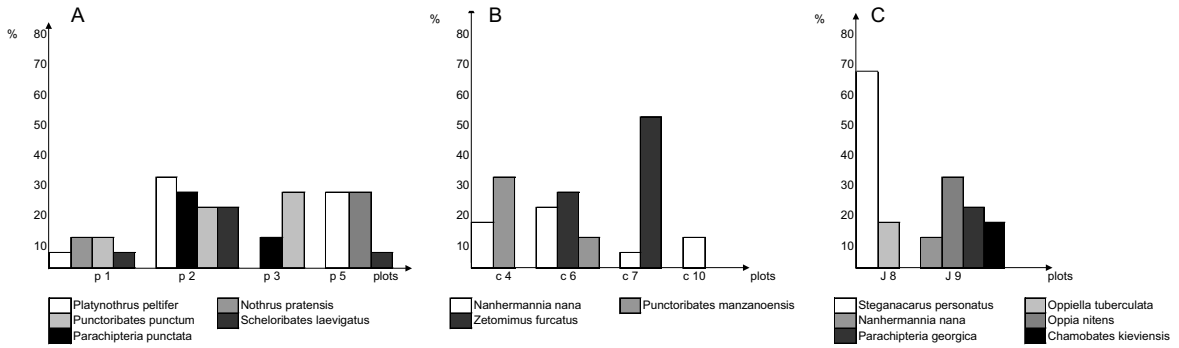

Figure 2 Distribution of dominant species of oribatid mites (%) in (A) peat-bogs, (B) cereal bogs, and (C) *Juncus*-bogs.

(70%), *Zetomimus furcatus* (50%), *St. serratus* (38%), *Eupelops acromios (33%s)* and *Platynothrus peltifer* (30%) (Table 1). The density was highest at site c6 (23,500 ind/m^2), where also the most species were found. However, in some sites where just a few species occurred, total density was high due to some dominant species. In the first plot, despite the high number of species (15), density was relatively low (5,640 ind/m^2), because the density of each of the species in this plot was low (Table 1).

The bogs of Colchic Lowland create a northern element in the subtropical area of the Black Sea coastal Ajara Region (Dokhturovski, 1936). For example, the boreal plant species *Drosera rotundifolia* is found here. The northern element is reflected in the oribatid fauna too. In our Georgian bogs we found *Z. furcatus*, a common species for bogs of Northern Europe and Northern America (Behan-Pelletier, 1996), Lithuania (Eitminavichute, 1966, 1969), Germany (Weigmann, 2004), and Denmark (Haarlow, 1957). Other northern elements in the oribatid fauna are *Oppia nitens* and *Oppiella neerlandica*.We also found a species typical for (sub)tropical bogs: *Pergalumna minor*.

Cluster analysis shows that there are two main groups: peat bog and cereal-*Carex* bog species. In plots p3, c10 and j9 *Carex* and *Juncus* species co-occurred, which makes these sites ecologically more similar – this is reflected in the oribatid fauna too (Fig. 1A). In the cluster analysis of dominance characteristics, dominant species of peat bogs and cereal-*Carex* bogs form one large group and the dominant species of *Juncus* and cereal bogs form a second group (Fig. 1B). The oribatid mites from site j8 appeared to stand apart from both clusters.

The dominant species of peat bogs are *P. peltifer, Nothrus pratensis, Parachipteria punctata*, and *Scheloribates laevigatus*. Relatively high values of dominance indexes were found in plot p2: *P. peltifer* (30%), *P. punctata* (26%), *P. punctum* (19%), and *S. laevigatus* (22%). The lowest index values for these dominant peat bog species were found in plot p1: *P. peltifer* and *S. laevigatus* (3%), *N. pratensis* and *P. punctum* (6%) (Fig. 2A).

In cereal-*Carex* bogs the dominant species were *Z. furcatus, Nanhermannia nana*, and *P. manzanoensis*. The dominance value of *Z. furcatus* is high in plot c7 (50%). All three dominant species are found in plot c6: *N. nana* (18%), *Z. furcatus* (24%), and *P. manzanoensis* (11%). In plot c10 only *N. nana* was found, with a low dominance value (8%) (Fig. 2B).

The two plots of *Juncus* bogs did not share dominant species. In plot j8 a high coefficient of dominance was obtained for *S. personatus* (70%) and, in plot j9, this applied to *O. nitens* (29%) and *Parachipteria georgica* (21%). The dominance indexes of the other species in these plots are lower (Fig. 2C).

DISCUSSION

Our inventory shows that the fauna of oribatid mites is characteristic for each type of the bogs under study. The similarity between the oribatid communities of cereal and *Juncus* bog emerged probably because they share *Juncus* species.

In bog ecosystems, invertebrates decompose only part of the vegetation. The main part remains non-decomposed and turns into peat, in which form it is conserved. Early studies on the invertebrate fauna of Colchic Lowland showed that species diversity of oribatid mites is much higher than that of Lumbricidae and insects (Kurashvili, 1984). For the whole area only four species of earthworms were found at a density 1-20 ind/m^2. Two species of soil and two species of water beetles were found. Their larval forms and imagines feed on young fish. The high number of species and their density demonstrate that oribatid mites are the main representatives of soil invertebrates that act as decomposers of plant material in bog ecosystems.

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The soil mites of buttongrass moorland (Tasmania) and their response to fire as a management tool

David Green
School of Geography and Environmental Studies, University of Tasmania, Private Bag 78, Hobart, Tasmania 7001, Australia.
E-mail: d.green@utas.edu.au

The buttongrass blanket moorlands are a unique habitat that constitutes a major landscape feature within the Tasmanian Wilderness World Heritage Area (TWWHA) of southwestern Tasmania. They are a highly pyrogenic vegetation type that has had a long history of regular burning by aborigines. Buttongrass may require a fire frequency of 5-20 years in order to maintain its suitability as habitat for many endemic vertebrates. The present knowledge of the Acari in the peat/litter accumulations in buttongrass is very poor and their response to fire is not understood. This study addresses the following questions: (1) How do soil mite communities in recently burned buttongrass compare with those that have not been burned in several decades? and (2) Are fire frequencies of between 5-20 years sufficient for soil mite populations to recover? A survey of soil mites was undertaken in a chronosequence of age classes of buttongrass at two study areas in the TWWHA. Six soil-cores were removed from up to four sites of each age class at 3-months intervals for 1 year, a total of 870 soil cores. All mature mites were identified to morphospecies with many individuals identified to genus or species. The soil mite community within buttongrass was found to be rich both at family and species level, in Oribatida, Prostigmata, and Mesostigmata. This community was significantly affected by fire, with a reduced number of species for many years post-fire. Density and diversity of these populations are positively correlated with age and many communities only changed significantly after 30-40 years post fire, which is in conflict with the prevailing management practice of burning every 5-20 years.

Key words: Soil mites, moorland, peat, fire, management

This study focuses on the little known community of mites (Acari) that reside in litter, peat, and soil within buttongrass blanket moorland in Tasmania. The Tasmanian Wilderness World Heritage Area (TWWHA) occupies approximately 20% of the State of Tasmania and was first listed as World Heritage in 1982, with a further expansion in 1989 (Anon., 1999a). The TWWHA consists of a patchwork of buttongrass, rainforest, wet sclerophyll forest, scrub, alpine and sub-alpine environments, and due to its remoteness is relatively undisturbed by post-European development (Mallick & Driessen, 2005).

The buttongrass moorlands are a unique and major landscape feature within Tasmania. Although buttongrass does occur in other parts of South Eastern Australia, it is nowhere as dominant as in Tasmania. In Tasmania it occupies around 1 million ha of land, predominantly in the southwest of the state, about 40% of which is within the TWWHA (Brown, 1999). Buttongrass blanket moorlands are associated with the wet, oligotrophic environments of the lowland to sub-alpine areas within this area (Brown, 1999). This vegetation gains its name from the dominant tall sedge *Gymnoschoenus sphaerocephalus* (buttongrass), but up to 165 vascular plant species from 46 families have been identified as being typical of this vegetation type, including 53 species (32%) that are endemic to Tasmania (Jarman et al., 1988). Buttongrass moorlands are a highly pyrogenic community with a history of regular burning by aborigines over thousands of years (Brown, 1999). It has long been argued that the current extent of buttongrass moorland is a result of past aboriginal activity and that it extends far beyond its natural limits (Jackson, 1968). Post-European settlement has seen a marked reduction in fire activity within most Tasmanian environments including buttongrass moorland, and it has been argued that this has had a detrimental effect on biodiversity within this community (Marsden-Smedley & Kirkpatrick, 2000).

Studies in other vegetation communities have indicated that soil invertebrates (including Acari) are adversely affected by fire and that it may take many years to recover from a severe fire (Coy, 1996; Kudryasheva & Laskova, 2002). Organic content within these soils and even complete peat layers which can take thousands of years to accumulate can be destroyed and converted into ash and mineralised nutrients in intense fires (Anon., 1999a). This can result in an intense pulse of nutrients that can change the soil pH and can easily be leached, leaving a nutrient- and humus-poor soil, with a significantly different structure (Brown, 1999). Buttongrass fire can result in the loss of peat depth and may even result in the complete loss of peat, leaving just the quartzite gravel substrate (Brown & Podger, 1982).

The prevailing management plan for buttongrass blanket moorland in the TWWHA is for the maintenance of a mosaic of differing aged stands (Anon., 1999a). Buttongrass is burnt for three main purposes: (1) to reduce fuel levels, (2) to create fire breaks, and (3) to create suitable habitat for several threatened species. It is recommended that suitable buttongrass moorlands be burnt at frequencies of between 3-13 years to facilitate the recovery of certain threatened species (Anon., 1999b).

The invertebrate fauna of this community remains poorly known (Driessen & Greenslade, 2004; Mallick & Driessen, 2005), especially the soil/litter Acari that reside in the peat accumulations in buttongrass. Their overall response to fire is not understood and so this study will address the question of how the soil Acari of this habitat are affected by fire and how quickly they successionally 'drift' back to their original composition. An understanding of how soil and litter mite populations are affected by fire will give insight into fire management for conservation and diversity management of invertebrates.

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METHODS

Study areas

This study was conducted in two large areas of buttongrass in southwestern Tasmania, at an area around Lake St. Clair (42°10'S, 146°8'E) and Lake Pedder (42°51'S, 146°12'E) (Fig. 1). In each area, sites were selected for varying age since the last recorded fire. The sites were selected from a series of sites identified and recorded by the Tasmanian Parks and Wildlife Service that have been used for a variety of flora and faunal studies. Table 1 outlines the environmental features of the two areas.

Climatic data was collected by the Bureau of Meteorology (Canberra), from two weather stations situated within the study areas, one at Lake St. Clair, the other at Strathgordon (Table 1). Chemical analysis of the peat was performed for each site. Per site five peat samples were bulked together, to obtain data on the following soil chemical parameters: exchangeable nitrogen, potassium, phosphate, calcium, magnesium, manganese, zinc, copper, boron, pH, organic matter (loss on ignition), and conductivity. Altitude, slope and aspect, peat depth, soil moisture, and vegetation height and density were also recorded.

The two areas are situated on different substrates, and this is reflected in the peat chemistry (Table 1). The vast majority of buttongrass blanket moorland in Tasmania is situated on pre-Cambrian quartzite bedrock; this is typically very low in nutrients. The Lake Pedder area is a good example of this, as reflected in the soil analysis for the area. The Lake St. Clair area constitutes an unusual situation in that it is situated on a Jurassic dolerite substrate, which weathers to a more nutrient-rich soil. This is reflected in the peat at Lake St. Clair, where eight of the nine nutrients tested were significantly higher. The Lake Pedder area also exhibits a slightly higher acidity (pH 3.8) compared to Lake St. Clair (pH 4.1).

Experimental design

Six peat-cores were removed from four sites of each age class at 3-months intervals over 1 year. Mite diversity has been shown to be seasonal in temperate environments (Block, 1966). Therefore sampling was performed at 3-months intervals to detect as many species as possible. Because the localised fire history is different within each of the two areas, these areas have had to be treated in a slightly different way. The Lake Pedder area has a longer documented fire history than the Lake St. Clair area that has a more complete series of young (<10 years) burns. Figure 2 indicates the fire chronosequence at both sites.

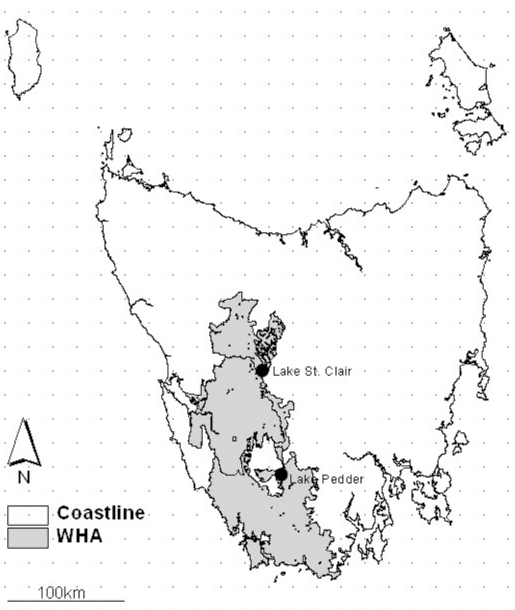

Figure 1 Map showing location of the Tasmania's World Heritage Area and the location of the two study areas.

Sampling

Soil cores of 5.2 cm deep and 7.3 cm in diameter (218 cm^3 volume) were removed from the sampling sites and taken to the invertebrates' laboratory in the School of Geography and Environmental Studies, University of Tasmania (SGES-UT). The mites were extracted from the soil cores using a Tullgren funnel apparatus; extraction was for 7 days and sample surface temperature was approximately 25 °C. The mites were collected and stored in a 70% ethanol solution. The mites were initially sorted using a 40× stereo dissecting microscope. Those mites used for identification were cleared using lactic acid and then permanently mounted on glass using Hoyer's Medium. All specimens are currently archived in the SGES-UT.

Acari community data

Data for Oribatida, Mesostigmata, and Prostigmata was collected at varying resolutions from species/morphospecies by genus or family, since these levels of resolution can all adequately reveal community structure (Osler & Beattie, 1999). Mites were identified using a variety of sources including manuals from the Acarology Summer School Program 2004 (Ohio State University, Columbus, OH, USA), Krantz (1978), Hunt et al. (1998), and Walter & Proctor (2001). Population density was calculated as the mean number of individuals per sampling unit, site or treatment (age class). Species richness or diversity refers to the mean number of species per sampling unit, site, or treatment. Relative abundance of individual species refers to a species proportional representation in a particular sampling unit, site, or treatment.

Analysis

To account for any aggregation in distribution of soil mites, relative abundance of species in samples was log-transformed and to reduce the variance heterogeneity associated with these data, the population data was also log-transformed. Community analysis using cluster analysis and non-metric multidimensional scaling (NMS) ordination were performed using the PC-Ord program (v.4, MjM Software Design). The cluster analysis used a Bray-Curtis similarity matrix and was sorted using complete linkage, to identify similarities between treatments, and to examine the relationship between mite community structure and age classes. Relationships between the various environmental factors and mite diversity were analysed using NMS autopilot routine, again using the Bray-Curtis similarity matrix. The community data was combined with a second matrix of environmental data in order to see how they associated with the community data. Analysis of variance was conducted using JMP 5.1 to test for differences among the means of the mite populations in the different age classes. Having no a priori reason for testing particular groups, a Tukey's HSD test was employed to compare all possible pairs of treatments.

Table 1 Physical, climatic, and chemical parameters at the two study areas (± SE, if appicable).

Attribute	Lake Pedder	Lake St. Clair
Number of sites	18	25
Age classes (years post fire in brackets)	LP1(<10), LP2 (10-20), LP3 (20-30), LP4 (30-40), LP5 (>40)	LSC1 (<5), LSC2 (5-10), LSC3 (10-20), LSC4 (>30)
Altitude range (m)	305-365	730-800
Mean annual rainfall (mm)	2,541	1,520
Mean summer max temp (°C)	18.3	17.7
Mean summer min. temp (°C)	9.1	6.9
Mean winter max. temp (°C)	9.4	7.2
Mean winter min. temp (°C)	3.4	0.8
Max. summer temp (°C)	36.3	35
Min. winter temp (°C)	-4.1	-11.5
pH	3.76 (± 0.02)	4.05 (± 0.05)
N (ppm)	0.76 (± 0.12)	1.24 (± 0.11)
P (ppm)	8.38 (± 0.67)	12.15 (± 0.77)
K (ppm)	150.13 (± 7.64)	203.08 (± 38.82)
Ca (ppm)	373.53 (± 64.85)	822.45 (± 85.52)
Mg (ppm)	568.53 (± 100.60)	440.40 (± 37.78)
Mn (ppm)	6.64 (± 0.11)	58.28 (± 23.93)
Zn (ppm)	1.03 (± 0.05)	6.28 (± 0.78)
Cu (ppm)	0.35 (± 0.03)	5.66 (± 0.36)
B (ppm)	1.77 (± 0.11)	2.93 (± 0.15)
Organic matter (LOI) (%)	50.53 (± 6.14)	61.94 (± 5.65)
Conductivity (µs/cm)	248.07 (± 12.14)	320.27 (± 41.11)

RESULTS

A total of 12,820 mites were identified belonging to 146 species and 72 families, while another 2,640 juveniles could not be adequately identified. This equates to a mean of 15 mites per sample or approximately 68,000 mites/m³, although the range of mites per sample was between 0 and 112 (or 502,000 mites/m³).

Five taxa were super abundant in this study: a species of Carabodidae (*Austrocarabodes* sp.) accounted for 11% of all mites, a species of Nanorchestidae (*Nanorchestes* sp.) for 8.5%, a species of Stigmaeidae for 7.6%, and two species of Oppiidae (*Brachioppiella* sp. and *Lanceoppia* sp.) each for 4.5%. Forty-nine species only occurred as 10 or fewer individuals during the study and of these there were 10 species that occurred only once.

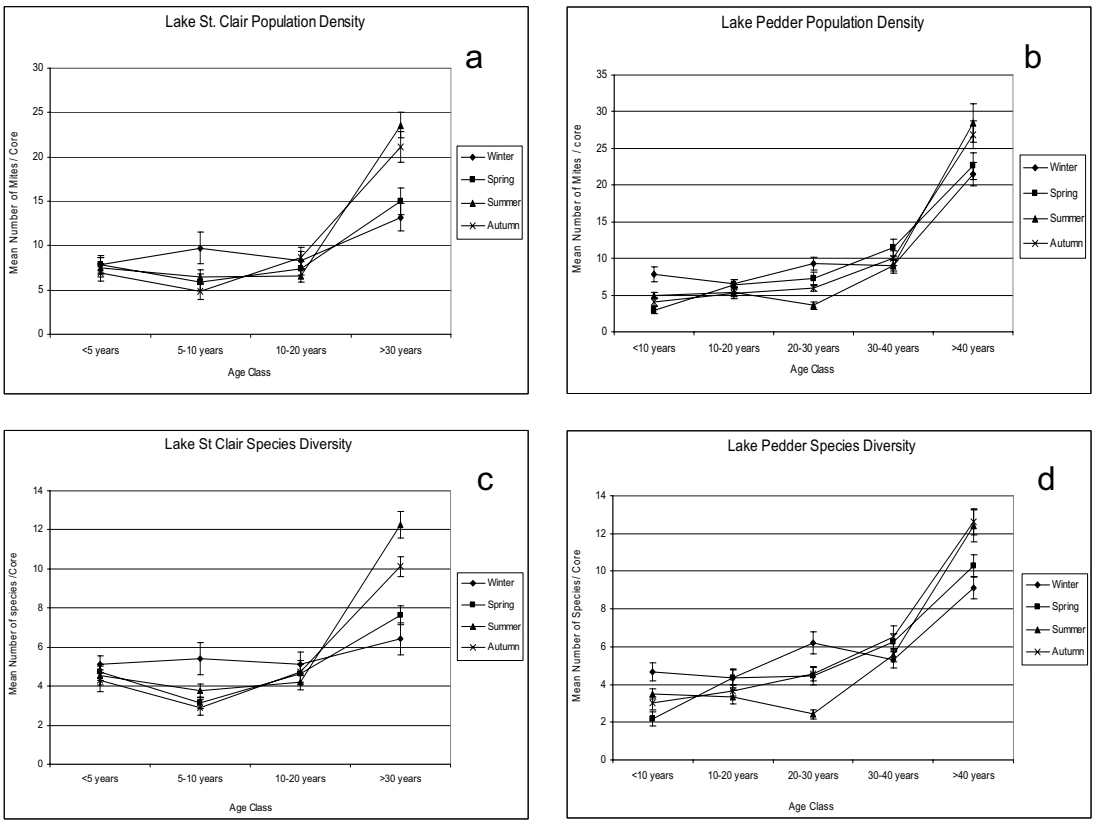

Figure 2 Population densities in the different age classes at (A) Lake St. Clair and (B) Lake Pedder, and species diversity at (C) Lake St. Clair and (D) Lake Pedder.

Patterns in species distribution show a separation between Lake Pedder and Lake St. Clair, on the cluster analysis (Fig. 3), but this is not altogether clear cut. Five of Lake Pedder's oldest age classes have been included with the Lake St. Clair branch. Interestingly, the older sites at both Lake Pedder and Lake St. Clair cluster around single nodes. Other patterns are somewhat more difficult to identify.

The ordination (Fig. 4) indicates much the same as the cluster analysis. Lake Pedder and Lake St. Clair sites appear to separate out quite well, along axis 1. The axis 2 separates out the age classes, with the general pattern being that younger age classes move to older age classes. Several of the environmental variables correlate with the ordination axes. pH, and several of the nutrients including Cu, Zn, N, P, and Ca are all positively correlated with axis 1. Vegetation density is positively correlated with axis 2, whereas organic matter and Mg are negatively correlated along this axis.

Comparison of population density and species diversity indicates that there are large differences in the populations of mites among the sites and this is supported by the analysis of variance ($F_{8,855}$ = 120.66, P<0.0001) Lake St Clair (Fig. 2A) averaged about eight mites per core in all the age classes except at the sites aged over 30 years, which averaged about 18 mites per core. A similar pattern is also seen in the number of species at Lake St. Clair (Fig. 2C), the younger three sites averaged approximately five species compared with nine in the oldest age class. Lake Pedder exhibits the same pattern except it appears to take much longer, the population density does not significantly rise until approximately 40 years after fire (Fig. 2B), and a similar pattern is found with the species diversity at lake Pedder (Fig. 2D).

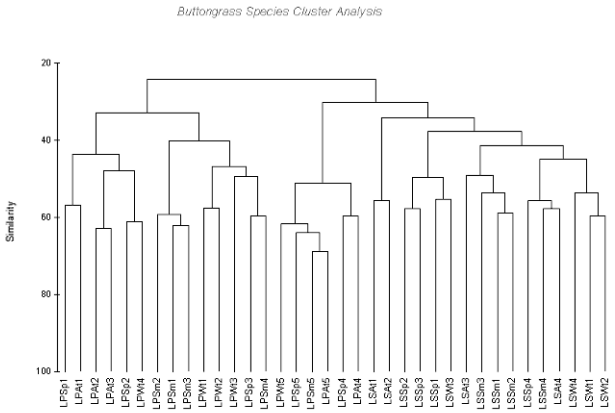

Figure 3 Cluster analysis of species. LP = Lake Pedder, LS = Lake St. Clair, Wt = winter sample, Sp = spring sample, Sm = summer sample, At = autumn sample, 1-5 = respective age class at each area.

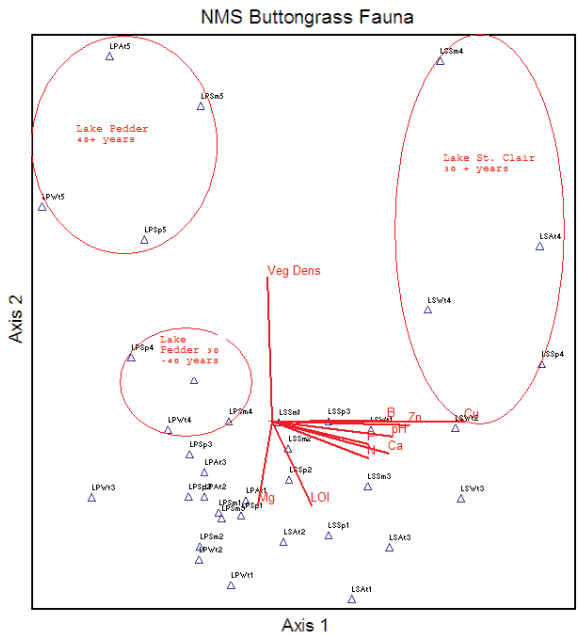

Figure 4 NMS cluster analysis of species and environmental variables. See Figure 3 for explanation of acronyms.

Table 2 Mean number of mites per core in each age class. Means followed by different letters are significantly different (Tukey-Kramer HSD).

Level	Mean
LP5	24.87a
LSC4	18.31b
LP4	9.85c
LSC3	8.08cd
LSC1	7.54cde
LSC2	6.72de
LP3	6.54de
LP2	5.91de
LP1	4.93e

The pairwise comparisons of population densities using Tukey-Kramer HSD (Table 2) reinforces these observations with only the last age class at each site identified as being different from the rest.

DISCUSSION

Although the two study areas had a slightly differing profile of age classes, it is obvious from these data that the soil mite communities in both areas respond similarly, although over a slightly longer time frame at Lake Pedder. There, the soil mite communities remain similar in their population density and number of species until 30-40 years post-fire, after 40 years there is a significant increase in both population density and species number. Lake St. Clair exhibits similar population densities and species numbers up to about 20 years, after 30 years post-fire there is again a large increase in population density and species diversity.

Yates & Lee (1997) reported a large reduction in many taxa, including mites in tussock-grassland soils in New Zealand up to 3 years post-fire, the length of the study, whilst Kudryasheva & Laskova (2002), who studied the effects of fire on Oribatida in peat and podsolic soils, found that although the fire did not have a catastrophic effect on the mites, it did reduce their abundance and species diversity. Zaitsev et al. (2002) found a marked delay of >95 years in the recovery of Oribatida populations to strong changes in environmental conditions. Webb (1994) reported that it took at least 15 years for oribatid mites in *Calluna* heathland to completely recover post-fire and that this was closely associated with the growth cycle of the heathland vegetation. Authors such as Ruf (2000) suggest that these patterns in soil fauna act as a type of 'memory' in soils, and that they can last for several decades. This study would confirm this idea and suggests that in buttongrass peat that memory is about 30-40 years.

It would appear that buttongrass peat accumulations greater than 30 years post-fire around Lake St. Clair, and greater than 40 years around Lake Pedder, are optimal peat habitats for soil mites. This may be a result of the slightly higher nutritional status of the Lake St. Clair area. It may be

a case of the better the resources, the faster the recovery. It could be assumed that with higher nutrients in the underlying substrate both the vegetation and the fauna should benefit. If the vegetation can accumulate faster then the resulting necromass will also accumulate at an elevated rate. This would ultimately result in greater recourses for the soil mites, leading to an accelerated recovery in population density compared to the relatively infertile peat of the Lake Pedder area. This increase in resources may also provide increased niche habitat, which would then account for the rapid increase in species numbers.

The NMS analyses indicate that the older age classes separate into distinct communities compared to the younger age classes that appear to be very similar in the constituent species. It seems that it is only with the longer fire periodicity that unique and highly speciose mite communities develop. The NMS also shows that these communities are correlated with vegetation density and organic content of the peat. The several mineral nutrients that correlate across the horizontal axis are likely to be a consequence of the differing nutritional status across the two areas reflecting their underlying geological substrate. The vegetation density and organic matter accumulations are highly correlated with buttongrass moorland age.

If it is necessary to burn buttongrass blanket moorland for the management of certain species and for wild-fire control, then this study would indicate that the burning at intervals of less than 30-40 years, would be highly detrimental to the maintenance of diverse communities of soil mites living in peat accumulations in this habitat. It would be recommended that buttongrass blanket moorland be burnt at varying time and space intervals to produce a mosaic of old and young buttongrass that adequately protects and responds to all the requirements laid down in the TWWHA Management Plan 1999, as well as protecting communities of soil invertebrates.

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The water mite genus *Torrenticola* (Hydrachnidia: Torrenticolidae) in Costa Rica – ecology, diversity, and bioindicator potential

Tom Goldschmidt
Zoologische Staatssammlung München, Münchhausenstraße 21, D-81247 München, Germany.
E-mail: tomgoldschmidt@web.de

The genus *Torrenticola* is found in springs and running waters on all continents except Antarctica, with the highest diversity in the Tropics and Northern Temperate regions. In Costa Rican streams and springs it is the most frequent and abundant water mite genus In a recent study 36 new species were described, raising the number of species known from Costa Rica to 42; furthermore, ecological data of >3,000 specimens from >200 sample sites were analysed. Based on these data, the first ecological analyses on Neotropical water mites are presented. *Torrenticola* spp. were found in all types of springs and running waters all over Costa Rica, but at species level differential habitat demands and distribution patterns are apparent. Analyses of habitat preferences of the species revealed linkages of particular species to certain habitat types (springs, large rivers, etc.), different sensitivity of the species towards pollution, and a distinct altitudinal zonation. These results demonstrate the great potential of this group of mites in the monitoring of aquatic habitats. The finding of up to seven species of the genus at a single locality allows the study of microhabitat specificity and niche-differentiation, as well as general patterns of species assemblages. High mountain regions could be determined as main centres of diversity and endemism. Furthermore, idiosoma size was found to differ among species and between sexes, and to vary with habitat and elevation. Finally, the state of knowledge on the diversity and general distribution patterns of the genus in the New World is described and discussed.

Key words: *Torrenticola*, Hydrachnidia, Neotropics, Costa Rica, ecology, habitat preference, bioindicator, stream monitoring, spring, running water

Within the wide morphological variety of water mites, the genus *Torrenticola* Piersig comprises a group of typical, heavily sclerotized stream-dwellers. The species are characterised by a complete dorsal and ventral armour, in general bearing dorsally a single large postero-central plate and four small anterior platelets. In some species groups the anterior platelets are partly or completely fused with the main dorsal plate (Goldschmidt, 2007). The Latin American species of *Torrenticola* are subdivided in 10 species groups (Goldschmidt, 2007); besides the state of fusion of the antero-dorsal platelets these groups are mainly separated by the structure of the gnathosoma. In detail, the main differences are found in the shape of the more or less elongated rostrum and the characteristic structure of the five-segmented palps. Especially the size of the palp segments and the shape of the ventral margin of the second and third palp segments – being either smooth or bearing cones, lamellae, etc. – differentiate the species (Goldschmidt, 2007). The morphological variability of the mouth parts indicates that differences in the feeding ecology might be an important factor driving the evolution and separation of species. However, nothing is known of fundamental differences in feeding ecology among species. As far as known, the adult stages are predators, mainly on Ostracoda and Cladocera (Antonio Di Sabatino, unpubl. data).

Torrenticolid water mites are found in springs and running waters on all continents, except Antarctica. In Central America, as well as other tropical regions *Torrenticola* is one of the major genera in these habitats. About 300 species are described worldwide, with the highest diversity in the Tropics and Northern Temperate regions. In Europe, a significant increase in diversity is documented from Northern and Central Europe towards the Mediterranean (Viets, 1978; Cicolani & Di Sabatino, 1991; Gerecke, 2002; Di Sabatino et al., 2003). Similar comparative data unfortunately are still very rare from the New World (Böttger, 1980; Cook, 1980). Until recently, 21 species of the genus were known from Central and South America, four of them from Costa Rica. Studies on extensive material from Costa Rica, revealed 36 new species, raising the number of known species from the Neotropics up to 57, with 42 of them known from Costa Rica (Goldschmidt, 2007).

Several studies in temperate regions already showed the bioindicator potential of water mites (Schwoerbel, 1964; Young, 1969; Angelier et al., 1985; Gerecke & Schwoerbel, 1991; Martin & Brinkmann, 2003; Gerecke & Lehmann, 2005; Proctor, 2007). However, until now, the use and development of this great potential has been hampered mainly by a lack of tradition to use this group of aquatic invertebrates in the assessment of the ecology of streams (Proctor, 2007). A similar potential of the Neotropical water mite fauna in general has already been shown (Goldschmidt, 2004). Therefore, besides the documentation of the ecology and diversity of the genus *Torrenticola*, this paper aims to show the great potential of the group as biological indicator. At the same time, due to the detailed analysis of habitat binding in Costa Rican species, the first data for the development of biomonitoring programmes are provided.

MATERIALS AND METHODS

The water mite samples, that served as a basis for the present study, were taken during field trips in 1995, 1996, and 1997. At 204 sample sites in various regions, habitats, and elevations in Costa Rica, 3,096 specimens of the genus *Torrenticola* were collected. Furthermore, detailed habitat data were taken on elevation, temperature, habitat type, substrate, pollution, conductivity, shading, vegetation, and velocity (for further details see Goldschmidt, 2004).

Canonical correspondence analysis (CCA) is used to analyse relationships between species and environmental

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parameters (Ter Braak, 1988; Ruse, 1994; Glavac, 1996; Clausen, 1998). In CCA graphs the length of a vector represents its significance for the interpretation of the faunal distribution. The nearness of a species to a vector (especially its tip) represents the importance of the respective parameter for the presence of the species (for details see Goldschmidt, 2004).

One-way ANOVA was performed to test the significance of body size differences of sexes and habitat types, Pearson's product-moment correlation was applied to test the correlation between body size and elevation (Dytham, 1999).

A cluster analysis was based on Ward's minimum-variance method in order to find and document species assemblages. Only species collected at more than one site were included in this analysis; within these 27 species, several groups were distinguished.

RESULTS

Significance of ecological parameters

Canonical correspondence analysis (CCA) revealed several main factors determining the variation of the Costa Rican torrenticolid fauna. The first axis of the CCA (eigenvalue 0.846) is the more important one, explaining the main variability; the second axis (eigenvalue 0.469) is less significant, yet still providing important information. According to this analysis, altitude and temperature are the dominating parameters (with a strong negative correlation between them). Also shade (or its absence), water depth, and (negatively correlated on the opposite side of the diagram) several shallow habitat types (such as springs, springbrooks, and water falls) are very important (Fig. 1). Within this general pattern, the occurrence of several species is clearly bound by certain parameters.

Most isolated and therefore most clearly defined are three species restricted to springs: *Torrenticola amalgamada* and *T. fontinale*, mainly found in rheopsammocrene springs on the Peninsula de Osa. *Torrenticola altifontana* was only found in a rheocrene spring in the Paramó region and is therefore completely isolated in the upper right corner of the diagram. Furthermore, there is a group of high-mountain species, only found in small streams in the Central Cordillera de Talamanca (*baderi*, *chirripoensis*, *cumbrensis*, and to a lesser extend *alticola* and *delgada*). These are represented closely to the tip of the altitude-vector. Mainly bound to shallow, small water bodies – therefore close to the upper half of the second axis – are two other species groups: (1) *conipalpis*, *brevicoxalis*, *ambigua*, and *harpagophora* are found in springs and springbrooks (with a bias towards springs), and (2) *alexandra*, *ratoncitoi*, *menudopalpis*, *costaricense*, and *alargada* are also found in springs, but more often in small running waters. On the lower right of the diagram, opposite to the shade-vector, there are some species mainly found in open habitats: *monticola*, *fastigata*, and *esferica*.

Habitats of the Costa Rican species

In Costa Rican streams and springs, *Torrenticola* is the dominant water mite genus in terms of frequency and abundance. Species of the genus were found in 62% of all sampled running waters (184 out of 295) and in 50% of all springs (20 out of 40). In both habitat types, *Torrenticola* is the most abundant genus, representing 21% of all specimens from running waters and 25% of all specimens from springs. In running waters as well as in springs, only four genera (out of 65 in running waters and 34 in springs) represent more than half of all specimens: *Torrenticola* is followed by the genera *Hygrobates* (16%), *Corticacarus* (13%), and *Sperchon* (12%) in running waters, and by *Eupatrella* (18%), *Stygarrenurus* (10%), and *Hydrodroma* (8%) in springs. Hence, *Torrenticola* is the only genus that is among the 'very abundant' genera in both habitat types. Nearly all types of running waters (from hygropetric zones and water falls to large rivers) were inhabited by various species of *Torrenticola*. However, within the great variety of habitats, most species and by far most specimens from Costa Rica have been collected in the mesolithal of riffle zones in fast flowing, small streams from the lowland rain forest up to the Paramó regions in the highest mountains.

Although several species were found in a great variety of habitats, most of the 42 Costa Rican species were bound to

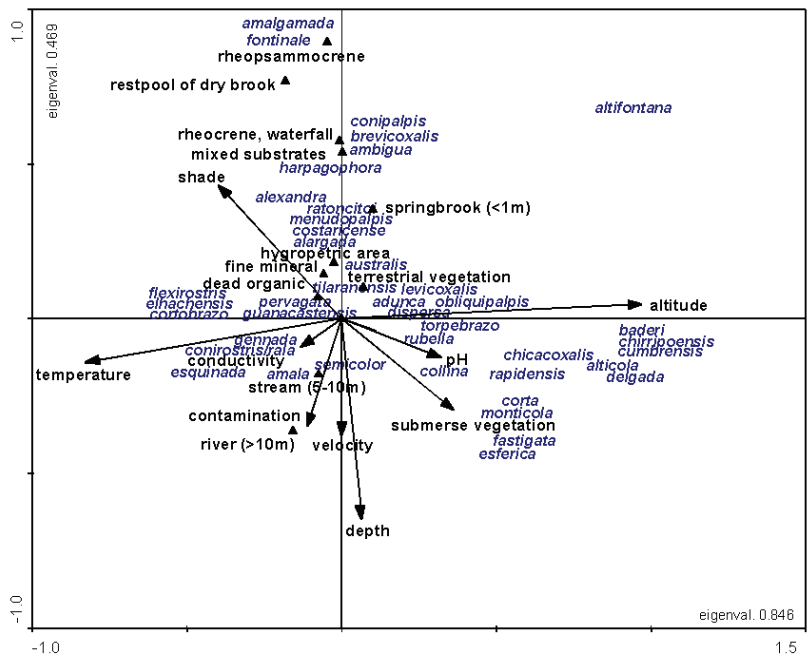

Figure 1 Canonical correspondence analysis (CCA)-biplot of the Costa Rican species of *Torrenticola* and the investigated environmental parameters.

certain habitat types. Twenty-six species were restricted to running waters, 13 species were found in running waters and springs. By far the most (34 species, including most of the very rare ones) were found in small streams, 12 of them exclusively. Just three species were collected in larger rivers (*amala*, *conirostris-rala*-complex, and *guanacastensis*), only *T. amala* showed a clear preference for rivers, as it was found in 90% of all samples from that habitat type. Most species collected in spring brooks or in streams were as often found in small streams, only four rare species (*alticola*, *corta*, *levicoxalis*, and *esquinada*) were restricted to streams. In hygropetric zones, no specialised species assemblages were found, only single specimens of seven species relatively common in various stream types were found in this habitat and, similarly, in waterfalls only one common species was found. Within the 13 species collected in running waters and springs, three are mainly concentrated in springs (*ambigua*, *conipalpis*, and *brevicoxalis*). Another three species (*altifontana*, *amalgamada* – both only found in singletons –, and *fontinale*) are pure spring specialists. Thirteen species of the genus *Torrenticola* were found in rheohelocrene springs, six of them also in rheopsammocrene springs (two species were exclusively found here), two in rheohelocrene springs, and one in a helocrene spring. Only *T. costaricense* was found in all four spring types (for details see Goldschmidt, 2007).

Elevation

In total, torrenticolid water mites in Costa Rica were collected at all elevations, from 10 to 3,500 m above sea level (asl). Most individual species of importance show much smaller ranges. Therefore a clear altitudinal zonation of the Costa Rican species can be observed. In five species the median of their sample sites lays below 500 m asl and they are followed by a large group of species mainly found between 500 and 1,500 m asl. A distinct separation can be found at 2,000 m asl. Only very few species with the median of their distribution below 2,000 m are still found higher, whereas seven species with the median of their distribution above 2,000 m are not found lower. Five species are mainly found even above 3,000 m asl, four of them (*baderi*, *cumbrensis*, *chirripoensis*, and *altifontana*) exclusively. Consequently, the clearest differentiation exists between several high-mountain species and the lowland-to-mid-elevation species with a clear limit at 2,000 m asl (detailed figure in Goldschmidt, 2007).

Body size patterns

The idiosoma length has been measured as a general estimate of the body size of the torrenticolid species. It varied from 500 to 1,089 µm. Several patterns can be drawn:

Sexual dimorphism – Within the 37 Costa Rican species of which both sexes were found, the females were significantly larger than the males (P = 0.006) (Fig. 2).

Elevation – Body size and median elevation of sample site are positively correlated (Pearson correlation r = 0.762; P<0.01) (Fig. 3).

Habitat types – Several species were collected in springs and running waters. Within *T. costaricense*, the specimens from springs are clearly larger than those from running waters (P = 0.004) (Fig. 4).

Naturalness of habitats

Several relationships can be observed with variables characterizing the habitat.

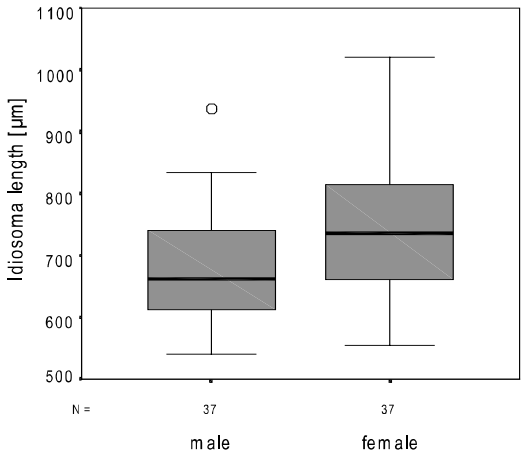

Figure 2 Comparison of the means of the idiosoma length of males and females of the Costa Rican species of *Torrenticola* present in both sexes (n = 37).

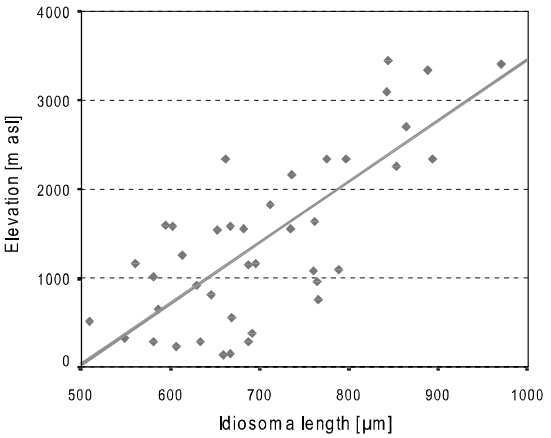

Figure 3 Correlation of the mean idiosoma length of the Costa Rican species of the genus *Torrenticola* with the median elevation of their sample sites (n = 42).

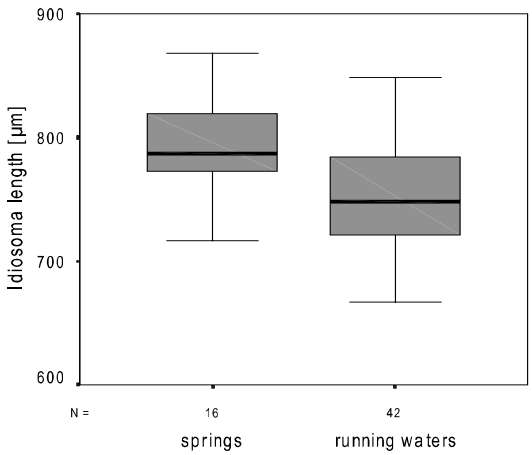

Figure 4 Comparison of the means of the idiosoma length of specimens of *Torrenticola costaricense* from springs (n = 16) and running waters (n = 42).

Shade

The sample sites of *Torrenticola* in Costa Rica are mainly found in forest regions or at least are accompanied by gallery forests. Most species are bound to more or less naturally preserved habitats. Consequently, 82% of all specimens were collected at partly or completely shaded habitats, only 18% in open habitats. Nearly half of the species (20 out of 42) were restricted to shaded sample sites, 13 species were mainly found in shaded habitats. Just nine high-mountain species were exclusively (six) or mainly (three) found in open habitats, mainly collected in open Paramó regions in the Cordillera de Talamanca. Within most of the species preferring shaded habitats, 73-94% of all specimens were collected there; only three of them were also found in relatively high abundance in un-shaded sites: *T. amala* (30%), *T. rubella* (36%), and *T. semicolor* (46%).

Pollution

Since most species depend on shading of their habitats, one would expect to find them in unpolluted habitats. Indeed, most species are clearly bound to clean, unpolluted water bodies. In total, 91% of all *Torrenticola* specimens were collected in clean habitats, only 9% were found in (heavily) polluted water bodies, mainly burdened by domestic or agricultural sewage. These 9% represent 14 of the 42 species known from Costa Rica, the remaining 28 species were restricted to clean sample sites. Within the more abundant species, three are obviously more tolerant towards pollution, as they were found clearly above average in polluted streams and rivers: *T. amala* (27%), *T. semicolor* (19%), and *T. rubella* (15%). The first is a typical species in lowland rivers, the others were found in various mountain streams. Interestingly, these three species also show the highest abundance in un-shaded habitats (see above). Apparently, the same species more tolerant towards pollution are also found in more open habitats (i.e., absence of forest as the natural riparian vegetation).

Species assemblages

As the various Costa Rican *Torrenticola* species display a highly differentiated ecology, the question arose whether certain species groups – maybe sharing the same ecology – could be distinguished. In order to analyse such species assemblages, a cluster analysis (Ward's minimum-variance method) has been performed including all species present at more than one sample site. The clusters are formed according to the variance of the samples, distances between two clusters are squared (Fig. 5).

The 27 species included in the analysis are divided in two groups: the lower part of the diagram represents all species found at sample sites at and above 2,000 m asl, whereas in the upper part all species are present that are restricted to sample sites below 2,000 m. There are two 'high mountain clusters' – *T. alticola* together with *T. chirripoensis*, and *T. delgada* with *T. fastigata* – of which the species were only found in fast-flowing small streams between 2,000 and 3,500 m asl. *Torrenticola chirripoensis* is even restricted to brooks in the Chirripó National Park above 3,000 m. The species forming the two remaining clusters in the lower part of the diagram are also found at lower elevations: *T. collina* and *T.*

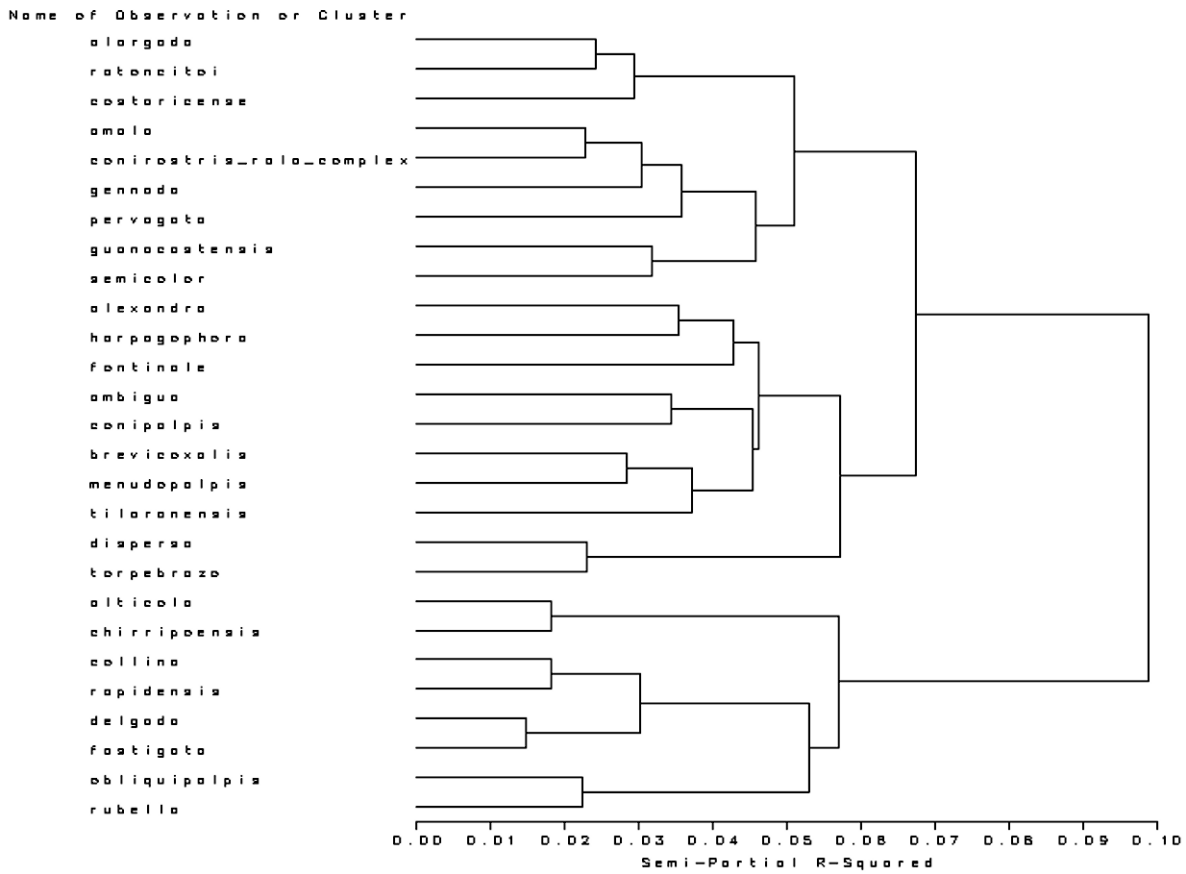

Figure 5 Cluster analysis (Ward's minimum-variance method, clusters are based on the variance of the samples, distances between two clusters are squared) of all species of *Torrenticola* from Costa Rica present at more than one sample site (n = 27).

Table 1 Number of specimens and sample sites of each species of *Torrenticola* collected in Costa Rica and percentage of the individual species in the total number of specimens collected and analysed in the present study.

Species	No. specimens	No. sample sites	%
conirostris/rala-complex	807	124	26.07
costaricense	550	68	17.76
amala	517	71	16.70
pervagata	209	28	6.75
gennada	193	27	6.23
tilaranensis	159	16	5.14
chirripoensis	104	6	3.36
semicolor	91	18	2.94
rubella	66	19	1.55
alargada	63	17	2.03
rapidensis	48	8	1.55
delgada	42	4	1.36
ratoncitoi	34	15	1.10
ambigua	32	13	1.03
guanacastensis	30	13	0.97
alexandra	29	12	0.94
obliquipalpis	18	11	0.58
fontinale	18	3	0.58
fastigata	11	5	0.36
menudopalpis	11	5	0.36
collina	11	4	0.36
alticola	7	4	0.23
australis	7	1	0.23
torpebrazo	6	2	0.19
conipalpis	5	3	0.16
dispersa	4	4	0.13
harpagophora	4	4	0.13
brevicoxalis	3	2	0.10
flexirostris	3	1	0.10
monticola	2	1	0.06
adunca	1	1	0.03
altifontana	1	1	0.03
amalgamada	1	1	0.03
baderi	1	1	0.03
chicacoxalis	1	1	0.03
corta	1	1	0.03
cortobrazo	1	1	0.03
cumbrensis	1	1	0.03
elhachensis	1	1	0.03
esferica	1	1	0.03
esquinada	1	1	0.03
levicoxalis	1	1	0.03

rapidensis mainly in fast-flowing small streams at 1,200-2,400 m in the Central Cordillera de Talamanca, *T. obliquipalpis* and *T. rubella* mainly in fast-flowing mountain streams at 640-2,400 m in the Cordillera de Tilarán, Cordillera Central, and Cordillera de Talamanca.

In the upper part of the diagram, *T. dispersa* and *T. torpebrazo* form a clearly separated cluster. These two species are mainly found in fast-flowing small mountain streams at 1,500-1,600 m asl in the Cordillera de Tilarán and Cordillera de Talamanca. The species group *T. alargada*, *T. ratoncitoi*, and *T. costaricense* are found in slow- to fast-flowing smaller water bodies, such as spring-brooks, small streams, and springs mainly in the central and northern mountain ranges at 500-1,600 m. The next group (*amala*, *conirostris-rala*-complex, *gennada*) mainly represents the 'lowland species', mostly found below 1,000 m asl on both sides of the central mountain ranges going down to nearly sea level. Within this group, *T. amala* and the *T. conirostris-rala*-complex again are separated. These are the only Costa Rican torrenticolid species regularly found in rivers. Finally there is a weakly differentiated larger group of species (*alexandra*, *harpagophora*, *fontinale*, *ambigua*, *conipalpis*, *brevicoxalis*, and *menudopalpis*), that are mainly found below 1,000 m asl. The whole group is characterised by the fact, that the species are also regularly found in springs. Clearly isolated within this group is *T. fontinale*, which is exclusively found in springs on the Peninsula de Osa.

Patterns of diversity

α-Diversity

In total 42 species of *Torrenticola* are known from Costa Rica at the moment. However, the abundance of the species is very different. While three dominant species (*conirostris-rala*-complex, *costaricense*, and *amala*) sum up to nearly 61% of all specimens, the 12 rarest species were only found in singletons (Table 1).

Correspondingly, also the α-diversity of the habitats is very different: at most sample sites just 1-3 species were found – only at 13 'diverse' sample sites 5-7 species were found (Fig. 6). These diverse sites are mainly streams in the mountain regions at 500-2,500 m asl. Only one site is located on the Peninsula de Osa, at 110 m asl. One site is a rheocrene spring. By far, most of the diverse sites were located in primary forest regions and, in all cases, the headwaters were in primary forests and no human impact could be found. As in the total fauna, also the *Torrenticola* assemblages of the individual sample sites are dominated by very few species. At most of the diverse sites, one or two species (different species at each site) are representing 60-90% of all specimens. No microhabitat specificity was found within these species assemblages.

γ-Diversity

The overall high γ-diversity of the Costa Rican torrenticolid fauna is not regularly distributed over the country. Many species were only found at very few localities and also the more abundant species are not regularly scattered, most are rather restricted to certain areas. Consequently, the number of species found in different regions of Costa Rica varies from two on the Peninsula de Santa Elena and the Nicoya, to 24 in the Central Cordillera de Talamanca. In general, the lowlands accommodate relatively few species, whereas the highest diversity is found in the central mountain regions. The most diverse region within the lowlands is the Peninsula de Osa with nine species. The Central Cordillera de Talamanca is not only the most diverse region within the country, the fauna of this region also contains the highest number of endemic species (12 out of 24). For details see Goldschmidt (2007).

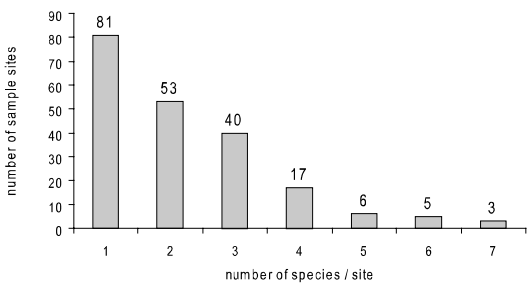

Figure 6 Distribution of sample sites in Costa Rica, where 1-7 species of *Torrenticola* have been found.

DISCUSSION

The dominant parameters explaining the Costa Rican water mite faunal distribution were elevation and temperature, besides habitat type and stream velocity (Goldschmidt, 2004). Correspondingly, variations in the *Torrenticola* fauna of running waters and springs in Costa Rica are mainly determined by elevation and temperature, followed by depth, habitat type, and shade. Especially the elevation of 2,000 m asl seems to limit the distribution of several species and marks an apparent faunal turn-over. At higher elevations, not only a different torrenticolid fauna was found in mountain regions, the fauna is also more diverse and more distinct, as these regions bear the highest numbers of endemic species. Whereas chemical parameters in general showed minor influence on the Costa Rican water mite fauna (Goldschmidt, 2004), most species of *Torrenticola* seem to depend on naturally preserved habitats. Some species are clearly more tolerant towards pollution than the vast majority.

Based on the distinct ecology of many species of the genus *Torrenticola*, several species assemblages could be determined. Again, mainly the high-mountain species are clearly separated, but also the 'river species' form a separate cluster.

An interesting correlation was found between body size and median elevation of the habitat, with species at higher elevations being significantly larger. As nothing is known of the life cycle of any Costa Rican water mite species, interpretation of this phenomenon is speculative. Because habitat elevation is also clearly separating species, this could be seen as some kind of 'island effect'. Higher elevation is associated with lower temperature, which might also be a proximate factor for size differences: cooler habitats provide better oxygen supply, hence it might be easier to grow bigger, it might stimulate egg production, and/or it might be easier to remain active for a bigger animal. Each of these ideas could form a starting point for further investigation.

The finding of up to seven species of the genus at the same locality raises questions of niche differentiation. However, because nothing is known of life cycle, host range, or feeding ecology of the species, further research is necessary before a discussion on any kind of microhabitat specificity or niche building can be started. General analyses of diversity patterns and geographical distribution of *Torrenticola* species in the New World unfortunately are also not possible at the present state of knowledge. Actually, Costa Rica seems to be the centre of diversity, as it is the only region in the New World where the torrenticolid fauna has been analysed in detail. Nevertheless, due to the very diverse geography of the country and its central position on the Central American land-bridge, a relatively high (and dense) γ-diversity of the torrenticolid fauna can be expected in Costa Rica (Goldschmidt, 2007). At the moment, the Costa Rican fauna has one species in common with Mexico and three with Guatemala, though probably only very small parts of the torrenticolid fauna of these countries are known. Apparently the diversity of the genus *Torrenticola* is significantly reduced towards southern South America: three species are described from Colombia, one of these is also known from Argentina, and none from Chile. As the water mite fauna of Chile is relatively well known, most probably the genus really has not reached the southern tip of the continent. Most likely, *Torrenticola* is also absent on the Caribbean islands (for details see Goldschmidt, 2007).

Conclusions

The findings on the ecology, habitat preferences, and distribution patterns of the Costa Rican species of *Torrenticola* allow two main conclusions: (1) the small areas of distribution of many species and the strict habitat binding of several high-mountain species to certain elevations result in a great threat of extinction, as is makes them extremely vulnerable to even small-scale habitat destruction and climate changes, and (2) the high diversity and differentiated ecology of the species of this genus illustrate the great potential of this group of water mites as bio-indicators for monitoring of aquatic habitats. The first necessary data (such as the differential tolerance of several species towards pollution, binding of particular species to certain elevations and habitat types) are provided in this paper. Their usefullness as bio-indicators is enhanced by the fact that determination keys are now available to the Neotropical species of the genus (Goldschmidt, 2007).

This general analysis of the Costa Rican species of the genus *Torrenticola* also revealed the great need for further research. On the local scale, further research in Costa Rica should be performed on the following subjects: (1) The Paramó-regions in the Cordillera de Talamanca should be further investigated, as most probably more endemic (and the most endangered) species are to be expected here and very special mountain faunas (until now only based on the Chirripó region) may be encountered; (2) detailed comparisons of the fauna in the dry forest region on the pacific slope in Guanacaste and the rain forest on the Caribbean slope at La Selva should be made, and (3) the life cycle and aut- and syn-ecology of individual species should be analysed, in order to enhance the database for the assessment of Costa Rican springs and running waters. On the regional scale, already available collections from North, Central, and South America (Canada, USA, Mexico, Ecuador, Argentina) should be analysed, in order to clarify large-scale patterns of the diversity and distribution of the genus *Torrenticola* in the New World and provide databases for comparisons of the fauna with other biogeographical regions.

Acknowledgements

I would like to thank Prof. Dr. Norbert Rieder (Univ. Karlsruhe, Zoological Inst. I) for hosting me at his department during the project on Costa Rican torrenticolid water mites. I also thank my former colleagues at the Dept Limnology at the Univ. Münster (Germany), especially Dr. Norbert Kaschek, and my friends in the Area de Conservación Guanacaste (Costa Rica), especially Maria Marta Chavarria Diaz and Roger Blanco, for their help and encouragement during the field work in Costa Rica. Finally, I thank Dr. Helga Bültmann (Univ. Münster) for the CCA analysis and Dr. Klaus Braune (Univ. Karlsruhe) for the cluster analysis. Part of this study has been financed by a postdoc-grant of the foundation Pro Acarologia Basiliensis in Basel, Switzerland. This study is mainly based upon material collected during my PhD research, throughout collecting trips to Costa Rica financed by grants from DAAD, Boehringer Ingelheim Fonds, Stifterverband für die deutsche Wissenschaft. Additional collecting trips to Costa Rica in 2002 and to Cuba in 2004 were financed by Deutsche Forschungsgemeinschaft (grants no. GO 1037/2-1 and GO 1037/3-1).

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Stage distributions of cunaxids in soil and litter at Chamela, Jalisco, Mexico

Blanca E. Mejía-Recamier & Gabriela Castaño-Meneses
Ecología y Sistemática de Microartrópodos, Depto. Ecología y Recursos Naturales, Facultad de Ciencias, UNAM, 04510 México, DF. E-mail: tellarecamier@yahoo.com.mx

Cunaxid mites are considered to be important predators in soil communities, but little is known of the distribution over their development stages. We studied the stage distribution of edaphic cunaxid mites in a deciduous dry forest in the Pacific region of Mexico. Monthly soil and litter samples were taken from June 1991 to July 1992 in two watersheds at the Chamela Biological Station (19°30'N, 105°03'W, 150 m asl), Jalisco, Mexico. A total of 4,720 individuals were collected from 43 species, including adults and immature forms. The proportion of adults was 74% (females 53%, males 21%), 10% tritonymphs, 1% deutonymphs, 6% protonymphs, and 9% larvae. Immature stages were more abundant in the soil than in the litter. Seasonal dynamics was recorded for the various stages. These data are the first on the seasonal distribution of developmental stages in edaphic cunaxid mites.

Key words: Cunaxidae, ontogenetic development, seasonal dynamics, soil, litter

The family Cunaxidae represents an important group of predatory mites, capable of living in edaphic and other environments, and of regulating prey populations (Muma, 1960; Schruft, 1971; May, 2001). Cunaxid species can also be used for biological control of many agricultural mite pests, mainly phytophagous mites on grape, peach, strawberry, and citrus crops (Muma, 1960; Schruft, 1971; Kethley, 1982; May, 2001; Petrova et al., 2004).

Although cunaxid predators are considered to be effective in the control of agricultural pests, as well as an important link in trophic webs, little is known about their life cycle. Currently, there are only descriptions of some stages of their development. Den Heyer (1979, 1980) describes the tritonymph of *Bonzia halacaroides* and *B. sphagnicola*, as well as the deutonymph, protonymph, and larva of *Pulaeus pectinatus*, and the protonymph of *P. glebulentus*, whereas Swift (1996) describes the tritonymph and deutonymph of *Dactyloscirus hoffmanea* and the larva of *D. smileyi.* Since there is little knowledge available about the life cycle of cunaxids, we collected data on the distribution of cunaxids over their development stages in a deciduous dry forest in the Pacific area.

MATERIAL AND METHODS

The Biological Station Chamela belongs to the Universidad Nacional Autónoma de México (UNAM) and is located in the Chamela municipality at the coast of Jalisco State (19°29'-19°32'N; 104°58'-105°05'W). The area around Chamela has low hills (20-250 m) and its climate is warm and subhumid, but subject to marked seasonality (Awoi, according to Köppen's classification, as modified by García, 1988). This is the most dry type of the subhumid climates. It has an average annual precipitation of 788 mm but between-year variation is large. In 1992, for example, 1,393 mm precipitation was recorded as result of tropical cyclones in the central part of the Pacific coast (García-Oliva et al., 2002).

The dominant vegetation is of a tropical dry forest type. There are >1,036 species of vascular plants with two of the most representative families being Leguminosae (57 species) and Euphorbiaceae (26 species). There are >227 species of trees, apart from bushes (216 spp.), lianes (187 spp.), herbs (366 spp.), and epiphytes (47 spp.) (Lott & Atkinson, 2002). Dominant soil types are luvi-eutric and eutric regosols, eutric cambisols, and haplic lixisol (Cotler et al., 2002).

The study area comprises 10,000 ha, a system of five hydrological watersheds, from which watersheds 1 and 4 (Cervantes et al., 1988) were selected because they are similar in size and have a mean annual productivity of 7,642 kg of fallen leaves per ha (Patiño, 1990; Martínez-Yrizar et al., 1996). On each watershed an area of 50 × 50 m was demarcated. Within this area, 10 points were selected randomly for soil (SW1 and SW4) and litter (LW1 and LW4) sampling. Samples were taken monthly from June 1991 to July 1992.

Extraction of fauna was made by means of Berlese-Tullgren funnels. Mites were collected in bottles with 75% alcohol. To identify species, semi-permanent slides were prepared in Hoyer's solution. Species were determined with the help of specialized literature (in absence of a comprehensive taxonomic synthesis).

Densities of various stages in each biotope and watershed were calculated. For each community, Shannon's diversity (H'), Pielou's evenness (J'), and Sörensen's similarity indices were calculated, as described by Ludwig & Reynolds (1988). To compare Shannon's diversity indices, a modified t-test was carried out (Zar, 1984). Relative abundances of the several developmental stages were compared among communities by means of a χ^2 test (Zar, 1984).

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Table 1 Temporal variation of species with the most development stages and the highest density in soil and litter in Chamela. ad, adults; tn, tritonymphs; dn, deutonymphs; pn, protonymphs; lv, larvae.

	1991						1992					
Species	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
SOIL						lv						
Pseudobonzia sp. nov. 2	ad tn pn	tn	tn			ad tn dn pn lv	ad tn lv	ad tn pn lv	ad tn pn	ad tn dn pn lv	ad tn dn pn	ad tn pn lv
Cunaxa potchensis	ad tn dn	ad tn ln	ad tn lv	ad pn	ad lv	ad lv	ad	ad lv	ln	ad lv	ad	ad lv
Dactyloscirus sp. nov. 2	ln	ln	ad pn			ad tn pn	ad tn	ad tn pn	ad tn pn ln	ad tn pn	ad tn pn	ad tn lv
Armascirus harrisoni		tn pn	ad tn		tn	ad tn pn	ad tn	ad tn dn	ad tn pn	ad tn lv	ln	tn dn lv
Cunaxoides sp. nov. 1	ad tn pn ln	ad pn lv	ad tn	ad lv	ad	ad tn pn lv	ad tn		ad tn lv	ad tn pn lv		
Cunaxoides sp. nov. 2		pn	ad	ad tn pn	ad tn pn	ad dn lv	ad		tn pn	tn dn pn lv		tn lv
Pulaeus sp. nov. 1						tn lv		tn pn				
Pulaeus sp. nov. 13		tn pn	tn	ad	ad	ad tn pn		ad tn	ad tn	ad tn pn lv		ad tn pn
Pulaeus sp. nov. 14	tn pn	ad pn lv				ad lv	ad	ad tn lv	tn pn			lv
Pulaeus sp. nov. 15				ad	ad	ad tn dn lv			tn pn	ad tn pn lv		
LITTER												
Cunaxa potchensis	ad lv	ad pn lv		pn lv	ad td	tn pn lv	ad pn lv	ad tn pn lv		ad tn pn lv		ad
Dactyloscirus sp. nov. 2	ad pn lv	ln	ad	lv		pl	ad lv	ad lv	ad	tn		
Armascirus harrisoni		lv	ad td	ad tn pn	ad	ad tn	ad tn pn	ad tn pn	ad tn pn	ad tn pn	tn	ad pn
Cunaxoides sp. nov. 1	ad tn pn lv	ad tn	ad tn lv	ad tn pn lv	ad tn pn	ad tn lv	ad tn dn	ad pn lv			ad	

RESULTS

A total of 4,634 individuals were collected (43 species), including adults and immatures. Annual density in the soil was higher for females (53%) than for males (21%), followed by tritonymphs (10%), larvae (9%), protonymphs (6%), and deutonymphs (1%). All cunaxid stages were more abundant in the soil of the two watersheds, than in the litter (χ^2 = 241.76, d.f. = 15, P<0.001; Fig. 1). Adult females were more abundant than males, especially in the soil of watershed 4 (Fig. 1).

Species with the highest density and of the most developmental stages in the soil were *Pseudobonzia* sp. nov. 2, *Cunaxa potchensis*, *Cunaxoides* sp. nov. 1 and 2, *Dactyloscirus* sp. nov. 2, *Armascirus harrisoni*, and *Pulaeus* sp. nov. 1, 13, 14, and 15. Only *C. potchensis*, *Cunaxoides* sp. nov. 2, and *Pulaeus* sp. nov. 14 and 15 showed all five developmental

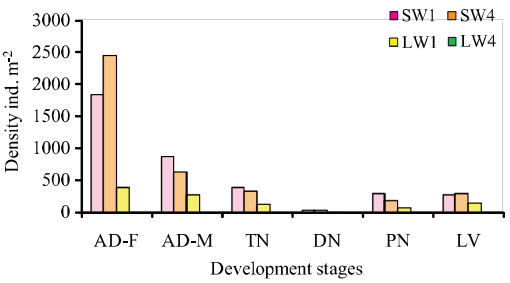

Figure 1 Total density (no. individuals m^{-2}) per development stage of mites in the family Cunaxidae. AD-F, female adults; AD-M, male adults; TN, tritonymphs; DN, deutonymphs; PN, protonymphs; LV, larvae; SW1, soil watershed 1;SW2, soil watershed 4; LW1, leaf litter watershed 1; LW4, leaf litter watershed 4.

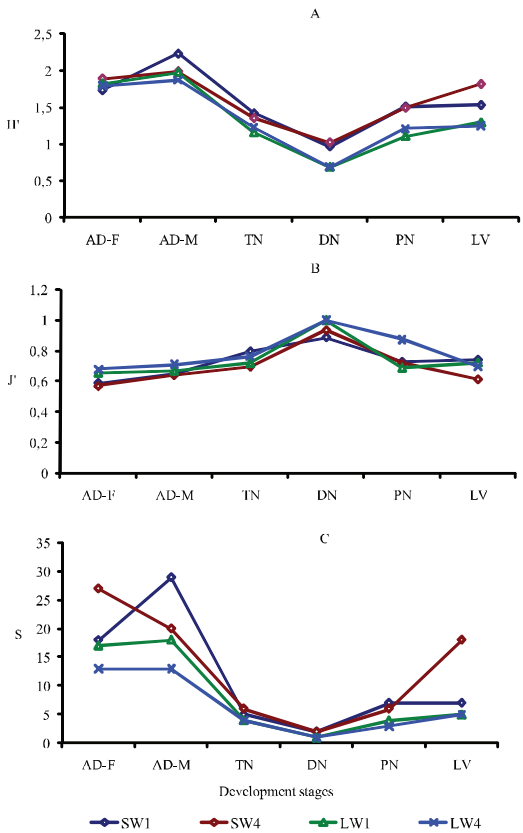

Figure 2 Shannon's diversity (H'), Pielou's evenness (J'), and Sörensen's similarity (S) in the various development stages found in Chamela, during a year of collection. Abbreviations as in Fig. 1.

stages, while *Cunaxoides* sp. nov. 1 was found in the soil and litter in all five stages in both (Table 1).

Diversity, species richness, and evenness showed a similar pattern among the developmental stages in all four communities. Deutonymphs were least diverse and rich, and they had a high evenness, suggesting that their populations were more homogeneous. The density was very low and there were no dominant species (Fig. 2). This is confirmed by the findings based on Sörensen's index, which allowed to compare the composition similarity between larvae and adults, while protonymphs were similar in composition to tritonymphs. Deutonymphs were the least similar to larvae, with only 1% (Table 2); this is because adults, larvae, tritonymphs and protonymphs occurred in relatively high densities.

Table 2 Sörensen's similarity index in all five stages of cunaxids in Chamela, Jal. ad, adults; tn, tritonymphs; dn, deutonymphs; pn, protonymphs; lv, larvae.

		ad	tn	dn	pn	lv
ad	*	23	21	15	23	85
tn	*	*	13	78	87	18
dn	*	*	*	19	13	1
pn	*	*	*	*	75	12
lv	*	*	*	*	*	17

Temporal variation

The temporal pattern of densities observed in adults was homogeneous and the densities were higher than those of the immature stages, having peaks of highest abundance in SW1 in December, in SW4 in August, in LW1 in November, and in LW4 in January. In May and June, the density of all stages and species decreased (Fig. 3A). Seasonal fluctuation in densities of the various immature stages, for the four environments, are depicted in Figures 3B-E.

Only the temporal distribution and the density of the five species that showed all developmental stages were taken into account. Peaks of highest abundance in the litter were found for *Cunaxoides* sp. nov. 1 in July, August, and October, whereas the highest peak of *C. potchensi* was found in February and April. In the other species the abundance was very low and there was an increase in density only in December (Fig. 4A). In the soil, in April, most of the species were found at a high density and *C. potchensis* showed the steepest increase; *Pulaeus* sp. nov. 14 showed a lower but uniform abundance throughout the year (Fig. 4B). In litter, only *Cunaxoides* sp. nov. 1 showed all five stages, with the adults as the most abundant (616 individuals m^{-2}), then tritonymphs (349), and larvae (212). Of *Cunaxoides* sp. nov.2, only adults and tritonymphs were observed in the litter, in very low densities. In the soil all stages were found, and *C. potchensis* (Fig. 4C-D) had the highest abundance of each stage.

Ontogenetic development

In general, of the cunaxid species under study the adult male is very similar to the female, but the male is smaller, and its genital plate is small and circular, with four genital rows of setae, located near the anal plate. Legs I-IV are shorter. Tritonymphs are smaller than females, but larger than males, with propodosomal plate, dorsal chaetotaxy similar to adult female, four pairs of genital setae on the genital plate, without agenital setae, and with two well developed genital papillae. Deutonymphs are as big as males, whereas the chaetotaxy of the propodosomal plate is identical to that of the adult female, with three pairs of genital setae on the genital plate, without agenital setae, and with two well-developed, genital papillae. Protonymphs are smaller than deutonymphs, also with chaetotaxy of propodosomal plate identical to that of the adult female, with two pairs of genital setae in longitudinal rows on the genital plate, without agenital setae, with a single genital papilla. Larvae are smallest, without genital setae, nor genital plate, nor agenital setae.

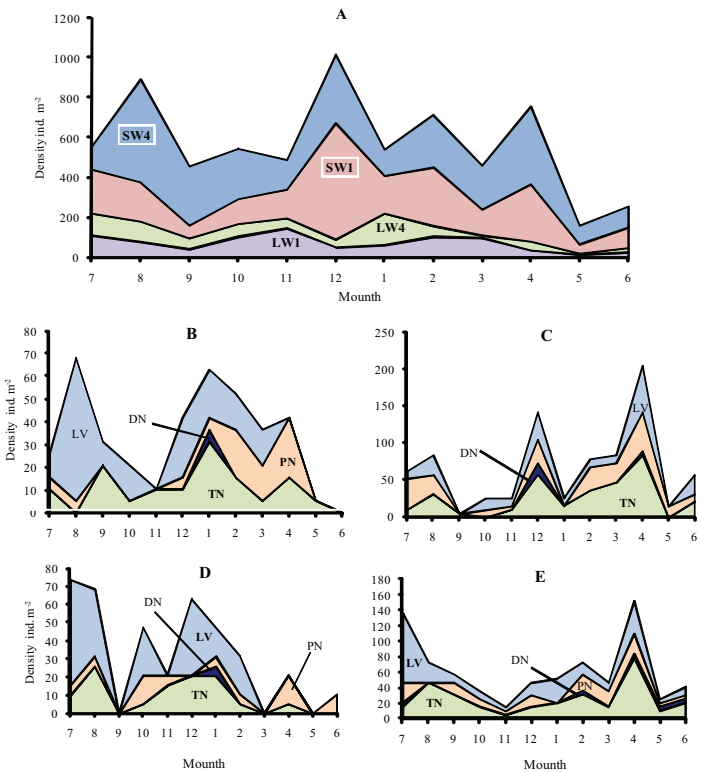

Figure 3. Temporal variation in the density (no. individuals m^{-2}) of development stages of cunaxid mites in Chamela: (A) Adults in litter watersheds (LW1, LW4) and soil watersheds (SW1, SW4), and juveniles in (B) litter watershed 1, (C) soil watershed 1, (D) litter watershed 4, and (E) soil watershed 4. Abbreviations as in Fig. 1.

DISCUSSION

Among the cunaxids studied females were always more abundant than males. This pattern may be due to differential sex allocation or differential mortality among the sexes. Walter & Kaplan (1991) observed that males move slower than females, leaving them more vulnerable to cannibalistic females and intraguild predators. The abundance of adults, especially the high density of females found after rains, suggests that they either survive rains better or benefit from humid conditions otherwise, e.g., in terms of food.

The peaks of highest abundance of the dominant species were related with the abundance of Collembola of families such as Isotomidae, Sminthuridae, Entomobryidae, and Sminthurididae (Gómez-Anaya & Palacios-Vargas, 2004). This suggest that they feed on collembolans. Walter & Kaplan (1991) state that *Coleoscirus simplex*, *Dactyloscirus inermis*, and the genera *Cunaxa* and *Pulaeus* feed on Collembola from families Isotomidae and Onychiuridae. Some groups of predatory mites from the Prostigmata are known to increase their populations when food type, temperature, and humidity are optimal for development (Kethley, 1982, 1990; Fox, 1999; Lee & Ahn, 2000; Fu et al., 2002; Landeros et al., 2004; Escalona & Vázquez, 2005; Tsoukanas, 2006).

Deutonymphs of cunaxids were not abundant in the soil and the litter of Chamela, compared to species of the family Bdellidae collected from the same site and present in relatively large numbers throughout the year (Mejía-Recamier, 1997). Temperature, food quality, and food quantity may explain this, because deutonymphs of cunaxids are known to be more sensitive to abiotic and biotic changes. It has been observed that temperature and diet have a significant effect on the development and longevity of deutonymphs of Phytoseiidae, which are predators of Tetranychidae (Lee & Ahn, 2000; Fu et al., 2002; Escalona & Vázquez, 2005; Tsoukanas et al., 2006). Another factor decreasing the density of duetonymphs could be intraspecific competition. However, it should be noted that the method of collection may not have been adequate for this immature stage, because deutonymphs are known to enter a quiescent peri-

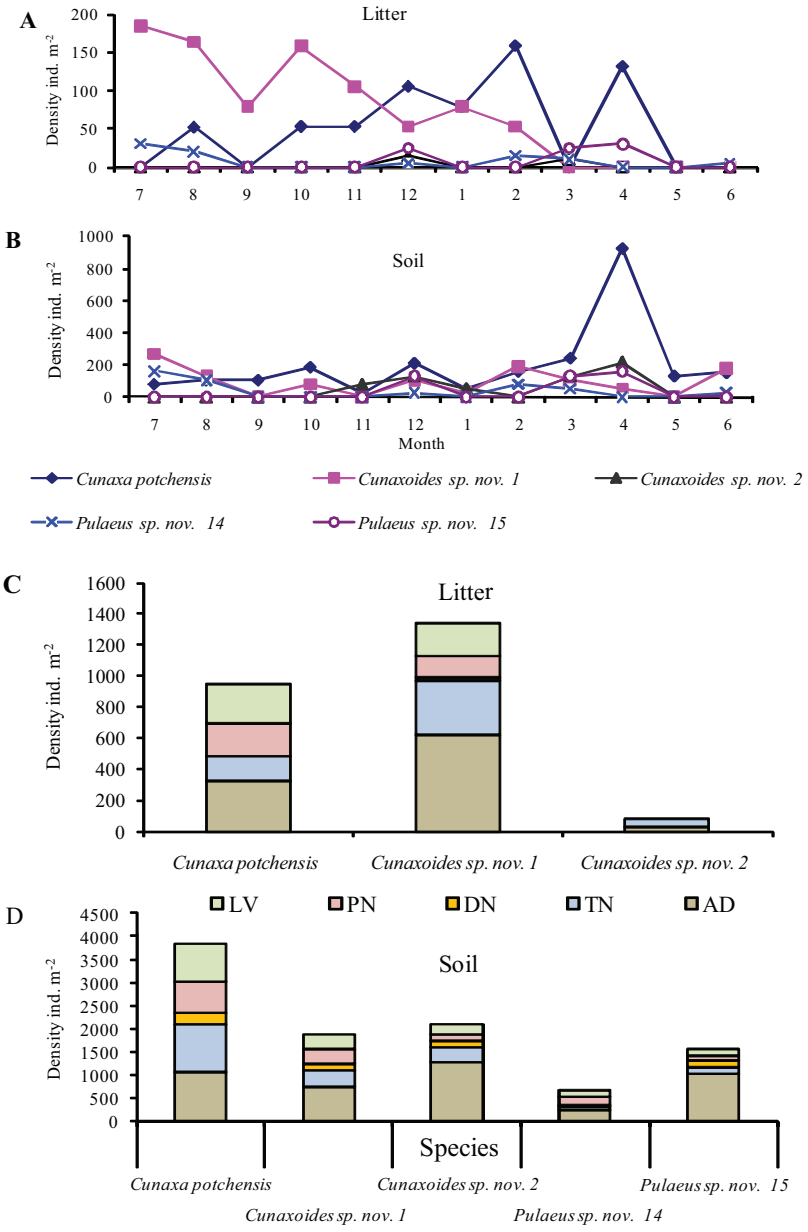

Figure 4 Variation in density (no. individuals m^{-2}) per month (A-B) and overall density (C-D) for various species that showed all immature stages in the soil and in the litter in Chamela. AD, adults; TN, tritonymphs; DN, deutonymphs; PN, protonymphs; LV, larvae.

od for hibernation and this period may take almost 64% of the total time of development. During this period they are highly vulnerable to attacks by other predators and they hide in places which offer them protection such as fissures, holes, and cracks in trees and rocks. All these biotopes were not sampled in our study. Still, as Sorensen et al. (1983) point out, they may show vertical movements on foliage throughout the day to hunt phytophagous prey.

We observed that cunaxids can produce several generations per year. Walter & Kaplan (1991) found that *C. simplex* has a generation time of 14 days under optimal temperature, moisture, and food conditions. An increase in density was observed after the rains in January for reasons that remain to be explained. Moreover, after the January rains (648 mm), tritonymphs and larvae showed an increase of density in the litter and a decrease in the months thereafter, whereas protonymphs showed the opposite pattern. However, in the soil the density of all three immature stages decreased in January and increased in the months thereafter. Species of Bdellidae showed a similar pattern of abundance in each of the developmental stages, but they occurred in lower densities (Mejía-Recamier, 1997).

Focusing on the immature stages, *Cunaxoides* sp. nov. 2 and *Pulaeus* sp. nov. 14 and 15 showed affinity to the soil environment, whereas *C. potchensis* and *Cunaxoides* sp. nov. 1 had affinity to both the soil and the litter. The highest abundance of *Cunaxoides* sp. nov. 1 was observed in the litter and during the humid season, whereas the abundance of *C. potchensis* was more uniform throughout the year in litter and soil. In the soil the species diversity was higher than in the litter. Possibly, the litter represents a more unstable habitat, allowing fewer species to coexist, whereas the more stable soil could harbour more niches and offer better conditions for coexistence of cunaxids by niche specialization. Also spatial variation in availability of nutrients in the soil (Huante et al., 2002) may create more opportunities for coexistence of cunaxid species in the soil.

Acknowledgements

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Mites (Mesostigmata) inhabiting bird nests in Slovakia (Western Carpathians)

Peter Fend'a
Department of Zoology, Faculty of Natural Sciences, Comenius University, Mlynská dolina B-1, 842 15 Bratislava, Slovakia.
E-mail: fenda@fns.uniba.sk

In Slovakia, 229 species of mesostigmatic mites (more than 230,000 specimens) have been collected from more than 2,500 nests of birds from 110 bird taxa. Most (97%) of the mite specimens were parasitic, representing 26 species, of which only six were typical avian parasites. All blood parasites had a wide host range, yet were specific for nest type (except for *Dermanyssus chelidonis* and *Eulaelaps novus*). Their abundance peaked in the nesting period. The 3% of the mite specimens that were non-parasitic were the most speciose (203 species). The composition of the non-parasitic mite fauna in the nests was dependent on nest location (habitat) and nest environment (biotope).

Key words: Mesostigmata, Aves, nest fauna, ectoparasites, Slovakia

Many arthropods seek refuge, food, and suitable microclimate for reproduction or hibernation in bird nests or they occur there by chance. Hence, the species composition in nests reflects the many taxa present in their direct environment. Heselhaus (1915) designated animals living in the nests as Nidicola. Nests harbour a miniature ecosystem, subject to density-regulating processes (Mulyarskaya, 1953).

The first record of mesostigmatic mites from birds and their nests in Slovakia was published by Mrciak & Rosický (1956), but a more comprehensive, pioneering inventory of Mesostigmata in Slovakian bird nests was published by Ambros et al. (1992). Since then, 34 studies have been published, complementing our knowledge of mesostigmatic mites inhabiting bird nests in this geographic area.

The purpose of this article is to synthesize published and unpublished information on mesostigmatic mites in bird nests, and propose a division of mesostigmatic nidofauna based on nest location, nest environment, and bird species.

MATERIALS AND METHODS

Mite material was collected from 2,572 bird nests in Slovakia in the period 1981-2005. The nests were kept in plastic polyethylene bags. Mites were extracted from the nests using a Tullgren's funnel with a 40-W light bulb as a heat source and they were collected in 70% ethylalcohol solution. The material was processed to yield microscopic preparations using a medium of chloralhydrate, called Liquid de Swan.

Apart from own data, also published data were used in a cluster analysis (Podani, 1988) performed to assess associations with bird hosts and their nests. The results of this analysis are not shown here, only preliminary conclusions are provided.

RESULTS

Including literature data, 229 species of mesostigmatic mites were identified from a sample of more than 230,000 specimens, collected from nests of birds from 110 taxa in Slovakia (Table 1).

Avian ectoparasites

Parasitic mites constituted 97% of all mesostigmatic mites found in the nests. Among the 26 species of obligatory or facultative blood-sucking mites, six species are typical avian parasites: *Dermanyssus hirundinis* was the eudominant species in nests of European penduline tit (*Remiz pendulinus*; 92.3%) and House martin (*Delichon urbica*; 84.6%). High numbers were also found in nests of Barn swallow (*Hirundo rustica*; 17.5%) and Sand martin (*Riparia riparia*; 7.9%). This species also occurred in nests in nest boxes (2.0%), in free nests of Passeriformes (0.9%), in the nests of birds of prey (4.1%), and also in nests on the ground (0.9%), or in nests on the water surface (1.0%). A total of 26 bird host species was recorded.

Dermanyssus gallinae was a typical parasite in the nests of Chicken hens (*Gallus domesticus*; 100%) and feral Rock pigeons (*Columba livia*; 99.9%). This species occurred in higher numbers in the nests of *H. rustica* (0.2%), *D. urbica* (1.0%), *R. riparia* (7.6%), and in nest boxes (0.7%), especially in the countryside in the vicinity of human settlement. In total 20 bird host species were recorded.

Dermanyssus chelidonis was a typical parasite in nests of *D. urbica* (14.1%). Sporadically, it was recorded in nests of Linnet (*Carduelis cannabina*), Blue tit (*Parus caeruleus*), and *R. riparia*.

Ornithonyssus sylviarum was eudominant in free nests of Passeriformes: 51.5% and even 92.1% in nests of Warblers (*Acrocephalus* spp.), *H. rustica* (82.1%), *R. pendulinus* (7.2%), and *R. riparia* (1.4%). However, in nests of *D. urbica* this

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Table 1 List of species from bird nests in Slovakia (+, present; #, dominant species).

mite species / host	Acc*	Ans	Cha	Cic	Col	Cor	Fal	Gal	Gru	Pas	Pic	Pod	Psi	Str
GAMASINA														
Ameroseiidae														
Ameroseius apodius Karg, 1971	+						+			+				
Ameroseius corbiculus (Sowerby, 1806)	+	+								+				
Ameroseius lidiae Bregetova, 1977		+							+	+				
Ameroseius longitrichus Hirschmann, 1963	+													
Ameroseius plumea Oudemans, 1902	+									+				
Ameroseius plumosus (Oudemans, 1902)						+								
Epicriopsis horridus (Kramer, 1876)	+	+												
Epicrius tauricus Bregetova, 1977										+				
Ascidae														
Arctoseius cetratus (Sellnick, 1940)	+	+							+					
Arctoseius semiscissus (Berlese, 1892)	+	+		+					+	+				
Asca bicornis (Canestrini et Fanzago, 1887)	+	+								+				
Blattisocius keegani Fox, 1947	+	+				+				+				
Blattisocius tarsalis (Berlese, 1918)					+					+			+	
Cheiroseius borealis (Berlese, 1903)		+								+				
Cheiroseius cassiteridium (Evans et Hyatt, 1960)	+	+							+	+		+		
Cheiroseius curtipes (Halbert, 1923)	+	+										+		
Cheiroseius mutilus (Berlese, 1916)		+		+						+		+		
Cheiroseius necorniger (Oudemans, 1903)										+				
Cheiroseius serratus (Halbert, 1915)		+												
Cheiroseius viduus (CL Koch, 1839)		+												
Iphidozercon gibbus (Berlese, 1903)		+												
Lasioseius berlesei (Oudemans, 1938)		+												
Lasioseius confusus Evans, 1958	+	+		+					+	+		+		
Lasioseius mirabilis Christian et Karg, 1993										+				
Lasioseius ometes (Oudemans, 1903)			+		+					+				
Lasioseius penicilliger Berlese, 1916 sensu Hughes, 1961										+		+		
Leioseius bicolor (Berlese, 1918)	+	+								+				
Leioseius minusculus (Berlese, 1905)	+	+		+					+	+				
Neojordensia levis (Oudemans et Voigts, 1904)		+								+				
Neojordensia sinuata Athias-Henriot, 1973				+						+		+		
Paragarmania dentritica (Berlese, 1918)		+				+	+			+			+	
Plesiosejus italicus (Berlese, 1905)		+												
Plesiosejus major (Halbert, 1923)		+		+								+		
Proctolaelaps fiseri Samšinák, 1960		+												
Proctolaelaps pini Hirschmann, 1963	+									+				
Proctolaelaps pomorum (Oudemans, 1929)										+				
Proctolaelaps pygmaeus (J Müller, 1860)	+	+		+		+				+			+	+
Proctolaelaps scolyti Evans, 1958						+				+				+
Proctolaelaps ventrianalis Karg, 1971						+								
Zerconopsis remiger (Kramer, 1876)										+				
Dermanyssidae														
Dermanyssus carpathicus Zeman, 1979										+				
Dermanyssus chelidonis Oudemans, 1939										+				
Dermanyssus gallinae (De Geer, 1778)		+			#	+		#		+	+			#
Dermanyssus hirundinis (Hermann, 1804)	+	+				#				#			#	+
Dermanyssus passerinum Berlese et Trouessart, 1889										+	+			
Digamasellidae														
Cornodendrolaelaps presepum (Berlese, 1918)	+					+								
Dendrolaelaps cornutus Kramer, 1886	+	+								+				
Dendrolaelaps punctum (Berlese)										+				
Dendrolaelaps zwoelferi Hirschmann, 1960	+	+												+
Digamasellus punctum (Berlese, 1904)			+			+				+				
Multidendrolaelaps bispinosus (Karg, 1971)	+													
Punctodendrolaelaps arvicolus (Leitner, 1949)		+								+				
Punctodendrolaelaps fallax (Leitner, 1949)		+				+				+				
Punctodendrolaelaps latior (Leitner, 1949)		+							+					
Eviphididae														
Alliphis halleri (G et R Canestrini, 1881)	+	+	+							+				
Crassicheles holsaticus Willmann, 1937										+				
Eviphis ostrinus (CL Koch, 1836)	+	+								+				
Scarabaspis inexpectatus (Oudemans, 1903)										+				
Halolaelapidae														
Halolaelaps porulus Hirschmann et Götz, 1968														+
Halolaelaps sexclavatus (Oudemans, 1902)										+				

Table 1 Continued

mite species / host	Acc*	Ans	Cha	Cic	Col	Cor	Fal	Gal	Gru	Pas	Pic	Pod	Psi	Str
Hirstionyssidae														
Echinonyssus butantanensis (Fonseca, 1932)						+				+				
Echinonyssus isabellinus (Oudemans, 1913)										+				
Echinonyssus musculi (Johnston, 1849)										+			+	
Echinonyssus pauli (Willmann, 1952)										+				
Laelapidae														
Androlaelaps casalis (Berlese, 1887)	#	+				#	+			#			+	#
Androlaelaps fahrenholzi (Berlese, 1911)	+	+				+				+				+
Eulaelaps novus Vitzthum, 1925										+				
Eulaelaps stabularis (CL Koch, 1836)	+	+				+				+				+
Haemogamasus ambulans (Thorell, 1872)										+				
Haemogamasus hirsutosimilis Willmann, 1952										+				
Haemogamasus hirsutus Berlese, 1889										+				
Haemogamasus horridus Michael, 1892		+												
Haemogamasus nidi Michael, 1892		+				+				+			+	+
Hypoaspis aculeifer G Canestrini, 1884	+	+				+				+				
Hypoaspis angustiscutata Willmann, 1951		+												
Hypoaspis astronomica (CL Koch, 1839)						+								
Hypoaspis austriaca Sellnick, 1935										+				
Hypoaspis cuneifer (Michael, 1891)										+				
Hypoaspis curtipilis Hirschmann, 1969		+												
Hypoaspis giffordi Evans et Till, 1966		+							+					
Hypoaspis heselhausi Oudemans, 1912										+				
Hypoaspis heyi Karg, 1962		+								+				
Hypoaspis hyatti Evans et Till, 1966										+				
Hypoaspis intermedia Hirschmann, 1969										+				
Hypoaspis kargi Costa, 1968		+				+				+				
Hypoaspis lubrica Voigts et Oudemans, 1904	+	+				#				+			+	+
Hypoaspis lubricoides Karg, 1971		+												
Hypoaspis marginepilosa Sellnick, 1940		+												
Hypoaspis miles (Berlese, 1892)		+				#				+				
Hypoaspis nidicorva Evans et Till, 1966										+				
Hypoaspis pini Hirschmann, 1969										+				
Hypoaspis praesternalis Willmann, 1949										+				
Hypoaspis sardoa (Berlese, 1911)		+								+				
Hypoaspis vacua (Michael, 1891)		+				+				+				
Laelaps agilis CL Koch, 1836						+				+			+	
Laelaps hilaris CL Koch, 1836						+								+
Laelaps muris (Ljungh, 1799)		+												
Pseudoparasitus myrmophilus (Michael, 1891)						+								
Pseudoparasitus placentulus (Berlese, 1887)		+	+							+				
Pseudoparasitus sellnicki (Bregetova et Koroleva, 1964)	+	+												
Pseudoparasitus venetus (Berlese, 1904)		+								+				
Macrochelidae														
Geholaspis longispinosus (Kramer, 1876)		+								+				
Geholaspis hortorum (Berlese, 1904)		+												
Holostaspella exornata Filipponi et Pegazzano, 1967		+												
Holostaspella neglecta Krauss, 1970		+							+					
Holostaspella ornata (Berlese, 1903)		+												
Holostaspella subornata Bregetova et Koroleva, 1960		+												
Macrocheles americana (Berlese, 1888)										+				
Macrocheles ancyleus Krauss, 1970	#						+							+
Macrocheles confusa (Foa, 1900)			+											
Macrocheles decoloratus (CLKoch, 1839)		+												
Macrocheles glaber (J Müller, 1859)	+	+	+	+		+			+	+				
Macrocheles matrius (Hull, 1925)						#				+				
Macrocheles merdarius (Berlese, 1889)			+	+		+				+				
Macrocheles montanus Willmann, 1951	+	+	+							+				
Macrocheles muscaedomesticae (Scopoli, 1772)			#			+			+	+				
Macrocheles nataliae Bregetova et Koroleva, 1960		+								+				
Macrocheles penicilliger (Berlese, 1903)	+	#	#	+			+			+				
Macrocheles punctoscutatus Evans et Browning, 1956		+								+				
Macrocheles recki Bregetova et Koroleva, 1960										+				
Macrocheles robustulus (Berlese, 1903)		+	+	+						+				
Macrocheles rotundiscutis Bregetova et Koroleva, 1960	+	+												
Macrocheles scutatus (Berlese, 1904)										+				
Macrocheles subbadius (Berlese, 1904)										+				
Macrocheles tardus (CL Koch, 1841)										+				

Table 1 Continued

mite species / host	Acc*	Ans	Cha	Cic	Col	Cor	Fal	Gal	Gru	Pas	Pic	Pod	Psi	Str
Macrocheles tridentinus (G et R Canestrini, 1882)		#												
Neopodocinum mrciaki Sellnick, 1968					+									
Macronyssidae														
Ornithonyssus bacoti (Hirst, 1913)													#	
Ornithonyssus pipistrelli (Oudemans, 1904)					+					+				
Ornithonyssus sylviarum (Canestrini et Fanzago, 1877)		+		+		+				#				+
Steatonyssus periblepharus Kolenati, 1858										+				
Myonyssidae														
Myonyssus rossicus Bregetova, 1956										+				
Pachylaelapidae														
Olopachys suecicus Sellnick, 1950		+												
Pachylaelaps ineptus Hirschmann et Krauss, 1965		+												
Pachylaelaps pectinifer (G et R Canestrini, 1882)		+												
Pachyseius humeralis Berlese, 1910		+								+				
Parasitidae														
Cornigamasus lunaris (Berlese, 1882)		+							+	+				
Eugamasus berlesei Willmann, 1935										+				
Gamasodes bispinosus (Halbert, 1915)									+			+		
Gamasodes spiniger (Trägårdh, 1910)		+				+			+	+				
Holoparasitus calcaratus (CL Koch, 1839)		+								+				
Holoparasitus excipuliger (Berlese, 1905)		+								+				
Holoparasitus tuberculatus Juvara-Bals, 1975										+				
Leptogamasus parvulus (Berlese, 1903)										+				
Leptogamasus succineus Witalinski, 1973		+												
Leptogamasus tectegynellus (Athias-Henriot, 1967)										+				
Lysigamasus cornutus (Schweizer, 1961)		+												
Lysigamasus lapponicus (Trägårdh, 1910)										+				
Lysigamasus orthogynellus (Athias-Henriot, 1967)		+												
Lysigamasus runcatellus (Berlese, 1903)		+												
Paragamasus similis (Willmann, 1953)										+				
Parasitus beta (Oudemans et Voigts, 1904)	+	+								+				
Parasitus coleoptratorum (Linnaeus, 1758) sensu Oud., 1908		+	+			+				+				
Parasitus fimetorum (Berlese, 1903)	+	#	#							+				
Parasitus hyalinus (Willmann, 1949)	+	+	+							+				
Parasitus loricatus (Wankel, 1861)		+								+				
Parasitus mammillatus (Berlese, 1904)	+	+								+				
Parasitus mustelarum (Oudemans, 1902)										+				
Pergamasus barbarus Berlese, 1904										+				
Pergamasus brevicornis (Berlese, 1903)		+	+							+				
Pergamasus crassipes (Linnaeus, 1758)	+	+								+				
Pergamasus mediocris (Berlese, 1904)										+				
Pergamasus norvegicus (Berlese, 1905)		+								+				
Pergamasus noster (Berlese, 1903)										+				
Pergamasus ruhmi Willmann, 1938										+				
Pergamasus septentrionalis Oudemans, 1902										+				
Poecilochirus austroasiaticus Vitzthum, 1930										+				
Poecilochirus carabi G et R Canestrini, 1882		+								+				
Poecilochirus davydovae Hyatt, 1980										+				
Poecilochirus necrophori Vitzthum, 1930		+								+				
Porrhostaspis lunulata J Müller, 1869			+							+				
Trachygamasus ambulacralis Willmann, 1949			+											
Trachygamasus gracilis Karg, 1965		+												
Vulgarogamasus kraepelini (Berlese, 1904)	+	+												
Vulgarogamasus oudemansi (Berlese, 1903)		+								+				
Vulgarogamasus remberti (Oudemans, 1912)		+				+				+				
Phytoseiidae														
Amblyseius andersoni (Chant, 1957)										+				
Amblyseius bidens Karg, 1970										+				
Amblyseius bicaudus Wainstein, 1962										+				
Amblyseius filixis Karg, 1970										+				
Amblyseius nemorivagus Athias-Henriot, 1961										+			+	
Amblyseius neobernhardi Athias-Henriot, 1966		+							+	+				
Amblyseius patrius Karg, 1970										+				
Amblyseius tubae Karg, 1970										+				
Dictydionotus pepperi (Specht, 1968)										+				
Euseius finlandicus (Oudemans, 1915)										+				
Neoseiulus alpinus (Schweizer, 1922)									+					
Neoseiulus cucumeris (Oudemans, 1930)		+							+	+				

Table 1 Continued

mite species / host	Acc*	Ans	Cha	Cic	Col	Cor	Fal	Gal	Gru	Pas	Pic	Pod	Psi	Str
Neoseiulus reductus (Wainstein, 1962)		+							+	+				
Neoseiulus umbraticus (Chant, 1956)									+	+				
Neoseiulus versutus (Begljarov, 1981)		+												
Neoseiulus zwoelferi (Dosse, 1957)						+				+				
Paraseiulus talbii (Athias-Henriot, 1960)										+				
Phytoseius juvenis Wainstein et Arutyunyan, 1970										+				
Proprioseiopsis levis (Wainstein, 1960)	+			+					+	+				
Proprioseiopsis messor (Wainstein, 1960)										+				
Proprioseiopsis okanagensis (Chant, 1957)										+				
Typhlodromus bakeri (Garman, 1948)										+				
Typhlodromus rhenanus (Oudemans, 1905)										+				
Typhlodromus richteri Karg, 1970										+				
Typhlodromus rivulus (Karg, 1991)										+				
Typhlodromus setubali Dosse, 1961										+				
Pseudolaelapidae														
Pseudolaelaps doderoi (Berlese, 1910)		+												
Rhodacaridae														
Cyrtolaelaps chiropterae Karg, 1971										+				
Cyrtolaelaps mucronatus (G et R Canestrini, 1881)		+								+				
Euryparasitus emarginatus (CLKoch, 1839)										+				
Gamasellus montanus (Willmann, 1936)										+				
Stylochirus fimetarius (J Müller, 1859)	+	+								+				
Veigaiidae														
Veigaia cerva (Kramer, 1876)		+								+				
Veigaia exigua (Berlese, 1917)		+												
Veigaia kochi (Trägårdh, 1901)		+												
Veigaia nemorensis (CL Koch, 1839)	+	+							+	+				
Veigaia planicola (Berlese, 1892)		+												
Zerconidae														
Prozercon carpathofimbriatus Masán et Fend'a, 2004										+				
Prozercon kochi Sellnick, 1943	+													
Prozercon tragardhi (Halbert, 1923)										+				
Zercon arcuatus Trägårdh, 1931										+				
Zercon berlesei Sellnick, 1958										+				
Zercon carpathicus Sellnick, 1958										+				
Zercon curiosus Trägårdh, 1910	+									+				
Zercon hungaricus Sellnick, 1958		+								+				
Zercon peltatus var. *peltatus* CL Koch, 1836	+	+							+	+				
Zercon romagniolus Sellnick, 1944										+				
Zercon triangularis CL Koch, 1836			+											
SEJINA														
Sejidae														
Sejus togatus CL Koch, 1836		+												
Uropodellidae														
Asternolaelaps querci Wisniewski et Hirschmann, 1984										+				
UROPODINA														
Polyaspidae														
Polyaspinus kovaci Mašán et Kalúz, 1999										+				
Polyaspinus schweizeri (Hutu, 1976)	+													
Uroseius hunzikeri Schweizer, 1922										+				
Uroseius infirmus (Berlese, 1887)	+					+	#			+				+
Uroseius trogicolis Mašán, 1999	+									+				
Trachytidae														
Trachytes aegrota (CL Koch, 1841)		+								+				
Trachytes baloghi Hirschmann et Zirngiebl-Nicol, 1969										+				
Trematuridae														
Nenteria breviunguiculata (Willmann, 1949)	+	+	+							+				
Nenteria dobrogensis Feider et Hutu, 1971						+								
Nenteria pandioni Wisniewski et Hirschmann, 1985	#	+					+			+				+
Nenteria stylifera (Berlese, 1904)		+								+				
Trichouropoda karawaiewi (Berlese, 1904)	+	#								+				
Trichouropoda longiovalis Hirschmann et Zirngiebl-Nicol, 1961						+				+				
Trichouropoda obscurasimilis Hirschmann et Zirngiebl-Nicol, 1961	+					+				+				
Trichouropoda orbicularis (CL Koch, 1839)										+				+
Trichouropoda ovalis (CL Koch, 1839)	+	#								+				
Trichouropoda patavina (G Canestrini, 1885)						+								
Trichouropoda rafalskii Wisniewski et Hirschmann, 1984		+												
Trichouropoda tuberosasimilis Hirschmann et Wisniewski, 1987										+				

Table 1 Continued

mite species / host	Acc*	Ans	Cha	Cic	Col	Cor	Fal	Gal	Gru	Pas	Pic	Pod	Psi	Str
Urodinychidae														
Dinychus bincheaecarinatus Hirsch., W.-A. et Zirn.-Nicol, 1984		+												
Dinychus carinatus Berlese, 1903										+				
Dinychus inermis (CL Koch, 1841)		+								+				
Dinychus perforatus Kramer, 1886		+	+							+				
Dinychus woelkei Hirschmann et Zirngiebl-Nicol, 1969	+									+				
Urodiaspis tecta (Kramer, 1876)										+				
Uroobovella fimicola (Berlese, 1903)	+									+				
Uroobovella minima (CL Koch, 1841) sensu Willmann, 1951	+													
Uroobovella pulchella (Berlese, 1904)		+								+				
Uroobovella pyriformis (Berlese, 1920)	#									+				
Uropodidae														
Discourella modesta (Leonardi, 1899)		+												
Uropoda minima Kramer, 1882	+									+				
Uropoda orbicularis (OF Müller, 1776)	+	+								+				
Uropoda subterrana (Schweizer, 1922)			+											

*Acc, Accipitriformes; Ans, Anseriformes; Cha, Charadriiformes; Cic, Ciconiiformes; Col, Columbiformes; Cor, Coraciiformes; Fal, Falconiformes; Gal, Galliformes; Gru, Gruiformes; Pas, Passeriformes; Pic, Piciformes; Pod, Podicipediformes; Psi, Psittaciformes; Str, Strigiformes.

species was not found and in nest boxes its occurrence was low (0.2%). In total 20 bird host species were recorded.

The taxonomic position of two other species reported to occur in Slovakia is doubtful: *Dermanyssus carpathicus* and *Dermanyssus passerinus*. Not (yet) known from Slovakia, but present in neighbouring countries, such as Poland and Austria, are the avian ectoparasites *Dermanyssus alaudae* (Schrank) and *Dermanyssus quintus* Vitzthum.

Nidofauna

Only 3% of the mite specimens were non-parasitic, but they are much more speciose (203 species). Their species composition is most influenced by nest location (habitat) and nest environment (biotope).

1. Synantropical nests (*C. livia* and *G. domesticus*). These nests typically harboured the blood-sucking mite *D. gallinae* (99.9 and 100%, respectively).

2. Nests of birds of prey (Accipitriformes, Falconiformes, and also Strigiformes; e.g., Long-eared owl [*Asio otus*] and Eurasian eagle-owl [*Bubo bubo*]). Typically, these nests had a low proportion of parasitic species, usually only *D. hirundinis* (4.1%). The mesostigmatic species typical for these nests were *Nentera pandioni*, *Macrocheles ancyleus*, *Uroobovella pyriformis*, and *Uroseius infirmus*. Fauna composition is relatively stable, even during the winter months.

3. Nests on the ground (Anseriformes). These nests typically had a very low proportion of avian parasitic species, usally only *D. hirundinis* (0.3%) and *D. gallinae* (0.01%). As a consequence of direct contact with the soil, the nidofauna contained many soil-inhabiting mites, such as *Macrocheles tridentinus*, *Trichouropoda karawaiewi*, and *T. ovalis*. Typical nidicolous species were *Macrocheles penicilliger* and *Parasitus fimetorum*. A relatively large proportion of mites were typically facultative blood-feeders on small mammals, such as *Eulaelaps stabularis* (1.0%) and *Haemogamasus* spp. (0.3%).

4. Nests on water (Podicipediformes, Gruiformes). These nests contained few avian parasites, such as *D. hirundinis* (0.7%), *D. gallinae* (0.1%), and *O. sylviarum* (1.4%). The nidofauna typically contained hygrophilous species, such as *Gamasodes bispinosus*. According to cluster analysis, nests on water group together with nests of various species of birds in the same habitat, such as Marsh harrier (*Circus aeruginosus*; Accipitriformes), Reed bunting (*Emberiza schoeniclus*; Passeriformes), and Little bittern (*Ixobrychus minutus*; Ciconiiformes).

5. Free nests of Passeriformes. [5.1] Nests on vegetation. The avian blood-sucking species *Ornithonyssus sylviarum* was dominant in nests on vegetation (51.5%) and in nests of *Acrocephalus* spp. it even reached 92.1%. A relatively large proportion of species belonged to the family Phytoseiidae. [5.2] Nests of *Turdus* spp. These nests had few species of avian parasites, but 10 species of parasites known from small mammals. A common species was *Androlaelaps casalis* (19.4%). [5.3] Nests of *Remiz pendulinus*. Eudominant species were *D. hirundinis* (92.3%) and *O. sylviarum* (7.2%). Again, a relatively great proportion of species belonged to the family Phytoseiidae. [5.4] Nests of *Delichon urbica*. Eudominant species were *D. hirundinis* (84.6%), *D. chelidonis* (14.1%), and *D. gallinae* (1.0%). Phytoseiidae species were absent. [5.5] Nests of *Hirundo rustica*. Eudominant species were *O. sylviarum* (82.1%) and *D. hirundinis* (17.5%). Phytoseiidae species were absent.

6. Nests in cavities (Passeriformes, Coraciiformes). [6.1] Nests in tree holes and nest boxes (Passeriformes). *Androlaelaps casalis* is a typical inhabitant of this nest type (75.7%). Even in tree holes without nests, *A. casalis* is a eudominant species. Another species typical in these nests is the predator *Hypoaspis lubrica*. The mite fauna in nests from nest boxes with Falconiformes or Strigiformes is enriched with species typical for nests of birds of prey. Nest boxes were sometimes also occupied by bats, which may explain the findings of bat parasites, such as *Ornithonyssus pipistrelli* (0.8%) and *Steatonyssus periblepharus* (0.3%). When previously occupied by small rodents, findings of *Echinonyssus pauli* and *E. musculi* are not rare. In nest boxes in an aviary with Zebra finches (*Taenopygia guttata*) in a village courtyard, *Ornithonyssus bacoti* (ectoparasite of rats) was a eudominant species. [6.2] Nests in burrows in the soil (Passeriformes, Coraciiformes). *Androlaelaps casalis* is also a typical inhabitant of burrow-nesting birds such as *R. riparia* (62.2%) and European bee-eater (*Merops apiaster*; 97.7%). In these nests, *D. hirundinis* and *D. gallinae* are also regularly found, whereas in the nests of *R. riparia*, *Eulaelaps novus* (2.2%) was recorded as a typical species. Six species of rodent ectoparasites were frequently found, but always in low numbers.

DISCUSSION

The nidofauna in Slovakia is quite similar to that found in neighbouring countries in nests of White-tailed eagle (*Haliaeetus albicilla*) in northern Poland (Gwiazdowicz et al., 2005), *C. livia* in Ukraine (Piryanik & Akimov, 1964), House sparrow (*Passer domesticus*) and Tree sparrow (*Passer montanus*) in Poland (Fenda & Pinowski, 1997; Krumpál et al., 2001), and White stork (*Ciconia ciconia*) from Poland (Bloszyk et al., 2005). The absence of the avian parasite *Ornithonyssus bursa* (Berlese) in Slovakia is striking, because this species is known from Western Europe and also from Poland and the Czech Republic (Kristofík et al., 2001).

The occurrence of *D. hirundinis* and *O. sylviarum* is probably correlated to air humidity. Ioff (1958) noticed that *D. hirundinis* prefers moderate humidity in nest material. In nest boxes from Slovakia, prevalence of *D. hirundinis* was 2.0% and *O. sylviarum* 0.1% (similar to nests in burrows, nests of *R. pendulinus* and *Turdus* spp.), but in free nests (including *H. rustica*) prevalence of *D. hirundinis* was 0.9% and *O. sylviarum* 51.5%. *Ornithonyssus sylviarum* spends most of its lifetime on the host's body (Borisova, 1977) and therefore it can better cope with low humidity in the environment. All avian blood-sucking mesostigmatic mites had a wide host range, but occurred in specific nest types (with the exception of *D. chelidonis*). Abundance of these mites was highest in the nesting period.

The first division of bird nests was proposed by Nordberg (1936): (1) nests on wet ground and on water, (2) nests on the ground, (3) nests above the ground, and (4) nests in burrows. Later, by subjecting all material to cluster analysis to compare the results with their intuitive ecological classification of the nests, Ambros et al. (1992) extended their division as follows: (1) free nests on the water and in wet habitats, (2) free nests above the ground, either (a) in nature, (b) on buildings (*Delichon*, *Hirundo*), or (c) both (a) + (b) (*Falco*, *Columba*, *Ciconia*), (3) nests on the ground, (4) nest in cavities, such as (a) nest boxes, and (b) nests in burrows (*Merops*, *Riparia*), and (5) nests from miscellaneous sites (*Motacilla*, *Phoenicurus* on buildings and on the ground). Since then, much more data are available, and a preliminary conclusion from cluster analysis is that especially the group of free nests above the ground requires more differentiation.

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Ereynetid mites (Tydeoidea: Ereynetidae) associated with garlic crops in Guanajuato, Mexico

Ignacio Mauro Vázquez-Rojas[1] & Edith G. Estrada-Venegas[2]

[1]Facultad de Ciencias, UNAM, Laboratorio de Acarología, México, DF 04510. E-mail: mauro112003@yahoo.com.mx; and
[2]Colegio de Postgraduados, Texcoco, México

Mexico is one of the 10 more important garlic producing countries in the world. Within Mexico, Guanajuato state is the main garlic producer, with 50-70% of the total garlic export. Recent data show that there are 19 genera and 169 named species in the Ereynetidae, more than half the number of species are in the Speleognathinae, all of them are parasites. In total 51 species of free-living ereynetids belong to the genus *Ereynetes*. Until now *Ereynetes* (*Opsereynetes*) *simplexus*, *E*. (*O*.) *robustus*, *E*. (*Anereynetes*) *tuberculatus*, *E*. (*A*.) *sabinensis*, *E*. (*Ereynetes*) *amplectorus*, *E*. (*E*.) *faini*, and *Riccardoella oudemansi* have been described and/or recorded from Mexico. Seven *Boydaia* spp., two *Neoboydaia* spp., and one *Trispeleognathus* species associated with birds are also cited. In this study we collected soil samples inside garlic crops in several localities of two municipalities in the state of Guanajuato from 2002 to 2005. Two species of Ereynetidae are recorded for the first time for garlic crops, *Ereynetes* (*E*.) *amplectorus* and *E*. (*E*.) *faini* with 179 and 81 specimens respectively; a total of 260 specimens were studied. Data on the presence of females, ovigerous females, males, and juveniles collected in the course of the garlic crop season are included. Females were more numerous than males in both species. The egg structure is described.

Key words: Ereynetidae, garlic, Guanajuato, Mexico

Mexico is one of the 10 more important garlic (*Allium sativum*) producing countries in the world. The cultivated surface is 18,737 acres (ca. 7,600 ha) with an average of more than 56 thousand tons of fresh garlic (2,988 kg/acre). Guanajuato state (more specifically the 'Bajio' area) is the main garlic producer at a national level: with a surface of 6,452 acres and with 18,548 tons, it takes 34% of the cultivated area and yields 33% of the national production. Of all Mexico's garlic export 50-70% comes from Guanajuato (Estrada-Venegas & Equihua-Martínez, 2005).

The family Ereynetidae comprises three subfamilies: Ereynetinae, Lawrencarinae, and Speleognathinae. Recent data show that there are 19 genera and 169 named species in the Ereynetidae, 94 species in the Speleognathinae, 18 in the Lawrencarinae, and 57 in the Ereynetinae. A total of 51 species of free-living ereynetids are in the genus *Ereynetes*. These are predators living on moss, lichens, litter, bat guano, in association with nests of scarabeids, birds and mammals, in decomposing wood, in coleopteran galleries, and under bark (André & Fain, 2000; OConnor & Klimov, 2004).

The following species have been described and recorded from Mexico: *Ereynetes* (*Opsereynetes*) *simplexus* (Baker), *E*. (*O*.) *robustus* (Baker), *E*. (*Anereynetes*) *tuberculatus* (Baker), *E*. (*A*.) *sabinensis* (Baker), *E*. (*Ereynetes*) *amplectorus* (Hunter), *E*. (*E*.) *faini* (Hunter), and *Riccardoella oudemansi* Sig Thor (Baker, 1945; Hunter, 1964). Seven species of *Boydaia* Womersley, two species of *Neoboydaia* Fain, and one species of *Trispeleognathus* Fain are also cited, all associated with birds (Hoffmann & López-Campos, 2000).

The present work is part of a multidisciplinary study on 'Integrated pests and diseases management on garlic crops in Guanajuato State'. To understand the role of the mite species associated with garlic crops and their management, we collected soil samples along the crop system in several localities of two municipalities in Guanajuato from 2002 to 2005.

MATERIAL AND METHODS

Soil samples (0-15 cm deep) were taken monthly from nine cultivated soil lots with garlic between February 2002 and September 2005. The experimental sites are listed in Table 1 – eight are in Salamanca municipality, one (El Zorrillo) is in San Luís de La Paz municipality, all in Guanajuato. Mites were extracted by Berlese funnels. All mites were sorted out but only results of the Ereynetidae are included here.

Specimens were studied under a compound microscope; images were taken with an AxioCam MRc digital camera mounted on a Zeiss Axioskope plus2 microscope using contrast phase and differential interference contrast (DIC). Measures are given in µm. The nomenclature of setae follows Hunter & Cross (1968). Data on garlic plant growth in each locality was recorded monthly for 4 years and used to describe the crop cycle (August to April of the following year). Monthly samples were also taken to establish the mite dynamics in each location. Abbreviations used are: F, female; Fo, ovigerous female; M, male; JV, juveniles; PN, protonymph; DN, deutonymph; TN, tritonymph.

RESULTS

A total of 179 specimens of *E.* (*E.*) *amplectorus* (114 females, 38 ovigerous females, 21 males, six juveniles) and 81 specimens of *E.* (*E.*) *faini* (34 females, 16 ovigerous females, 27 males, four juveniles) were identified (Table 1, Fig. 1). The juveniles were PN, DN, or TN.

Ereynetes (*Ereynetes*) *faini* (Hunter) (Fig. 2A)

Ereynetoides Fain et Nadchatram, 1962.
Ereynetoides faini Hunter, 1964: 188.
Ereynetes (= *Ereynetoides*) Fain, 1964: in Hunter & Cross (1966): 154.
Ereynetes (*Ereynetes*) *faini* (Hunter, 1964): in Hunter & Cross (1966): 155.

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Table 1 Sample locations and numbers collected of *Ereynetes* (*E*.) *amplectorus* and *Ereynetes* (*E*.) *faini*. F, female; Fo, female ovigerous; M, male; JV, juvenile stages.

	E. amplectorus					*E. faini*				
Locations	Total	F	Fo	M	JV	Total	F	Fo	M	JV
La Cuadrilla	60	30	24	6	0	0	0	0	0	0
La Cuchilla	4	0	0	0	4	8	3	1	3	1
El Fuerte	1	1	0	0	0	26	13	3	8	2
Pozo Felix	8	4	2	2	0	34	12	8	14	0
San Isidro	14	7	2	4	1	3	1	1	1	0
El Tajo	25	18	3	4	0	4	4	0	0	0
Las Torres	13	7	4	1	1	3	0	1	1	1
Los Razos	33	33	0	0	0	0	0	0	0	0
El Zorrillo	21	14	3	4	0	3	1	2	0	0

Diagnosis

Diagnosis was based on Hunter (1964). Setae *cc* positioned lightly behind sensory setae, the dorsal setae are thickly barbed compared with other species, and the propodosomal shield margin is indistinct anterior to setae *ca*. The shield pattern is distinct for the species (Fig. 3).

Material examined

All specimens were collected from soil planted with garlic in Mexico by E. Estrada and A. Equihua. Salamanca: San Isidro, 1 female, 1 male, 26-03-03; 1 female, 28-05-03; El Tajo, 2 females, 13-02-02; 1 female, 14-04-02; 1 female, 13-02-02; La Cuchilla, 3 female, 2 males, 13-01-05; 1 male, 1 female, 15-01-05; 1DN, 10-02-05; Las Torres, 1 male, 1 female, 23-04-03; 1 DN, 29-09-02; Pozo Felix, 17 females, 10 males, 15-05-02; 1 female, 15-03-02, 1 male, 15-05-02; 2 females, 14-06-02; 3 males, 19-06-02; El Fuerte, 9 females, 7 males, 2PN, 11-12-03; 2 females 1 male, 18-02-04; 1 female, 23-02-03; 1 female, 24-03-04; 3 females, 15-12-02. San Luis de La Paz: El Zorrillo, 2 females, 29-07-02; 1 female, 22-08-03.

Average size of adult specimens studied was: male, 307.22 µm (n = 25); female, 332.21 µm (n = 46); broken and damaged specimens were excluded.

Egg structure and genital opercula

The structure of the eggs is similar to that illustrated by André et al. (2004) for *Hanriccardoella faini* André, Ducarme & Lebrun. Ornamentation is on one half of the egg along its extension; the other half is rounded and smooth (Fig. 4A). Genital opercula of the female have a sclerotized edge on each flap (Fig. 5A).

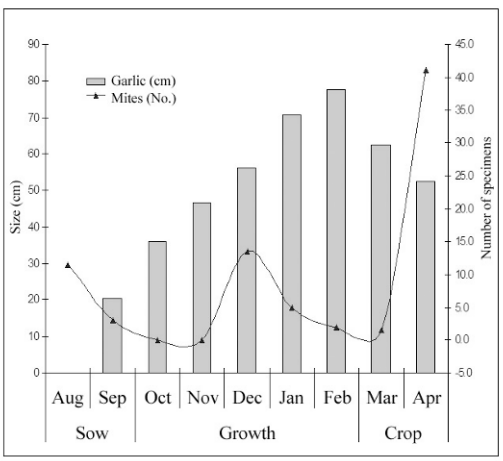

Figure 1 Average garlic plant growth throughout the culture cycle and corresponding ereynetid mite population development.

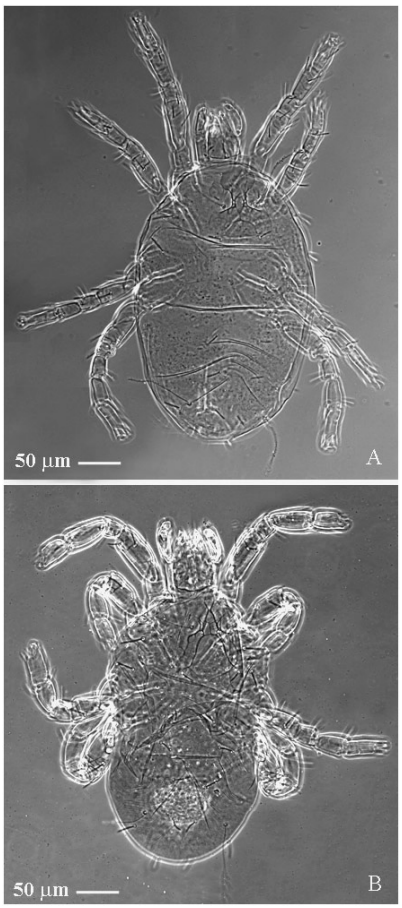

Figure 2 General aspect of female *Ereynetes* (*E*.) *faini* (A) and *Ereynetes* (*E*.) *amplectorus* (B).

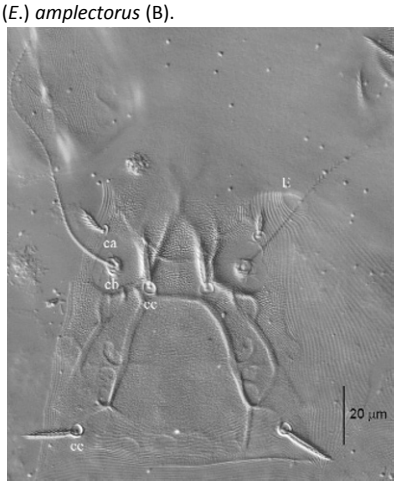

Figure 3 Prodorsal shield of *Ereynetes* (*E*.) *faini* female. Nomenclature of setae as in Fig. 6.

Ereynetes (*Ereynetes*) *amplectorus* (Hunter) (Fig. 2B)

Ereynetoides Fain et Nadchatram, 1962.
Ereynetoides amplectorus Hunter, 1964: 186.
Ereynetes (= *Ereynetoides*) Fain, 1964: in Hunter & Cross (1966): 154.
Ereynetes (*Ereynetes*) *amplectorus* (Hunter, 1964): in Hunter & Cross (1966): 155.

Diagnosis

Diagnosis was based on Hunter (1964). Circular striations around the lens-like eyes, delicate dorsal setae, and setae *cc* slightly in front of the anterior sensory setae. The heavy pattern on the propodosomal shield consists of a curved posterior portion separated from the more elaborated anterior part.

Material examined

Salamanca: San Isidro, 1 male, 28-03-03; 1 female, 28-05-03; 1 female, 29-07-02; 7 females, 3 males, 1DN 22-08-02; La Cuadrilla, 1 male, 26-03-03; 38 females, 23-04-03; 2 females, 23-04-02; 1 female, 23-04-05; 2 females, 01-01-03; 1 female, 10-02-05; 9 females, 5 males, 13-12-02; 1 female, 29-07-02; El Tajo, 1 female, 13-02-02; 3 females, 1 male, 14-06-02; 9 females, 2 males, 10-04-02; 8 females, 15-05-02; 1 male, 17-01-02; Los Razos, 33 females, 26-09-02; Las Torres, 8 females, 1 male, 22-08-02; 2 females, 1TN, 23-04-03; 1 female, 31-10-02; Pozo Felix, 2 females, 2 males, 15-03-02; 2

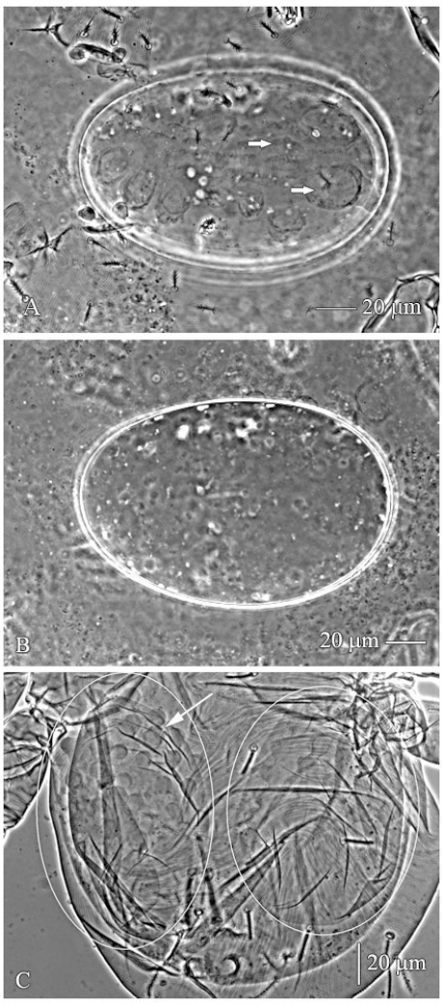

Figure 4 Female of *Ereynetes* (*E.*) *faini* with one egg, (A) ventral view, (B) dorsal view. (C) Female of *Ereynetes* (*E.*) *amplectorus* with two eggs, ventral view. Ellipses show position of eggs; arrows show projections.

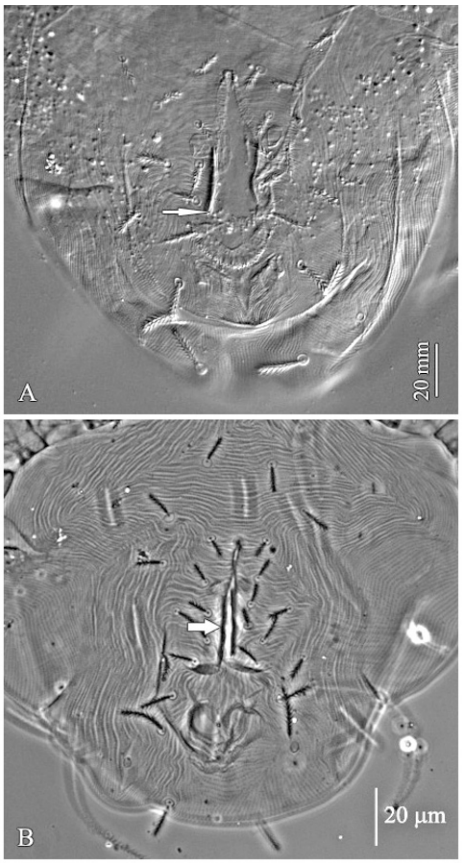

Figure 5 Female genital opercula, (A) *Ereynetes* (*E.*) *faini*, (B) *Ereynetes* (*E.*) *amplectorus*. Arrows show sclerotization (DIC images).

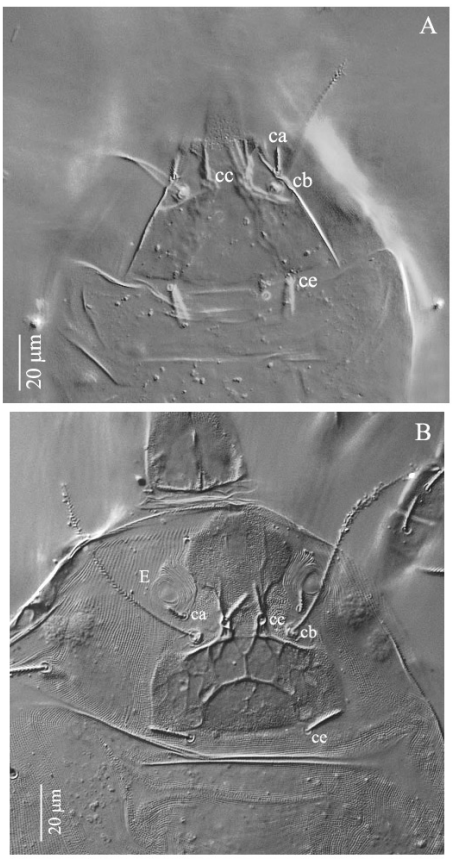

Figure 6 Prodorsal shield of *Ereynetes* (*E.*) *amplectorus*, (A) male, (B) female. Nomenclature of setae on right side of corresponding setae; E, eye (DIC images).

females, 14-03-02; 1 female, 19-06-02; La Cuchilla, 3PN, 1TN, 13-01-05; El Fuerte, 1 female, 15-12-02. San Luis de La Paz: El Zorrillo, 3 females, 26-09-02; 15 females, 3 males, 29-08-02.

Average size of the adult specimens studied is: male, 261.78 µm (n = 13); female, 296.95 µm (n = 133); broken and damaged specimens were excluded.

Egg structure and genital opercula

Egg structure was different from any other described species. Eggs are ovoid and approximately 120 µm long and 80 µm wide, with numerous projections over the entire surface (Fig. 4B). Genital opercula of the female have a sclerotized edge on each flap (Fig. 5B). The pattern of the propodosomal shield is slightly different between males and females; males have a spindle-like anterior end (Fig. 6A), females have a rounded anterior end (Fig. 6B). Those characteristics have not been mentioned in the original description although one allotype male of *amplectorus* is in the type series.

DISCUSSION

The two species were already known for Mexico, but this is the first time they have been found in soil with a garlic crop. They persist throughout the crop cycle. Study of (the biology and ecology of) ereynetid species in Mexico is needed, including the possible redescription of some species (André et al., 2004).

It has been stated that one apomorphic trait in the evolution of the Ereynetidae is egg ornamentation (André et al., 2004). Eggs of the studied specimens are also ornamented: all or part of the egg surface is covered with rounded projections. In *E. amplectorus* it is not clear whether these projections are along the surface or coiled around the surface (Fig. 4C). The egg structure of *E. faini* is slightly different because the rounded projections are located at just one side (Fig. 4A,B). Females of both *E. amplectorus* and *E. faini* carry one egg per gravid female, but in some cases two or three eggs were present (Fig. 4C); no information on this has been published as far as we know.

Mite population size depends on the development of the garlic crop. At the beginning of the garlic cycle (August, September) the mites move deeper in the soil and are less numerous in the samples. Then, as the plant develops the mites increase in numbers until 1 month before maximum plant size is reached, but both plant size and mite abundance are strongly affected by the use of pesticides and fungicides (December, January). At the end of the crop cycle, soil mites increase in numbers (Fig. 1). It appears that mite abundance is closely related to the garlic plant phenology and garlic crop management.

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Physiological Acarology

Nutritional biology of oribatid mites from different microhabitats in the forest

Jaroslav Smrž
Department of Zoology, Charles University, Vinicná 7, Praha 2, CZ-128 44, Czech Republic. E-mail: smrz@cesnet.cz

Two or three microhabitats were defined within each of several habitats within a forest. For example, if 'tree' is a habitat, its microhabitats are the moss cover on the tree, the moss at the foot of the tree in contact with the soil, and the soil around the tree. The microhabitat community structure of oribatid mites was recorded. The nutritional biology, especially the type of food and the digestion pattern, of the more dominant species was monitored. Mites were extracted in Berlese-Tullgren funnels, collected in modified Bouin-Dubosque-Brasil fixation fluid, sectioned in paraplast, and stained with Masson triple. Faecal pellets in the rectum were also stained with acridine orange and observed under fluorescent light. Mite microanatomy was evaluated based on nutritional parameters (food bolus, food type, activity of the walls of the intestine, faecal pellets, bacteria within the body, nutritive deposits, metabolites). Some species consume and digest a specific type of food in all microhabitats of one habitat. But others are able to shift diet according to current food supply in a particular microhabitat. A third type of species is ubiquitous and usually consumes a mixture of unspecified food types in every microhabitat studied. Obligate mycophagous oribatids (e.g., *Damaeus*, *Belba* or *Metabelba*) seem to be highly specialized. These genera inhabit mostly soil or microhabitats in contact with soil (e.g., mosses on soil), and are rarely found in moss cover on trees, away from the soil. Other species inhabiting soil microhabitats as well as moss covers on trees (*Achipteria coleoptrata*, *Hermannia gibba*) can adapt to the nearly pure fungal food in soil microhabitats, although they graze on a different type of food in a microhabitat consisting of moss covering a tree.

Key words: Microhabitats, moss, community structure, microanatomy, nutritional biology, Oribatida

The distribution of mites can be explained largely by the effects of abiotic factors, plant cover, and type of soil (Wallwork, 1970, 1976; Coleman et al., 2004). Hence, one may expect that each habitat harbours a specific type of mite community. The sampling of mites, however, can yield very different results in the same, restricted, and apparently uniform habitat. One partial explanation is that most soil organisms have an aggregated distribution, thereby increasing the chance of some species missing out in the samples (Wallwork, 1970, 1976; Anderson, 1977, 1978a,b; Usher 1982). Another explanation may be that a combination of the microclimatic environments and feeding biology determine the distribution of oribatids (Mitchell, 1978) and that the habitat is divided into microhabitats.

Different communities can be found in a single soil type, with similar soil moisture, but also with similar vegetation or moss cover. Therefore, we should take other factors into account, such as food availability. The role of food availability was discussed by Smrž (1992, 2005a, 2006b). Food preference can affect mite distribution via food searching behaviour (Smrž 2006b), especially for food specialists, such as mycophages and algivores. Food specialization was analysed in several papers from a morphological viewpoint (Schuster, 1956; Pauly, 1956; Kaneko, 1988) and based on enzymological tests (Luxton, 1972; Siepel & de Ruiter-Dijkman, 1993). The application of labelled isotopes of nitrogen is another useful method (Schneider et al., 2004). The combination of histology and micromorphology, microorganism plating including their identification, and enzymological tests was also used to evaluate the palatability of food for mites (Smrž, 1996, 2002; Smrž & Norton, 2004).

This paper aims: (1) to describe oribatid mite community structures in adjacent microhabitats within a single habitat, (2) to assess food selection or preferences of, at least, dominant species in the communities inhabiting the microhabitats under study, (3) to estimate the constancy or flexibility of food specialization or preference in species inhabiting two or more closely adjoining microhabitats simultaneously, (4) to determine the importance of food availability for the mite's ability to invade and settle in microhabitats under study, and (5) to apply feeding guild theory to the mite communities studied.

MATERIALS AND METHODS

The hornbeam tree (*Carpinus betulus*) was studied as an experimental habitat involving three microhabitats: (1) moss cover (*Hypnum cupressiforme*) on the trunk up to 2 m above the foot, (2) cover of the same moss species on the roots of the tree, contacting the soil, and (3) the soil around the roots of the tree. The tree was situated in a Central Bohemian forest near the village of Racice (Rakovník district). Three samples were taken over a period of 3 months (Sept-Nov).

The sampled cores were extracted using Berlese-Tullgren funnels and the mites were collected in modified Bouin-Dubosque-Brasil fluid (Smrž, 1989). The mites were identified to species. The separated mites were embedded in paraplast (Polysciences), sectioned (5 µm thick sections) on a Leica 2155 microtome, stained by Masson's triple, and observed under a light microscope including Nomarski interference contrast application. The mite excrement was smeared, stained with acridine orange, and observed under the fluorescence microscope AX-70 (Olympus) under WU and WB cubes.

The food palatability was estimated using techniques described earlier (Smrž, 1996, 1998, 2002; Smrž & Norton, 2004) and was characterized by: (1) food bolus present in two or all three parts of the alimentary canal (mesenteron, colon, rectum), (2) activity of the walls of the mesenteron and mesenteral caeca expressed by their vacuolisation and

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dark granules of enzymes, (3) haemocytes around the alimentary canal (Smrž, 1995, 2006a), (4) glycogeneous deposits around the alimentary canal (Smrž & Materna, 2000), (5) guanine crystalline deposits in the alimentary canal walls or in the mesenchyma tissue (Smrž, 2002), and (6) mesenchyma bodies containing bacteria, around the alimentary canal (especially in mycophagous mites) (Smrž, 2003).

The feeding preferences are expressed by type of food found in the mesenteron and degree of palatability according to the tests mentioned above:

Type of food. Fungi: high portion of fungal propagula (spores or fragments of mycelium) in the gut; plant litter: fragments of vascular plants in the gut; moss: fragments of moss in the gut; bacteria: clearly bacterial cells (based on staining) in the gut; varia: mixture of food types including the dominant mucous mass mixed with only scattered bacterial cells.

Palatability. +++: highly palatable, i.e., at least two parts of the alimentary canal filled with food bolus, very active mesenteral and caecal walls, glycogeneous deposits and haemocytes, bacterial bodies in the mycophages; ++: palatable, i.e., at least two parts of the alimentary tract filled with food bolus, active caecal walls, with no other palatability parameters; +: weakly palatable, i.e., only mesenteron filled with bolus of the same type in all studied specimens, with no other palatability parameters; h, so-called hermit's food (to indicate that the food is just sufficient for survival), i.e., only one part of the gut with loose food bolus consisting of mucous mass and no other indicators of palatability.

RESULTS AND DISCUSSION

The community structures in the three microhabitats were quite different (Tables 1-3). *Phthiracarus* sp. (unidentified) occurred in all three microhabitats, just as *Achipteria coloeoptrata* and *Tectocepheus velatus*. Their feeding specialization decreased in this order. Plant litter was present as food for *Phthiracarus* sp. in all three microhabitats and the parameters tested indicated palatability in all microhabitats. *Achipteria coleoptrata* shifted its feeding selection from polyphagy (microhabitat 1) to pure mycophagy (microhabitat 3). The food was clearly of hermit's type in microhabitat 1, but very palatable in 3. The same phenomenon was recorded in this species in the steppe biotope (Smrž, 2005b). Most ubiquitous was *T. velatus* (but see Hajmová & Smrž, 2001, for a critical discussion of this term), consuming 'hermit's food' in all three microhabitats. This 'hermit' nature of the food was supported by the absence of juveniles of *T. velatus* in these microhabitats in spite of their dominant occurrence in microhabitat 1 (cf. Hajmová & Smrž, 2001).

The occurrence of *Melanozetes mollicomus* correlated with the presence of a moss cover. Moss litter was palatable in both microhabitats, as supported by the presence of juveniles as well. In addition to eating moss fragments, however, this species also tends toward necrophagy (Behan-Pelletier & Norton, pers. comm.).

The feeding of *Liacarus coracinus* (microhabitat 1) appeared interesting in comparison with other polyphagous oribatids: palatable food was indicated by the mucous mass with conspicuously abundant bacterial cells. This species deposited glycogeneous granules in the mesenchyma tissue. The other polyphagous oribatids consumed this food as well, but it was probably hermit's food to them and their were no indications of palatability of this food.

Food selection was manifested by *Oribatula tibialis*. This species dominates in meadows and agroecosystems (Zilová, 1999). In microhabitat 1, it plays a mycophagous role, but the scattered fungal spores provided indications for weak palatability only. In the moss microhabitat, ca. 50% of the species consumed the food offered as 'hermit's food'.

Hermannia gibba occurred only in microhabitats 2 and 3, both containing soil (cf. Bäumler, 1969). This species is probably mycophagous, with clear indications of food palatability in microhabitat 3 only. Generally, contact with soil (microhabitat 2 compared to 1) resulted in an increase of community diversity in terms of mycophagy (*Damaeus auritus*) and simultaneously in a shift of the feeding by *A. coleoptrata* to mycophagy. Moreover, *Hypochthonius rufulus* – the apparently bacteriophagous mite (Smrž, 1989) – emerged in microhabitat 2 consuming microbial food, based on all measures of palatability. This palatability was confirmed by the presence of juveniles in microhabitat 3.

In the soil (microhabitat 3) the mycophagous part of the community increased up to four species. This was as much due to actual mycophagous species (*Damaeus auritus* and *Belba pseudocorynopus*) (cf. Pauly, 1956) as to the polyphagous species that exhibited a switch to mycophagy (*Achipteria coleoptrata*, *Hermannia gibba*) (cf. Bäumler, 1969; Smrž, 2005b). The increased diversity of microhabitat 3 was probably caused by the wider food offer in comparison with the moss cover. Hence, polyphagous species could utilize a wide variety of food types with high palatability. The occurrence of the desmonomate group (*Platynothrus*, *Nothrus*, *Heminothrus*) confirmed this. *Haffenrefferia gilvipes* showed no sign of acquiring palatable food in microhabitat 2, whereas it did show such signs in microhabitat 3.

The wide variety of palatable food types in microhabitat 3 was confirmed by the number of food specialists and the abundance of juveniles of the dominant species. Moreover, only 21% of the species used the food available as 'hermit's food'.

The feeding guilds, as defined in the literature (Luxton, 1972; Siepel & de Ruiter-Dijkman, 1993), were evident for food specialists, i.e., mycophages (*Damaeus*, *Belba*) and litter feeders (*Melanozetes*, *Phthiracarus*). However, classification was ambiguous for species that switched food preference (*Achipteria*, *Hermannia*). Maybe such species can be categorized only on a per microhabitat basis. Species that consumed only 'hermit's food' were hard to classify into one of the feeding guilds.

In conclusion, the three microhabitats studied exhibited different structures of oribatid communities as well as various feeding specializations of mites. The soil was the most diverse with probably the broadest array of foods and with palatable foods for 50% of the mite species . The highest proportion of palatable food consisted of microorganisms, i.e., fungi and bacteria. Palatability was also indicated by the occurrence of juveniles among the dominant mite species and the high number of feeding specialists, mainly mycophages. Palatability decreased towards the moss cover on the tree trunk, especially with respect to microbial and fungal food. Therefore, the oribatid community of tree trunk moss contained a high proportion of polyphagous mites and a low proportion of specialized, mycophagous mites. The available food played the role of 'hermit's food' (unpalatable) for 50% of the species in the moss on the tree trunk. The moss on the roots contacting the soil had an oribatid community intermediate between the soil and the moss on the tree trunk.

Table 1 The mite community structure and feeding in moss cover on the tree trunk.

Species	Dominance (SD)	Feeding	Palatability
Oribatula tibialis (Nicolet)	32.6 (3.2)	fungi	+
Tectocepheus velatus (Michael)	29.3 (1.8)	varia	h
Melanozetes mollicomus (CL Koch)+J	16.6 (2.2)	moss	++
Chamobates cuspidatus (Michael)	8.4 (1.4)	varia	h
Achipteria coleoptrata (L)	6.2 (1.9)	varia	h
Phthiracarus sp.	4.6 (1.6)	litter	++
Liacarus coracinus (CL Koch)	1.6 (1.1)	varia, bacteria	++
Chamobates subglobulus (Oudemans)	1.3 (1.1)	varia	h

Abbreviations used: J, presence with juveniles; +, level of palatability resulting from the mentioned system of parameters; h, hermit's food, only for survival, not palatable.

Table 2 The structure and feeding in moss cover on the tree roots.

Species	Dominance (SD)	Feeding	Palatability
Melanozetes mollicomus (CL Koch)+J	30.6 (2.3)	moss	+++
Hermanna gibba (CL Koch)+J	26.7 (2.2)	varia	+
Achipteria coleoptrata (L)+J	20.5 (2.9)	fungi	++
Damaeus auritus (CL Koch)	12.0 (2.2)	fungi	+++
Hafenrefferia gilvipes (CL Koch)	3.2 (0.9)	varia	h
Hypochthonius rufulus CL Koch	3.2 (1.0)	varia, bacteria	+++
Phthiracarus sp.	3.2 (1.0)	plant litter	+++
Tectocepheus velatus (Michael)	1.1 (0.2)	varia	h
Hermanniella granulata (Nicolet)	1.0 (0.3)	varia	h

Abbreviations used: J, presence with juveniles; +, level of palatability resulting from the mentioned system of parameters; h, hermit's food, only for survival, not palatable.

Table 3 The mite community structure and feeding in soil around the tree.

Species	Dominance (SD)	Feeding	Palatability
Hypochthonius rufulus CL Koch+J	20.0 (2.9)	varia, bacteria	+++
Platynothrus peltifer (CL Koch)+J	19.7 (2.0)	varia	+++
Phthiracarus sp.	15.4 (3.1)	plant liter	+++
Hermannia gibba (CL Koch)+J	10.6 (1.3)	fungi	+++
Achipteria coleoptrata (L)+J	9.3 (1.2)	fungi	+++
Tectocepheus velatus (Michael)	9.2 (1.3)	varia	h
Galumna lanceta Oudemans	4.5 (0.7)	varia	h
Oribatella quadricornuta (Michael)	4.3 (0.9)	varia	h
Nothrus palustris CL Koch	1.5 (0.4)	varia	++
Heminothrus targionii (Berlese)	1.5 (0.4)	varia	++
Haferefferria gilvipes (CL Koch)	1.5 (0.4)	varia	++
Hermanniella granulata (Nicolet)	1.5 (0.4)	varia	+
Damaeus auritus (CL Koch)	1.5 (0.2)	fungi	+++
Belba pseudocorynopus Märkel et Mayer	1.5 (0.2)	fungi	+++

Abbreviations used: J, presence with juveniles; +, level of palatability resulting from the mentioned system of parameters; h, hermit's food, only for survival, not palatable.

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Enzyme activities and internal bacteria of saprophagous soil mites (Acari: Oribatida, Acaridida)

Jaroslav Smrž
Department of Zoology, Charles University, Vinicná 7, Praha 2, CZ-128 44 Czech Republic. E-mail: smrz@cesnet.cz

Several saprophagous mites (Acari: Oribatida, Acaridida) were tested for chitinolytic activity of their enzymes. These mites were sampled in the field (*Damaeus*, *Belba*, *Metabelba*) or obtained from cultures (*Archegozetes*, *Scheloribates*, *Tyrophagus*). Mites were tested on several fungi from a fungal collection (*Alternaria*, *Fusarium*), as well as fungi isolated from mite-rearing boxes (*Penicillium*, *Mucor*). These tests involved (1) light and fluorescence microscopy of adequately stained mites to detect internal, but extra-intestinal, bacteria, (2) staining of mite homogenates to detect chitinase activity, and (3) plating of mite homogenates and purification to obtain pure cultures of the internal bacteria for identification. Mycophagy of the mites was classified into three types: (1) grazing and digesting fragments of fungi, including their chitinous cell wall, as proven by chitinase activity, (2) cutting and ingesting hyphae, but digesting only the cell content (no chitinase activity), and (3) piercing mycelium and sucking its content (chitinolytic bacteria were plated from the homogenate of those mites, but no chitinase activity).

Key words: Mycophagy, microanatomy, chitinolytic activity, chitinolytic bacteria, Oribatida, Acaridida

Consumption of fungi, i.e., mycophagy, seems to occur widespread in the animal kingdom. Yet, the evidence needed to infer mycophagy sensu stricto is often incomplete. For example, some oribatids or acaridids have been considered to be mycophages, only based on patterns of the mites' affinities to fungi. More frequently, mycophagy is inferred from recording fungal elements in the gut contents of mites cleared with lactic acid, or from laboratory feeding tests (one-way or cafetaria tests) in which mite contact with fungus, mite residence on fungus, and production of excrements was recorded (e.g., Czajkowska, 1970). A more useful method is testing for enzyme activity, and this has resulted in the classification of mites into feeding groups or guilds (Luxton, 1972; Dinsdale, 1974; Haq, 1981; Siepel & de Ruiter-Dijkman, 1993). Also, the application of labelled isotopes seems to be a very useful method in this respect (Schneider et al., 2004).

However, a narrower definition of mycophagy is needed. The consumption of cell contents rich in trehalose is not specific for a mycophagous organism. Trehalose can occur in many organisms, not only in fungi (Wigglesworth, 1974). The mobilisation of nitrogen from chitin is the most critical feature of mycophagy sensu stricto. Therefore, chitinolytic activity seems to be necessary for the digestion of the cell walls of fungi. However, this digestive activity is rare among animals under normal circumstances. It occurs only during some pathological accidents (invasion of nematodes into their host or during some metabolic disorder). The chitinolytic enzymes originate mainly from internal bacteria (Aktuganov et al., 2003).

This study aims to (1) provide a more strict definition of mycophagy, (2) search for the source of chitinolytic enzymes (bacteria, host), and (3) identify the internal bacteria involved.

MATERIALS AND METHODS

Several mites were selected for the analyses. Four mite species, *Damaeus onustus* CL Koch, *D. auritus* CL Koch, *Belba pseudocorynopus* Märkel et Mayer, and *Metabelba pulverosa* Strenzke, were sampled in forests of Central Bohemia. These are considered to be actual mycophages according to the literature (Luxton, 1972) and according to own studies (Smrž & Trelová, 1995). *Tyrophagus putrescentiae* Schrank, an acaridid mite, was collected from a mass rearing (220 cm^3 glass boxes with a plaster of Paris/charcoal bottom), that was started with mites from an alfalfa field near the city of Prague and showed fungal food in the gut (Smrž & Jungová, 1989). *Archegozetes longisetosus* Aoki and *Scheloribates laevigatus* (CL Koch) were also collected from a laboratory culture, where they were reared on algae (*Protococcus* sp.). After consumption of these algae, however, a mixture of fungi invaded our rearing boxes and these may also serve as food. Both these species are considered to be omnivorous (Hubert et al., 1999; Smrž & Norton, 2004).

The mites were analysed by histology: fixed in modified Bouin-Dubosque-Brasil fluid (Smrž, 1989), embedded in paraplast (Polysciences), sectioned on a Leica 2155 microtome (5 µm thick sections), stained by Masson's triple stain, and observed under a light microscope with a Nomarski interference contrast application. The mite excrements were smeared, stained by acridine orange, and observed under the fluorescence microscope AX-70 (Olympus). Some details were observed under a transmission electron microscope (TEM). For TEM, the mites were fixed in cacodylate-buffered glutaraldehyde (4%), postfixed in 1% osmium tetroxide, embedded in Spurr medium, and sectioned using an Ultracut ultramicrotome (Reichert). Sections were stained in lead citrate and uranyl acetate and observed under a Philips TEM 300.

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The live mites were rinsed in several steps: twice in 96% ethanol, followed by 10% Pur detergent to remove microorganisms from the body surface, washed twice in distilled water, and subsequently homogenized in Ringer's solution. The internal bacteria from the homogenate (per 1 larger *Damaeus* mite, 5 smaller *Archegozetes* mites, or 10 smaller individuals of other species) were plated on MPA (pH 7) (Smrž et al., 1991) and identified in the internationally certified microbiological institute (CCM Brno, Czech Republic). Only the presence of bacterial species was assessed, no quantitative relations. With regard to the plating of bacteria, species dominance could be assessed from the number of colony forming units (cfu) per dish: dominant bacterial species usually formed about 8 cfu per dish.

Chitinase activity of the same homogenate of mites, as well as the bacterial suspension, was tested on a carboxymethylchitin thin layer on a microslide after staining by basic fuchsine (Smrž, 2000).

The bacterial suspension was applied on the fungal colonies from a reference collection (*Alternaria alternata*) or plated from rearing boxes (*Penicillium* sp., *Mucor* sp.). All observations were done in triplicate and performed only to assess presence/absence of bacteria and chitinolytic activity. The results of histology, plating, and testing of homogenates and bacterial suspension were uniform; therefore, no statistical analysis was necessary.

RESULTS

Damaeus, *Belba*, *Metabelba*: mites from the forest, considered to be mycophages

These species had their alimentary tract (mesenteron, colon, rectum) full of fungal propagules (spores or fragments of mycelium). The cell walls of these propagules became thinner progressively from the anterior to the posterior parts of the gut. Markedly, no fungal fragments were visible in the rectum in spite of their presence in the mesenteron or colon. In excrements, only small dead, digested parts of fungi (red colour under the fluorescence microscope) could be recorded.

Free cells (hemocytes) occurred abundantly around the alimentary tract, especially around the mesenteral caeca and anterior part of the rectum. Groups of bacterial cells adjoined to the mesenteral caeca.

The homogenate of *Damaeus onustus*, as well as the bacterial suspension from this homogenate, exhibited chitinolytic activity. The dominant bacteria were identified as *Pseudomonas fluorescens*. The colony of *Penicillium* sp. was destroyed by the suspension of these bacteria.

Tyrophagus putrescentiae: the actual mycophagous mite reared in the laboratory

Similar phenomena were recorded as above. One group of these mites was reared on *A. alternate*, and the bacterium *Serratia marcescens* was plated and identified from them. This bacterium was lytic for *A. alternata*. Another group of mites was reared on *Fusarium oxysporum*, and *Alcaligenes faecalis* was plated and identified.

Archegozetes longisetosus and *Scheloribates laevigatus* reared in the laboratory

The consumption of green algae (*Protococcus* sp.) was confirmed by the presence of algal cells within the gut. They had no bacterial group adjoining the gut. Two weeks after complete consumption of the algae, some fungi infested the rearing box. Both mite species grazed progressively on these fungi and several weeks later, an increasing number of fungal particles was observed in the gut, with hemocytes around it. In this phase, there were also bacterial groups adjoining to the alimentary tract. For both mite species, the bacteria were plated and identified as *S. marcescens*. These bacteria were found to be capable of destroying the fungi *A. alternata*, *Penicillium* sp., and *Mucor* sp.

DISCUSSION

Under the strict definition of mycophagy, it may be possible to assess its 'functional apomorphy'. Thus, contact of mites with fungi is insufficient evidence for mycophagy sensu stricto. The same applies to piercing and sucking of the fungal cell content. So-called 'fungal sugar' is nothing else than trehalose and, indeed, this can be found in many organisms, not only in fungi (Wigglesworth, 1974). Moreover, chewing and gulping of fungi is not sufficient evidence to infer fungi as palatable food. Cell walls may not have been digested, they may have passed on to the mesenteron up to the rectum as intact, undigested particles. I propose that mycophagy sensu stricto involves the digestion of the chitinous fungal cell walls, thus chitinolytic activity is required. Chitinolytic activity was demonstrated in several oribatids by Luxton (1972) and Siepel & de Ruiter-Dijkman (1993).

Because chitinolysis is rarely found to be a capacity of animals themselves (Aktuganov et al., 2003; Roberts & Selitrennikoff, 1988), it seems more likely that chitinolytic activity is due to bacteria. Lysis by bacteria commonly acts against fungi in the soil (Aktuganov et al., 2003; Singh et al., 1999). For mites, such bacterial activity has been reported by Smrž & Trelová (1995) and Smrž et al. (1991). In the experiments reported here, bacteria were observed in mites as bodies or groups adjoining to the alimentary tract. Homogenates of mites yielded several bacteria that are considered to be producers of chitinolytic enzymes: *P. fluorescens*, *S. marcescens* (Downing & Thompson, 2000); *Alcaligenes* sp. (Vaidya et al., 2003). The suspension of these bacteria exhibited chitinolytic activities and they were observed to destroy the plated fungi offered in our experiments. Based on our experimental tests we conclude that chitinolytic activity in the actual mycophagous oribatid and acaridid mites resulted from the association of mites with bacteria adjoining the alimentary tract. This effect, of course, depends on mite species and available fungal food.

In addition to actual mycophages (*Damaeus*, *Belba*, *Metabelba*, *Tyrophagus*), there are omnivorous mites that are able to physiologically adapt to fungal food after consuming it for some time. This phenomenon involves first acceptance of the fungal food, followed by an increasing amount of fungal propagules in the gut, and finally continued feeding on fungi as if it is palatable food (Siepel & de Ruiter-Dijkman, 1993). However, the palatability has to be confirmed by assessing several characteristics: prevailing fungal propagules in the mesenteron and colon, high intensity of digestion as gut wall cells activity, hemocyte presence around the gut, deposition of nutrients (glycogeneous granules) in the body, and formation of bacterial groups around the gut. High digestive activity of substances that are especially rich in nitrogen, such as chitin, can be assessed by the deposition of crystalline excretions in the form of guanine, a feature of all arachnids (Vitzhum, 1940-43). Finally, the bac-

terial species adjoining the alimentary tracts of these omnivorous mites need to be tested for their specificity in chitinolysis of particular fungi.

In conclusion, the consumption and digestion of the chitinous cell walls of fungi is proposed as a defining characteristic of mycophagy sensu stricto. This was inferred from crowding of the gut by fungal propagules, gradual thinning (digestion) of their cell walls caudad in the gut, highly accelerated activity of the cells in the alimentary tract, hemocyte concentration around the gut, and bacterial groups or bodies around some parts of the gut. The palatability of fungi as food was inferred from the deposition of nutrients, glycogeneous granules, and crystalline excretions called guanine. The chitinolytic bacteria (*P. fluorescens*, *S. marcescens*, *A. faecalis*) were isolated from the mites. Mite homogenates and a suspension of these bacteria exhibited chitinolytic activity. A suspension of these bacteria was shown to destroy the plated fungi. If this ability to digest chitinous fungal cell walls holds for all genuinely mycophagous mites, one may ask how feeding on fungi can arise in omnivorous mites. These mites can physiologically adapt to this food after some time on a diet of fungi.

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Analysis of tissues for EcR and RXR nuclear receptor gene expression during vitellogenesis in the soft tick *Ornithodoros moubata*

Mari Horigane & DeMar Taylor
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan.
E-mail: taylor@sakura.cc.tsukuba.ac.jp

Vitellogenin (Vg) synthesis and oocyte development appear to be induced by ecdysteroids in the soft tick *Ornithodoros moubata*. Vg transcription in insects has been shown to be mediated by two nuclear receptors, an ecdysteroid receptor (EcR) and a retinoid X receptor (RXR). EcR and RXR expression in female ticks increase after engorgement. However, there is little information about EcR and RXR expression in tissues as related to reproduction. Therefore, the sites of EcR and RXR expression during vitellogenesis were determined. Expression levels of EcR and RXR were determined for salivary glands, midgut, ovary, anterior reproductive tract, fat body, muscle, and cuticle of mated females by reverse transcriptase RT-PCR and real-time PCR. Ovaries showed constant expression of EcR and RXR throughout the adult female stage. The fat body showed higher expression of EcR and RXR on one day after engorgement. The fat body of female ticks has been reported to be the organ of Vg synthesis. Therefore, increases in EcR and RXR of the fat body support the hypotheses that the site of Vg synthesis is the fat body and ecdysteroids regulate this synthesis in *O. moubata*. EcR and RXR in the ovary and anterior reproductive tract may also play roles in the regulation of oocyte development.

Key words: *Ornithodoros moubata*, soft tick, ecdysteroid, ecdysteroid receptor (EcR), retinoid X receptor (RXR), vitellogenesis

Most ticks require a blood meal in order to produce egg proteins that are incorporated into oocytes for the production of offspring. Vitellogenin (Vg), a precursor of the yolk protein vitellin is produced in large amounts and appears to be regulated by endocrinological processes (reviewed by Chinzei & Taylor, 1994; Taylor & Chinzei, 2002; Rees, 2004). A model for Vg synthesis in *Ornithodoros moubata* Murray by Chinzei & Taylor (1990) proposes that two factors are necessary for regulation of vitellogenesis in ticks. The synganglion secretes vitellogenin-inducing factor (VIF) (Chinzei et al. 1992), thought to be a neuropeptide, that stimulates an organ in the posterior half of the tick to secrete a fat body stimulating factor (FSF). FSF stimulates the fat body to synthesize Vg that is released into the hemolymph and incorporated into the oocytes. Injection of ecdysteroids into unfed females has been shown to induce Vg synthesis (Taylor et al., 1997). Moreover, Vg up-regulation and oocyte development occurs in only mated females in the presence of high titers of ecdysteroids in the hemolymph (Ogihara et al., 2007) indicating FSF is an ecdysteroid.

Other blood-sucking arthropods such as the mosquito *Aedes aegypti* show similar regulation of Vg synthesis (Hagedorn, 1985, 1989). After blood feeding, ecdysteroids secreted from ovaries of *A. aegypti* stimulate the fat body and Vg is synthesized in the fat body. Moreover, regulatory mechanisms of mosquito vitellogenesis are well understood at the molecular level. Two nuclear receptors appear in the fat body (Miura et al., 1999; Wang et al., 2000). One receptor is the ecdysteroid receptor (EcR) and the other is ultraspiracle (USP), a homologue of vertebrate retinoid X receptor (RXR) (Oro et al., 1990). EcR and USP are members of the nuclear receptor family and the molecular structure reveals specific domains (Henrich & Brown, 1995; Mangelsdorf & Evans, 1995) that include a DNA-binding domain to bind with the regulatory regions of target genes and a ligand-binding domain for ligand reception. The N-terminus domain of most nuclear receptors is a region that varies between isoforms by alternative splicing and determines the transactivation function of the receptor. EcR and USP have several isoforms and each isoform shows stage- and tissue-specific expression patterns indicating the isoforms have different functions (Henrich & Brown, 1995; Wang et al., 2002). EcR and USP form a heterodimer that can bind ecdysteroids (Yao et al., 1992, 1993; Thomas et al., 1993; Hall & Thummel, 1998). The ecdysteroid-EcR/USP complex binds to the ecdysteroid response element (EcRE) in the regulatory region of genes transactivated by ecdysteroids (Yao et al., 1992; Kapitskaya et al., 1996; Swever et al., 1996). The Vg gene of *A. aegypti* contains EcREs in the 5' upstream regions and is directly regulated by the ecdysteroid-EcR/USP complex (Kokoza et al., 2001; Martin et al., 2001).

The hard tick *Amblyomma americanum* L. has been shown to have three EcR (AamEcR) and two RXR (AamRXR) isoforms (Guo et al., 1997, 1998). Both receptors were shown to have the conserved structure of the nuclear receptor superfamily. AamEcR and AamRXR are expressed in the presence of high titers of ecdysteroids and the heterodimers of AamEcR/AamRXR can bind to *Drosophila* hsp27 EcRE (Guo et al., 1998; Palmer et al., 2002; Rees, 2004). From the soft tick *O. moubata*, one complete EcR sequence and one complete RXR sequence have been identified and these sequences have high homology with the hard tick EcRs and RXRs (Horigane et al., 2007a, 2008). EcR and RXR expression in *O. moubata* increases just after engorgement and decreases gradually during vitellogenesis (Horigane et al., 2007a, 2008). These results indicate that ecdysteroids regulate gene transcription in ticks similar to mosquitoes. However, the expression of EcR and RXR during vitellogenesis has not been clarified. Therefore, the objective of this study was to determine the sites of both EcR and RXR

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expression. In order to accomplish this, various tissues were separately collected by dissection and the expression of EcR and RXR analyzed by reverse transcription polymerase chain reaction (RT-PCR) and real-time quantitative PCR.

MATERIALS AND METHODS

Ticks

Ticks (*O. moubata*) were from a laboratory colony obtained from Professor Yasuo Chinzei, (Medical School, Mie University, Tsu, Japan) in 1994 and maintained in incubators at 30 ± 1 °C, 70 ± 10% relative humidity and total darkness. Ticks were fed on rabbits (*Oryctolagus cunniculus*) as described by Chinzei et al. (1983). The Ethical Committee for Animal Studies at the University of Tsukuba approved the experimental procedures and animal care.

RNA extraction for mRNA quantification

For analysis of EcR and RXR gene expression in *O. moubata*, fed and unfed mated females were dissected and seven tissues (salivary gland, midgut, ovary, anterior reproductive tract, fat body, muscle, and cuticle) collected. The dorsal cuticle was used as the tissue for the cuticle. The anterior reproductive tract included the vestibular vagina, lobular and tubular accessory glands, receptaculum seminis, uterus and oviducts. Fat body was collected with trachea because most of the fat body tissue of ticks is very faint and associated with the trachea. Total RNA was isolated with TRIzol reagent (Invitrogen) as described by the manufacturer. Total RNA (2 µg) was treated with DNase I Amp grade (Invitrogen) following cDNA synthesis with the SuperScript III First Strand Synthesis System (Invitrogen). The reverse transcription reaction was performed with Oligo $(dT)_{20}$ primers according to the manufacturer and used as a template for mRNA quantification.

Analysis of the sites of EcR and RXR expression by RT-PCR

RT-PCR was performed with Platinum *Taq* polymerase (Invitrogen) to determine expression patterns of EcR and RXR. Actin gene (accession no. AB208021) was used as an internal control (Horigane et al., 2007b). The primers for EcR and RXR were designed from sequences of the DNA binding domain to detect total EcR and RXR expression (Table 1). The PCR conditions for EcR and RXR were 94 °C for 2 min, followed by 37 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed at 72 °C for 7 min. The PCR conditions for actin were 94 °C for 2 min, followed by 24 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by elongation at 72 °C for 7 min.

Expression of EcR and RXR was confirmed by sequencing the RT-PCR products. PCR products were subcloned into a pGEM T-easy vector (Promega) and the vector containing the fragment was transformed into High Competent Cells, JM 109 (TOYOBO). The plasmids were purified with a Concert Rapid Plasmid Purification System (Invitrogen) as described by the manufacturer. The sequencing reaction was performed with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with 5'-TAATACGACTCACTATAGGG-3' (T7 primer) and 5'-TATTTAGGTGACACTATAG-3' (SP6 primer) primers. The sequences were confirmed with an ABI Prism® 310 Genetic Analyzer (Applied Biosystems).

Table 1 Primers used in this study.

Description	Sequences
OmEcR forward	5'-GCACTGACCTGTGAAGGGTGT-3'
OmEcR reverse	5'-GTACGGTCGATGTCGCAGTTATTGCCGTAC-3'
OmRXR forward	5'-GGCTGCAAAGGCTTCTTCAA-3'
OmRXR reverse	5'-GACAAGGATTGCGCTGCCTCTTGTC-3'
OmActin forward	5'-GGGAATGGAATCCTGCGGTA-3'
OmActin reverse	5'-GAGCTGGGACAGTGTTGGCGTACAGGTC-3'
OmEcR labeled reverse	5'-gtacggTCGATGTCGCAGTTATTGCCG5AC-3'
OmRXR labeled reverse	5'-gacaagGATTGCGCTGCCTCTTG5C-3'

Analysis of the sites of EcR and RXR expression by real-time PCR

Real-time PCR was performed to determine the expression of EcR and RXR in various tissues with 50 ng of cDNA. All primers for real-time PCR were LUX fluorogenic primers (Invitrogen) designed with the D-LUX™ program (Invitrogen). The primers for EcR and RXR were the same sequences for primers used in RT-PCR. Only the reverse primers for EcR and RXR were labeled with FAM (Table 1). OmEcR forward and OmEcR labeled reverse primers were used to determine EcR expression, and OmRXR forward and OmRXR labeled reverse primers for RXR expression. Real-time PCR reaction was performed with Platinum qPCR SuperMix-UDG (Invitrogen) as described by the manufacturer. Final concentrations of forward and reverse primers were 0.25 µM/sample for EcR and RXR. The program used for amplifying the reactions was as follows 2 min at 50 °C, 2 min at 95 °C and 45 cycles at 95 °C for 15 s and 60 °C for 30 s. Samples for EcR and RXR were prepared separately and run in 384-well PCR plates (Applied Biosystems) on an ABI Prism 7900HT (Applied Biosystems). Standard curves for EcR and RXR were determined with 10-fold dilutions of cDNA at concentrations from 0 to 100 ng and all slope values were 3.2 to 3.3. Expression of each sample was determined from two to four replications. Data analysis was performed by relative standard curve methods with SDS 2.0 (Applied Biosystems) and Excel (Microsoft). Quantities from unfed tissues were adjusted to 1 unit and the relative values of EcR and RXR are shown as compared to the value for each respective unfed tissue.

RESULTS

To determine the sites of EcR and RXR expression in *O. moubata*, RT-PCR was performed with cDNA of seven tissues (salivary gland, midgut, ovary, anterior reproductive tract, fat body, muscle, and cuticle) from mated females (Fig. 1). The PCR products were sequenced and confirmed to be EcR and RXR (data not shown). EcR and RXR expressions were lower than actin so PCR was performed at 37 cycles for these receptors and 24 cycles for actin. EcR was expressed in almost all tissues of females unfed and l, 5, and 10 days after engorgement, whereas RXR expression was limited to several tissues (Fig. 1). In unfed females, all seven tissues showed EcR expression, but RXR was weakly expressed in the ovary, midgut, and anterior reproductive tract. All tissues of 'day 5' females also showed EcR expression, but RXR was expressed only in the salivary glands, midgut, ovary, anterior reproductive tract, and fat body. The salivary gland showed strong RXR expression and EcR expression on day 1. The ovary showed expression of both EcR and RXR at all times assayed.

Anterior reproductive tract showed EcR expression with weak expression of RXR on days 5 and 10 after engorgement. Fat body showed EcR expression with weak RXR expression on day 1 and day 5. These results indicate both EcR and RXR have tissue- and stage-specific expression patterns.

However, changes in EcR and RXR expression levels remained unclear as seen by RT-PCR. Real-time PCR is a more suitable method to detect small changes in gene expression. Therefore, the expression levels of EcR and RXR receptors in the midgut, ovary, anterior reproductive tract, and fat body were investigated by real-time PCR. The values from each tissue are presented as relative expression levels of RNA standardized with unfed mated females as 1 unit (Fig. 2). The fat body showed the highest expression of both EcR and RXR on days 1 and 5 after engorgement. The midgut, ovary and anterior reproductive tract did not show increases in EcR and RXR expression except for a slight increase in RXR on day 1. Although EcR and RXR expression were observed in seven dissected tissues by RT-PCR, real-time PCR showed changes in EcR expression are highest in the fat body and indicates the fat body can be stimulated by a blood meal to upregulate Vg synthesis.

DISCUSSION

The vitellogenin gene of *A. aegypti* has been shown to have ecdysone response elements (EcREs) in the 5' upstream regulatory region that are a direct target for binding of the ecdysteroid-EcR/USP complex (Raikhel et al., 2002). Several studies provide evidence that the tick EcR/RXR (USP homologue) heterodimer also has transcriptional activity with EcRE (Guo et al., 1997, 1998; Mao & Kaufman, 1998, 1999; Sauer et al., 2000; Palmer et al., 2002). To understand the functions of EcR and RXR in ticks, determination of EcR and RXR expression sites were performed by RT-PCR and real-time PCR in *O. moubata* mated females. This study showed that EcR and RXR expression in *O. moubata* increased in the fat body on days 1 and 5.

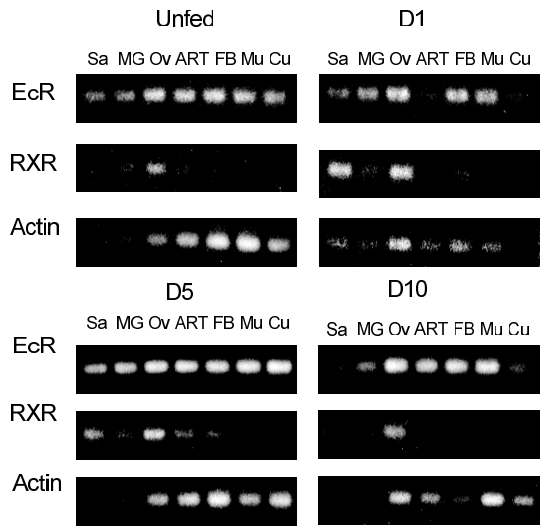

Figure 1 Expression patterns of EcR and RXR in seven tissues of mated females analyzed by RT-PCR. RT-PCR was performed with 37 cycles for total EcR and RXR and 24 cycles for OmActin. D1: 1 day after engorgement; D5: 5 days after engorgement; D10: 10 days after engorgement. Sa: salivary gland, MG: midgut, Ov: ovary, ART: anterior reproductive tract, FB: fat body, Mu: muscle, Cu: cuticle.

The fat body has been shown to be the synthetic organ of Vg in *O. moubata* and *O. parkeri* (Chinzei & Yano, 1985; Chinzei, 1986; Taylor et al., 1991) and high expression of EcR and RXR in the fat body coincides with high titers of ecdysteroids in the whole bodies and hemolymph of mated females just before Vg synthesis (Ogihara et al., 2007). James et al (1997) also showed a peak of ecdysteroids appears before the peak production of Vg and 20-hydroxyecdysone (20E) stimulates the fat body to produce Vg in *Ixodes scapularis* Say. In addition, Sankhon et al. (1999) showed that 20E stimulates Vg production in cultured fat bodies of *D. variabilis* and Taylor et al. (1997) showed that injection of high concentrations of ecdysteroids stimulate vitellogenesis in unfed *O. moubata* females. Moreover, Friesen & Kaufman (2002) showed that injection of 20E into non-vitellogenic *A. hebraeum* females induced accelerated Vg synthesis. Recently, Thompson et al. (2005) reported the injection of ecdysteroids into partially fed *D. variables* females initiated expression of the Vg gene, release of Vg into the hemolymph and Vg uptake into developing oocytes. Females of *O. moubata* also showed increases in EcR and RXR expression immediately after engorgement in whole body extracts (Horigane et al., 2007a, 2008) that coincide with increases in ecdysteroid titers in the hemolymph (Ogihara et al., 2007). All of these studies indicate that ecdysteroids are essential in the regulation of Vg synthesis by the fat body. The results of this study show that the receptors are also present in the fat body at the time of Vg synthesis to mediate regulation by ecdysteroids. In *A. aegypti*, the fat body has been demonstrated as the synthetic site of Vg and ecdysteroid titers increase after a blood meal (Wang et al., 2000). EcR expression has also been observed in the fat body of unfed mosquito stages and this expression increases soon after engorgement, whereas USP shows constant expression before and

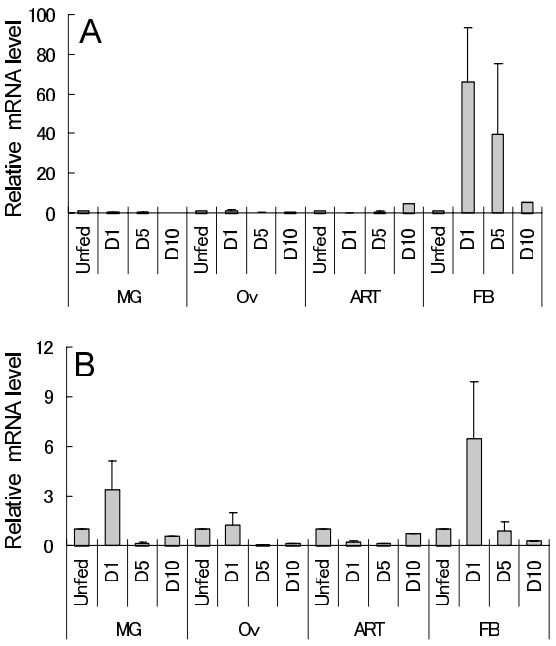

Figure 2 Analysis of gene expression of EcR (A) and RXR (B) in four tissues of mated females analyzed by real-time PCR. Expression levels in the unfed samples of each tissue were standardized as one unit and all other samples shown as the relative quantity of mRNA compared to the unfed standard. D1: 1 day after engorgement; D5: 5 days after engorgement; D10: 10 days after engorgement. MG: midgut, Ov: ovary, ART: anterior reproductive tract, FB: fat body.

during vitellogenesis. Similarities between the receptor expression patterns of *O. moubata* and *A. aegypti* indicate EcR and RXR can also provide transcriptional activation of the Vg gene in *O. moubata*.

In this paper, the reproductive tract was separated into two parts, the ovary and anterior reproductive tract (containing the vestibular vagina, lobular and tubular accessory glands, receptaculum seminis, uterus, and oviducts). Ovary and anterior reproductive tract showed different patterns of EcR and RXR expression. The ovary showed similar levels of both EcR and RXR expression, whereas the anterior reproductive tract showed EcR expression on only day 5 with a slight increase in RXR. Eggs mature in the ovaries of insects with the support of nurse cells, follicle cells, and other functional cells. *Bombyx mori* showed EcR and BmCF1 (USP of *B. mori*) expression in vitellogenic follicle cells (Swerver et al., 1995) and *D. melanogaster* also showed EcR and USP expression in germ line cells and somatic cells including the follicle cells of ovaries during all stages of oogenesis (Christianson et al., 1992; Buszczak et al., 1999). Ovaries of *A. aegypti* also show increased expression of EcR and USP after a blood meal (Wang et al., 2000). The expression of EcR and USP in insect ovaries and supporting cells appears to be closely related with oocyte development (McCall, 2004). However, the ovaries of ticks are tubular panoistic organs lacking nurse cells and follicle cells (Sonenshine, 1994). Very little is known about the endocrine regulation of oocyte development and the functions of the ovary and anterior reproductive tract in ticks but the expression of EcR and RXR in both tissues of *O. moubata* indicates similar functions for these tissues in oocyte development. Further research, such as in situ hybridization, is needed to clarify the roles of EcR and RXR in the ovary and anterior reproductive tract.

In addition, all seven tissues showed EcR expression in females unfed and after engorgement indicating each tissue may respond to ecdysteroid regulation. In the hard tick *A. americanum*, salivary gland degeneration after engorgement is regulated by ecdysteroids (Mao & Kaufman, 1999; Sauer et al., 2000). A high titer of ecdysteroids appears during engorgement through day 1 with EcR and RXR expression in the salivary glands, indicating the EcR/RXR heterodimer mediates ecdysteroid regulation of salivary gland degeneration. *Ornithodoros moubata* females also showed EcR expression on day 1 in the salivary glands indicating similar regulation of the salivary gland. As well as the salivary glands, ecdysteroids may regulate various phenomena in other tissues. However, there is little information about regulation by ecdysteroids in other tissues. Future research is needed to resolve these questions. In addition, expression patterns have only been analyzed for mated females in this study so the comparison of EcR and RXR expression between mated and virgin females may help elucidate the receptor functions.

Acknowledgements

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A cysteine protease inhibitor (cystatin) from the tick *Haemaphysalis longicornis* is involved in tick innate immunity

Jinlin Zhou[1,2] & Kozo Fujisaki[1]

[1]National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan. E-mail: tick@ms.kagoshima-u.ac.jp; and [2]Shanghai Institute of Animal Parasitology, Chinese Academy of Agricultural Sciences, 3 Lane 345, Shilong Road, Shanghai 200232, China

Proteins capable of selective and specific inhibition of cysteine protease have been identified as cystatins and are isolated from a variety of microbes and tissues of animals and plants. The physiological function of these proteins have been proposed in regulation of protein turnover and defending against pathogens, as well as in modulating the host's immune response. Genes encoding cystatins have been found in several kinds of ticks, but the function of cystatin in ticks is not understood. We cloned a gene encoding cystatin from the hard tick *Haemaphysalis longicornis*, and designated it Hlcyst-2 (*H. longicornis* cystatin-2). The full-length cDNA is 569 bp, encoding a putative 133 amino acids protein with an obvious signal peptide. The cystatin was expressed most in tick midgut and hemocyte. Blood feeding induced a significantly increasing expression in midgut. Real-time PCR confirmed that adult ticks injected with the immuno stimulant lipopolysaccharide (LPS), expressed Hlcyst-2 1.6× more than control ticks injected with phosphate-buffered saline. *Babesia gibsoni*-infected tick larvae expressed Hlcyst-2 1.8× more than uninfected larvae. The recombinant protein also showed a significant growth-inhibitory effect on *B. bovis* cultured in vitro. These results indicated this cystatin Hlcyst-2 is involved in tick innate immunity.

Key words: *Haemaphysalis longicornis*, cystatin, *Babesia gibsoni*, innate immunity

Cystatins are tight-binding inhibitors of papain-like cysteine proteases and are widespread in plants and animals. Based on amino acid sequence, this superfamily can be subdivided into three closely related families (Rawlings & Barret, 1990; Turk & Bode, 1991). The physiological function of cystatins is not well understood. However, regulation of protein turnover and protection against insects and pathogens have been proposed in plants (Turk & Bode, 1991). Mammalian cystatin C has also been suggested to participate in the defense against pathogen invasion (Olsson et al., 1999). Filarial cystatins are pathogenicity factors and are thought to play a key role in balancing the host-parasite immune relationship (Shierack et al., 2003). Genes encoding cystatins have also been found in several ixodid ticks (Valenzuela et al., 2002; Karim et al., 2005). However, the function of tick cystatins remains unknown.

Ticks are important vectors of a wide variety of disease-causing bacteria, viruses, protozoa, and other pathogenic organisms. Despite the importance of ticks as vectors of disease, very little is known of their basic biology, especially their immune system. Understanding vector immunity is important in determining the host-pathogen interactions that facilitate or limit disease transmission. The hard tick, *Haemaphysalis longicornis* Neumann, is distributed mainly in East Asia and Australia, where it transmits a wide range of pathogens; include bovine theileriosis (*Theileria* spp), bovine babesiosis (*Babesia ovata*), canine babesiosis (*Babesia gibsoni*), and human rickettsiosis (*Rickettsia japonica*) (Fujisaki et al., 1994; Jongejan & Uilenberg, 2004). In this study, we report the characterization of a secreted cystatin from *H. longicornis* and provide evidence that cystatin is involved in the innate immunity of ticks.

MATERIALS AND METHODS

Ticks and tissue collection

The parthenogenetic Okayama strain of the tick *H. longicornis* has been maintained by feeding on rabbits and mice for several generations in our laboratory (Fujisaki, 1978). For tissue collection, the ears of rabbits were infested with adult females of *H. longicornis*. Ticks were recovered from the rabbit after 4 days, and the tissues were immediately dissected under the microscope (You et al., 2001). The sample materials were stored at –80 °C until used.

Construction of the tick midgut full-length cDNA library by vector-capping and cDNA sequencing

The full-length cDNA library was made using the vector-capping method (Kato et al., 2005). Total RNA was prepared from the midgut of partially fed female adult ticks, which had remained attached to the rabbit for 4 days. The cDNA was synthesized with 5 μg total RNA by the G-Capping method, and ligated into plasmid vector pGCAP1, the resulting plasmids were transformed into electrocompetent cell DH12S (Invitrogen). A total of 10,000 recombinant transformants from the library were randomly selected for plasmid DNA purification and sequencing. Nucleotide sequences were determined using an automated sequencer (ABI PRISM 310 Genetic Analyzer). The cDNA clone encoding a secreted cystatin was chosen for further analysis.

Expression and purification of the cystatin in *Escherichia coli*

The open reading frame (ORF) of a cystatin gene in the pBluescriptSK(+) vector was subcloned into pGEX-4T-3 *E. coli* expression vector (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The resulting plasmid was checked for accurate insertion by sequencing and was designated as

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the pGEX-4T-3/cystatin plasmid. The gene was expressed as a glutathione S-transferase (GST)-fusion protein in the *E. coli* BL21 (DE3) strain according to the manufacturer's instructions (Amersham). The resulting *E. coli* cells were washed three times with phosphate-buffered saline (PBS), lysed in 1% Triton X-100-PBS, sonicated, and then centrifuged at 12,000 ***g*** for 10 min at 4 °C. Supernatants containing soluble GST fusion protein were purified with glutathione-Sepharose 4B beads (Amersham) according to the manufacturer's instructions. Analysis for recombinant protein expression and purification was carried out by standard SDS-PAGE.

Enzymatic assays and analysis on non-denaturated polyacrylamide gel with gelatin

The enzymes used were papain (EC 3.4.22.2; Sigma), cathepsin L (EC3.4.22.15, Sigma), and cathepsin B (EC 3.4.22.1, Sigma). The enzymatic assay buffer was 100 mM sodium phosphate, containing 1 mM DTT and 2 mM EDTA, adjusted to pH 6.5 for papain and to 6.0 for cathepsin L and B. The fluorogenic substrate used was Z-Phe-Arg-AMC (benzyloxycarbonyl-Phe-Arg-7-amido-4-methylcoumarin; Peptide Institute, Osaka, Japan). Protease (0.2 μM) was incubated with different concentrations GST-fused recombinant cystatin or control GST protein and 10 μM fluorogenic substrate. The reactions were allowed to proceed at 37 °C for 30 min in the black 96-well plate and then measured by fluorometry with an excitation at 355 nm and emission at 460 nm.

The inhibition of papain was also visualized on non-denaturated polyacrylamide gel containing gelatin substrate. Papain (2 μM) was incubated with cystatin (4 μM) or GST (4 μM) at 37 °C for 30 min. Samples were then applied to 8% polyacrylamide gels that had been set with 0.1% (w/v) gelatin (Roche et al., 1997). After electrophoresis at 4 °C, the gel was incubated overnight in sodium acetate buffer, pH 5.5, and then stained with CBB.

Real-time quantitative PCR

For estimation of mRNA abundance, we used one-step TaqMan real-time reverse transcription polymerase chain reaction (R/T RT-PCR) methods. Gene specific primers and fluorogenic probes were designed to target the cystatin gene or control tick actin gene (Accession number AY254898) using Primer Express software (Applied Biosystems, USA). The PCR primers and TaqMan probes used in this study are not shown. One-step R/T RT-PCR reaction was conducted using TaqMan one-step RT-PCR master mix reagents kit (Applied Biosystems) according to the manufacture's guidelines. Samples were amplified by using a program that included a reverse transcription procedure consisting of one cycle of incubation at 48 °C for 30 min and 94 °C for 10 min, followed by 45 cycles of denaturation at 94 °C for 15 s, and annealing/extension at 60 °C for 1 min. We established standard curves for cystatin and actin using serial dilutions (800-50 ng) of total RNA. Amplification and product detection were performed under the ABI PRISM 7900 HT sequence detection system (Applied Biosystems). A positive result was determined by identifying the threshold cycle (C_T) value at which reporter dye emission appeared above background. The relative amount of cystatin produced per unit of actin was calculated for each sample. Each analysis was done at least 3-fold.

Expression analysis of cystatin in tick developmental stages and tissues

In order to determine the cystatin gene expression in different development stages, total RNA extracted from egg (embryo), larval, nymphal, and adult ticks was used to purify the total RNA and then analyzed by Real-time PCR. To analyse the cystatin distribution in various tissues, salivary glands, midgut, fat body, ovary, and hemocytes were dissected from 4-day-fed adult ticks and total RNA was purified and subjected to real-time PCR.

Induced expression of cystatin in the midgut by blood feeding

A batch of adult ticks was fed on a rabbit. Thirty ticks from day 0 (before feeding), day 1 (attaching stage), day 4 (semi-fed stage), and day 7 (engorged stage) were collected and dissected, respectively; the dissected midgut was prepared to purify total RNA and then subjected to real-time PCR.

Induced expression of cystatin by lipopolysaccharide (LPS) injection in adult ticks

The injection was carried out using 50 μl microcapillaries (Microcap, Drummond Scientific, Broomall, PA, USA) drawn to fine-point needles by heating. The needles were connected to an air compressor. The solution was injected into the tick body from the fourth coxae. LPS is a major component of the outer membrane of Gram-negative bacteria and commonly used as an immune stimulant in insects (Goto et al., 2002). One microliter of LPS (Sigma) solution (0.1 μg/ml in PBS) or control PBS was microinjected into 30 unfed female ticks in the experimental and control groups, respectively. The ticks were allowed to rest for 6 h at 25 °C and then used to purify the total RNA for real-time PCR analysis.

Induced expression of cystatin by *Babesia gibsoni* infection in larval ticks

The NRCPD strain of *B. gibsoni* was experimentally infected in a splenectomized 1-year-old Beagle dog as described by our group previously (Fukumoto et al., 2001). When the parasitemia of the infected dog was up to 5%, adult ticks were placed on the dog. Individual engorged female ticks were incubated at 25 °C and 90% r.h. for oviposition. Hatched larval ticks from each individual adult were checked by the PCR method. The amplified fragment of *B. gibsoni* special gene was sequenced to determine whether they were infected or not (Fukumoto et al., 2001). Larval control ticks and *B. gibsoni*-infected larvae were used to purify total RNA and subjected to real-time PCR.

Growth-inhibitory assays of cystatin against *Babesia bovis* cultured in vitro

The in vitro growth-inhibitory assays were conducted as described previously (Bork et al., 2003). One hundred microliter of infected bovine red blood cells (RBCs) was diluted with non-infected RBCs to obtain 1% parasitemia in a 0.1-ml volume, and the mixture was subsequently suspended in 0.9 ml of a suitable growth medium supplemented with the indicated concentrations of recombinant cystatin. The suspension was added to 24-well culture plates (Nunc, Roskilde, Denmark), and the plates were incubated in a humidified multigas water-jacketed incubator at 37 °C for 4 days. During the incubation period, the overlaid culture medium was replaced daily with 0.9 ml of fresh medium containing cystatin at the concentrations indicated below. In parallel, GST

proteins were prepared as controls. All experiments were carried out in triplicate wells (for each concentration) and in three separate trials. Parasite growth in Giemsa-stained thin blood smears with approximately 1,000 RBCs was monitored daily and was determined on the basis of morphological appearance.

Nucleotide sequence accession number

The sequence of the Hlcyst-2 gene of *H. longicornis* has been submitted to the GenBank database under accession number DQ364159.

RESULTS

Construction of a full-length cDNA library using total RNA

The vector-capping method was applied to construct a cDNA library from the total RNA of *H. longicornis*'s midgut. After transformation of *E. coli* cells, transformants were grown on agar plates without amplification in a liquid medium. The library was composed of approximately 100,000 independent colonies, from which 10,000 were picked randomly, and the 5'-ends of the cDNA of these clones were sequenced. Readable sequences of 8,304 cDNA inserts were obtained, and an EST database was made (data shown elsewhere).

Cloning and sequence analysis of the full-length cDNA encoding *Haemaphysalis longicornis* cystatin

From the midgut library, three genes encoding different cystatins were cloned and sequenced, and the putative cystatins were designated Hlcyst-1 (*H. longicornis* cystatin-1), Hlcyst-2, and Hlcyst-3, respectively. In this paper, we focus on the secreted cystatin Hlcyst-2. The full-length cDNA of Hlcyst-2 is 569 bp, including an intact ORF encoding an expected protein with 131 amino acids. A hydrophobic region at the N-terminus of Hlcyst-2 had the characteristics of a signal peptide, and a cleavage site was predicted between amino acids 18 and 19. The mature protein consisted of 113 residues with a calculated molecular weight of 12.9 kDa and isoelectric point of pI 8.5. SMART analysis (Schultz et al., 2000) detected the cystatin-like domain in the putative amino acid sequence (position 23-130). BLASTP analysis of the predicted polypeptide sequence against all non-redundant databases accessed through NCBI revealed a significant score with members of family-2 cystatins of other species. The identities of putative amino acid of Hlcyst-2 with available tick cystatins are among 34-48%. The alignment of putative amino acids with several known tick cystatins was shown. Amino acid sequences of QxVxG (x can be one of several amino acids) are highly conserved in various cystatins (Brown & Dziegielewska, 1997). The other conserved regions also include a glycine in the N-terminal region and a PW motif in the second hairpin loop in the C-terminal region (Bjok et al., 1996; Hall et al., 1993), which were all found in this cloned cystatin Hlcyst-2 and most reported tick cystatins. In addition, the putative amino acid sequence of Hlcyst-2 gene contains four cysteine residues at the C-terminus, these residues were predicated to form two disulfide bonds in family-2 cystatins (Barrett et al., 1986). However, an active domain, SND, inhibiting legumain-like proteases, enzymes known to be involved in MHC class II antigen processing (Alvarez-Fernandez et al., 1999), was lacking in all tick cystatins.

Expression of the cystatin in *Escherichia coli*

The Hlcyst-2 gene was ligated into the bacterial expression vector pGEX-4T-3, and then successfully expressed as a GST-fusion protein with an expected size of 39 kDa. The recombinant Hlcyst-2 (rHlcyst-2) was expressed as a soluble form and then purified by affinity-chromatography. The yield of purified rHlcyst-2 was typically 1-2 mg/l bacterial culture. The recombinant protein was >95% pure, as estimated by SDS-PAGE under reducing conditions.

Inhibitory activity and heat stability of the recombinant cystatin

The purified rHlcyst-2 was assayed for inhibitory activity against the cysteine protease papain and the human cathepsin L and B proteases. As measured by Z-Phe-Arg-AMC hydrolysis, 0.4 μM of rHlcyst-2 strongly inhibits (>90% inhibition) 0.2 μM of papain and cathepsin L, whereas the activity of cathepsin B was less effectively inhibited by rHlcyst-2. The inhibition of gelatinolytic papain activity by rHlcyst-2 was also shown in polyacrylamide gel under non-denaturating conditions. In the gel, the control lane (papain treated with GST) migrates as a smear and a corresponding broad band of gelatinolytic activity in a polyacrylamide gel containing gelatin, but the test lane (papain treated with rHlcyst-2) completely inhibits its gelanolytic activity. The heat stability of rHlcyst-2 at different temperatures was also investigated and rHlcyst-2 remained the inhibition activity at 50 °C, but lost significant inhibitory activity when treated at 60 °C, and completely lost its activity at >70 °C.

Expression analysis of cystatin in different tick stages and tissues

The relative amount of Hlcyst-2 mRNA per unit of actin, measured as relative expression rate (%), gradually increased while ticks develop from larva to adult. The tissue distribution results of Hlcyst-2 showed that this gene expressed most richly in the tick midgut (1051.7%) and hemocytes (69.9%), while only a few copies were detected in salivary glands (5.7%), ovary (0.2%), or fat body (2.3%). These results indicated that the Hlcyst-2 gene was expressed throughout the developing stage, but its distribution showed clear tissue specificity.

Induced expression of cystatin gene

The relative expression of Hlcyst-2 induced in the midgut by blood-feeding in 1-, 4-, and 7-day-fed ticks was 5.6, 17.0, and 13.3× higher than that of non-fed ticks, respectively. Transcription of the Hlcyst-2 gene was induced by LPS injection in adult ticks. Six hours after injection, the relative expression for the LPS group is 1.6× that of the control PBS group. Transcription of the Hlcyst-2 gene was induced by *B. gibsoni* infection in tick larvae. The relative expression for infected ticks is 1.8× that of non-infected ticks.

Growth-inhibitory assay of cystatin against *Babesia bovis* cultured in vitro

The in vitro growth of *B. bovis* was significantly inhibited by rHlcyst-2 at 1, 2, and 3 μM in the second- and third-day culture ($P<0.05$), compared with the control 3-μM GST. As observed by light microscopy the parasites exposed to rHlcyst-2 showed some morphological changes.

DISCUSSION

The present study describes the sequence of a novel cystatin Hlcyst-2 from the tick *H. longicornis*. The putative amino acid sequence indicates that it belongs to a member of family-2 cystatins. Family-2 cystatins, represented by human cystatin C and chicken egg cystatin, are secretory proteins with one cystatin domain and two characteristic disulphide bridges (Rawlings & Barrett, 1990). Various cystatins have been characterized based on their capacity to inhibit the activity of cysteine proteases. In this study, the GST-fused recombinant Hlcyst-2 cystatin effectively inhibited the activity of papain and human cathepsin L, whereas the human cathepsin B activity was only moderately inhibited. Similar features were also found for the cystatins of nematodes (Hartmann & Lucius, 2003) and of snake venoms (Briliard-Bourdet et al., 1998). It was thought to be a general feature of all cystatin inhibitors, owing to the occluding loop in the cathepsin B active site that limits the access of both substrates and inhibitors to the active site (Musil et al., 1991). The heat stability test showed that rHlcyst-2 was somewhat stable against thermal denaturation, but less heat-stable compared to bovine and human cystatin C that is stable at 60 °C (Olsson et al., 1999). This inhibition profile demonstrated that rHlcyst-2 has a potency similar to known cystatins, suggesting that the recombinant rHlcyst-2 is produced as a functional, correctly folded protein. Taken together, this is the first report on the identification of the tick cystatin although several tick genes encoding cystatins are available from database and a preliminary test using tick cystatin gene for RNAi was published recently (Karim et al., 2005),.

The expression of mRNA for Hlcyst-2 was detected in the four developmental stages, which indicated the important physiological role of this molecule throughout the tick's life cycle. The mRNA of Hlcyst-2 was detected in the midgut (most) and in hemocytes, and it was present in very low amounts in all other tissues examined. The restricted expression was also found in mammalian and insect cystatins (Agarwala et al., 1996; Freije et al., 1991; Goto et al., 2002). The tissue specificity expression suggested a distinct role in different tissues.

Cystatins have been proposed to function as defense proteins in plants and mammals (Turk & Bode, 1991; Olsson et al., 1999). In insects, a cystatin from the silk moth, *Bombyx mori*, acts in the same way as defense protein against invading pathogens and parasites, many of which use cysteine proteases to enter their hosts (Yamamoto et al., 1999). Similarly, a cystatin from the horseshoe crab, *Tachypleus tridentatus*, showed antimicrobial activity against Gram-negative bacteria (Agarwala et al., 1996). Our transcript study of Hlcyst-2 mRNA may help to elucidate the possible functions in tick innate immunity. The significant up-regulation of the Hlcyst-2 gene has been detected in LPS-injected ticks. LPS, a major component of the outer membrane of Gram-negative bacteria, is commonly used as an immune stimulant in other insects (Goto et al., 2002). More importantly, *B. gibsoni*, a protozoan naturally transmitted by ticks (Zhou et al., 2002), also induced a significant increase in expression of the Hlcyst-2 gene. Blood ingestion by the ticks is the major pathway for pathogens to invade the tick. Hence, the ability to respond to microbial challenge, possibly via defensins as observed in the midgut of malaria mosquitoes and soft ticks, was up-regulated by blood feeding (Nakajima et al., 2001; Richman et al., 1996). The fact that the Hlcyst-2 gene was up-regulated during tick feeding, suggests that cystatin Hlcyst-2 is involved in the innate immunity of ticks. This suggestion is supported by the observation that the in vitro growth of *B. bovis* was significantly inhibited by rHlcyst-2.

An additional function of cystatin may be the modulation of host immune responses. A recent report using the cystatin gene of the tick *Amblyomma americanum* for an RNAi study suggested that cystatin might be involved in disrupting normal antigen processing in antigen presenting cells of hosts like nematodes (Karim et al., 2005). Parasitic nematode cystatins are involved in immune evasion processes such as inhibition of antigen presentation (Dainichi et al., 2001; Manoury et al., 2001). However, the inhibition of host antigen presentation by nematode cystatins is mediated by the legumain inhibitory motif within the cystatin. Since the Hlcyst-2 domains are devoid of any legumain inhibitory motif, we suggest that the Hlcyst-2 is not involved in the inhibition of host antigen presentation.

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Chemical Acarology

Oil gland secretions in Oribatida (Acari)

Günther Raspotnig
Karl-Franzens-University, Institute of Zoology, Universitätsplatz 2, A-8010 Graz, Austria.
E-mail: guenther.raspotnig@uni-graz.at

The chemical ecology of Oribatida is tightly integrated with a distinct exocrine system in the opisthosoma, known as 'oil glands' (syn. opisthonotal glands). Representing homologous structures, oil glands characterize the four more derived cohorts of Oribatida (Parhyposomata, Mixonomata, Desmonomata, and Brachypylida), but also the Astigmata, as the monophyletic unit of 'glandulate Oribatida'. Generally, oil glands constitute large intima-lined sacs that are located in the dorso-lateral regions of the idiosoma and that open to the body outside via a single (frequently flapped) pore on either side of the notogaster. Secretions of more than 20 oribatids have so far been analyzed. They consist of hydrocarbons, terpenes, aromatics, and alkaloids. Many components occur in specific combinations; secretion profiles characterize groups (on any taxonomic level) and have emerged as tools for phylogenetic analyses: Parhyposomata, e.g., produce phenolic- and naphthol-rich secretions, whereas a distinct set of terpenes and aromatics (the so-called 'astigmatic compounds') is considered synapomorphic for middle-derived Mixonomata and all groups above ('astigmatic compounds-bearing Oribatida'). In some subgroups of the 'astigmatic compounds-bearing Oribatida', these components are not easily traced as they tend to be reduced and replaced by others. Functionally, oil glands produce various allomones against predators and fungi, and alarm pheromones for intraspecific communication. Pheromonal properties of oil gland compounds probably evolved early in ancient oil gland-bearing oribatids from purely defensive functions, culminating in a radiation of semiochemical roles (alarm, aggregation, sex) in oil glands of the Astigmata.

Key words: Oil glands, opisthonotal glands, Oribatida, semiochemicals, Astigmata

The chemical ecology of many arthropod groups, including mites, has been studied extensively in the last decades, but chemical communication has remained poorly investigated in certain acarine taxa, e.g. in the Oribatida. The Oribatida is a vast (paraphyletic) assemblage of mainly soil-dwelling mites, comprising about 10,000 hitherto described species (Balogh & Balogh, 1992; Schatz, 2002). For many years, chemical communication in oribatids has been inferred from behavioural evidence only, including early observations of enhanced spermatophore deposition in the presence of females (Woodring, 1970) or the possibly pheromone-related pairing behaviour in *Collohmannia gigantea* (Schuster, 1962). Further evidence for semiochemical interactions is seen in the rich configuration in terms of multiple epidermal glands that underlie the areae porosae of the notogaster. These are supposed to produce not only pheromones for intraspecific chemical communication (Alberti et al., 1997), but also protective compounds against micro-organisms such as fungi and bacteria (Raspotnig & Krisper, 1998).

Currently, however, the main focus in oribatid chemical ecology is directed towards a large exocrine system that is homologously present in most Oribatida (and also in Astigmata). This system is represented by so-called oil- or opisthonotal glands. These glands are thought to have evolved only once in the oribatid stem-line – after the branching-off of the two basal groups of Oribatida: Palaeosomata and Enarthronota – and are thus present (though in different character states) in the four more derived oribatid cohorts, i.e., the Parhyposomata, Mixonomata, Desmonomata, and Brachypylida. Their occurrence in astigmatic mites, for instance, is one of the major arguments for the evolutionary origin of Astigmata in an ancestral oil gland-bearing oribatid group (Norton, 1998). Thus, oil gland-bearing oribatids (including astigmatic mites) very likely represent a large monophyletic unit, the 'glandulate Oribatida' (sensu Norton, 1998). Generally, oil glands comprise paired opisthosomal sacs that may reach conspicuous dimensions (up to 25% of body length). Moreover, enhancing their conspicuousness, oil glands produce noticeably scented secretions in certain species, such as lemon scents in *C. gigantea* (Raspotnig et al., 2001) and phenolic scents in *Parhypochthonius aphidinus* (Sakata & Norton, 2001). The production and emission of scents from oil glands, e.g., 'minthy smells', is also known from certain astigmatic species (Bull, 1970; Kuwahara et al., 1987, 1988, 1991a,b; Leal et al., 1988).

Despite these distinctive features and in contrast to the increasing knowledge on oil glands of astigmatic mites (Kuwahara, 1991, 2004; Kuwahara et al., 1975), oil glands of oribatids have remained obscure until recently. Known to Acarology from the early work of Michael (1884) – who designated them as 'expulsory vesicles' and found them to contain an oily liquid of unknown chemical composition – it was not before 1995 that an oribatid oil gland secretion was first analyzed (Sakata et al., 1995). Subsequently, oil glands were re-discovered as central systems in life and survival strategies of Oribatida, with recent reports on their chemistry, functional morphology, and biological roles (Raspotnig, 2006; Raspotnig et al., 2001, 2003, 2004, 2005a,b, 2008, 2009; Sakata & Norton, 2001, 2003; Sakata et al., 2003; Shimano et al., 2002). The present paper reviews recent advances in oil gland research in Oribatida.

MATERIAL AND METHODS

Oil gland secretion analysis: an overview

Oil gland secretions of Oribatida and Astigmata have been analyzed by various working groups. Here emphasis is on

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procedures followed by Raspotnig and colleagues (e.g., Raspotnig et al., 2004, 2005a,b, 2008, 2009). Chemical investigations into oil gland secretions mainly rely on whole-body extractions of live individuals that discharge their secretions directly into a solvent. Crude extracts, containing a mixture of oil gland secretion components (but potentially also components from other parts of the body), are separated by capillary gas chromatography (GC). Mass spectrometric (MS) fragmentation patterns of single compounds (mainly electron impact spectra) are used for structure determination, leading to propositions for the identity of extract components. For a final identification of extract components, GC retention times (and MS fragmentation) of synthetic reference compounds have to be compared to those of extract components. Only compounds with matching spectra *and* matching retention times are positively identified.

A possible technique for discriminating oil gland components from components of other body parts in the extracts is outlined in Raspotnig et al. (2001). By now, however, there is corroborative evidence that the extraction technique described below, provides chromatographic access to constituents of oil gland secretions only (see also Sakata & Norton, 2001, 2003; Raspotnig et al., 2001, 2004, 2005a,b).

Extraction procedure

Briefly, extraction and analysis of oil gland secretions (as carried out by the author) involved the extraction of freshly collected, live individuals in hexane (1-10 individuals per 50 µl depending on body size) for a maximum of 30 min. Crude extracts were used for analysis.

Gas chromatography–mass spectrometry

The analytical instruments included a Fisons 8000 GC coupled to a Fisons MD 800 MS from Thermo-Quest (Vienna, Austria). The GC-column (a DB-5MS fused silica capillary column: 30m x 0.25 mm i.d., 0.25 µm film thickness from Fisons) was directly connected to the ion source of the MS. The splitless Grob injector was kept at 260 °C; helium was the carrier gas. The following temperature program was used: initial temperature 50 °C for 1 min, followed by an increase of 10 °C/min to 200 °C, with 15 °C/min to 300 °C, and an isothermal hold for 5 min. The ion source of the MS and the transfer line were kept at 200 °C and 310 °C, respectively. Electron impact (EI) mass spectra were recorded at 70eV.

Scanning electron microscopy and histology

Scanning electron micrographs (SEM) were taken after routine procedures of fixation, cleaning, dehydration, and mounting of specimens on small dishes, prior to sputtering with gold. Micrographs were taken on different instruments, mainly on a Philips ESEM. For histology, semithin sections (1-3 µm thickness) were cut on a rotatory microtome, following embedding in the methylmethacrylate resin LR-White (Raspotnig et al., 2003).

Bioassays

Bioassays for clarification of semiochemical properties of oil gland secretions were performed by presenting small filter paper pieces loaded with different amounts of single synthetic oil gland components to individuals or groups of individuals of the species under concern. For *C. gigantea*, behavioural reactions were recorded by a digital video camera and subsequently analysed (Raspotnig, 2006).

RESULTS AND DISCUSSION

Oil gland morphology

Oil glands of Oribatida generally open to the body outside via one large orifice on either of the dorso-lateral sides of the notogaster. Orifices are frequently inconspicuous, being surrounded by moderately projecting smooth cuticular lips as is the case in many mixonomatan, desmonomatan, and brachypylid oribatids (e.g., Sakata & Norton, 2001; Raspotnig et al., 2003). In Parhyposomata, e.g., in *Parhypochthonius* species, oil gland pores are elongated to form small lateral projections, or are located on distinct, moderately projecting cuticular plates such as in *Gehypochthonius*. In certain Brachypylida, namely in the Hermannielloidea, oil gland orifices (convergent to Parhypochthonioidea) are located atop conspicuous tubercles. Frequently, oil gland pores are provided by cuticular flaps that represent closure mechanisms under muscular and nervous control. Flaps may be externally visible such as in species of *Oribotritia* or may be sunk into the pore such as in *Collohmannia* (Raspotnig et al., 2003). In *Platynothrus*, pore orifices are sickle-shaped and narrow and may lack additional closing mechanisms. Externally-located cuticular flaps are also known from oil glands of astigmatic mites (Howard et al., 1988).

Generally, the main part of a typical oil gland is represented by a voluminous reservoir that is covered by a fine intima. The reservoir may be disc-shaped (e.g., *Collohmannia*) to spherical (e.g., *Platynothrus peltifer*; juveniles of *Hermannia convexa*); it may comprise a single-layered epithelium, responsible for the deposition of the intima only or the epithelium may be multilayered. Thus, in the latter case – realized in, e.g., juvenile *H. convexa* – the epithelium also contributes to the production of the secretion. In the former case – true for, e.g., *C. gigantea* – secretory cells are located around the reservoir. In Astigmata, possibly as a highly derived feature, oil glands may be represented by a single cell on either side of the body, each containing an intracellular (also intima-covered) reservoir; this situation is true for *Dermatophagoides* species (Brody & Wharton, 1970; Tongu et al., 1986). In this case, the production of a complex secretion as well as its storage and emission is managed by one cell.

The muscular configuration of oil glands has been studied in *C. gigantea* in some detail: muscle bundles, crucial for a controlled emission of the secretion in pulses, attach to the intima of the reservoir and to the inner side of the notogaster. Muscular contraction leads to traction on the reservoir itself, and, consequently, to an opening of the oil gland-closure flap. Flap closure may be provided by the self-elasticity of the cuticle.

In the majority of glandulate oribatid groups, oil glands are well developed and represent medium to large glands. In many Mixonomata however, especially in *Collohmannia* and in Euphthiracaroidea but also in certain (basal) Brachypylida (e.g., in Hermanniellidae), oil glands are indeed 'hypertrophied' (Raspotnig et al., 2003). In contrast, trends to size-reduction are apparent in different lineages of Desmonomata, e.g., in Camisiidae and in Hermanniidae (Raspotnig et al., 2005a, 2009), as well as in certain Brachypylida. In *Ceratozetes cisalpinus*, e.g., oil glands are tiny structures (Woodring & Cook, 1962). (Nearly) complete reduction of oil glands is known from mixonomatan Phthiracaridae and desmonomatan Malaconothridae.

Table 1 Chemistry of oil gland secretions in Oribatida: status quo.

species	secretion profile	ref
Parhyposomata		
Parhypochthonius aphidinus Berlese	3-ethylphenol, C11, C13, C13:1	[1]
Gehypochthonius urticinus (Berlese)	1-methyl-2-naphthol, C13, C13:1, C17:1, C17:2,	[1]
Mixonomata		
Nehypochthonius porosus Norton & Metz	C13, C15 + two unknowns (M=150, M=204)	[1]
Perlohmannia sp. (not described)	γ-acaridial, neral, geranial, C15	[1]
Collohmannia gigantea Sellnick	2,6-HMBD, γ-acaridial[1], neral, geranial, neryl formate, C13, C15	[2]
6 spp. Oribotritiidae (Oribotritia, Mesotritia)	chrysomelidial, ß-springene + other components (including C17:1, C17:2; in *Oribotritia banksi* also neral, geranial)	[3]
Desmonomata		
Trhypochthoniellus crassus (Warburton & Pearce) (= *Hydronothrus crispus*)	2,6-HMBD, γ-acaridial[2], neral, geranial, neryl formate[2], C13, C15, C15:1, C17:1, C17:2	[4,5]
Trhypochthoniellus sp. (not determined)	γ-acaridial, neral, geranial, neryl formate, geranyl formate, C17:1, C17:2	[5]
Trhypochthonius japonicus Aoki	2,6-HMBD[3], γ-acaridial, geranial, (Z,E)-farnesal, (E,E)-farnesal, C17:1, C17:2 + two unknowns	[5]
Trhypochthonius tectorum (Berlese)	2,6-HMBD, γ-acaridial, neral, geranial, (Z,E)-farnesal, (E,E)-farnesal, C15, C17:1, C17:2	[6]
Archegozetes longisetosus Aoki	2,6-HMBD, γ-acaridial, neral, geranial, neryl formate, C15, C15:1, C17:1, C17:2	[7]
Nothrus palustris CL Koch	neral, geranial, dehydrocineole, C21:2	[8]
Platynothrus peltifer (CL Koch)	γ-acaridial, rhizoglyphinyl formate, neral, geranial, neryl formate, C17:1, C17:2	[9]
Heminothrus targionii (Berlese),	neral, geranial, C17:1, C17:2	[3]
Heminothrus ornatissimus (Berlese)	id.	
Camisia horrida (Hermann)	neral[4]	[3]
Camisia spinifer (CL Koch),	no components detectable	[3]
Camisia biurus (CL Koch),	id.	
Camisia solhoeyi Colloff	id.	
Hermannia convexa (CL Koch)	γ-acaridial[4], neral[4], geranial[4], 1,8-cineole, C17:1[4], C17:2[4]	[10]
Brachypylida		
Scheloribates azumaensis Enami, Nakamura et Katsumata	γ-acaridial[4], geranial[4], 2-(2-pentenyl)-2-cyclopenten-2-one (?), precoccinelline 193C, coccinelline-type alkaloid (?), pumiliotoxin 251D + unknowns	[11]
Scheloribates sp. (not determined)	pumiliotoxin 237A, 8-deoxypumiliotoxin 193H, 6,8-diethyl-5-propenylindolizidine, 1-ethyl-4-pentenynylquinolizidine (?) + seven unknowns	[11]
diverse families (no species determined)	about 80 different alkaloids	[12]

[1]Not mentioned in [2], but inconsistently present. [2]Profiles given in [4] and [5] differ with regard to neryl formate and γ-acaridial. [3]2,6-HMBD was absent in one (of two) populations investigated. [4]Compounds present in juveniles only. Compounds marked with '(?)' were only tentatively identified. References: [1] Sakata & Norton, 2001, [2] Raspotnig et al., 2001, [3] Raspotnig et al., 2008, 2009, [4] Sakata et al., 1995, [5] Sakata et al., 2003, [6] Raspotnig et al., 2004, [7] Sakata & Norton, 2003, [8] Shimano et al., 2002, [9] Raspotnig et al., 2005b, [10] Raspotnig et al., 2005a, [11] Takada et al., 2005, [12] Saporito et al., 2007.

Chemistry and evolution of oil gland secretion profiles

Even though a large amount of chemical data was published on several brachypyline families most recently (Saporito et al., 2007), detailed profiles of secretions are only known for about two dozen oribatid species (see Table 1). So far, two species of Parhyposomata (genera *Parhypochthonius* and *Gehypochthonius*), about 10 species of Mixonomata (mainly genera *Nehypochthonius*, *Perlohmannia*, *Oribotritia*, and *Collohmannia*), about one dozen species of Desmonomata (mainly *Trhypochthonius*, *Thrypochthoniellus*, *Platynothrus*, *Heminothrus, Camisia, Nothrus*, and *Hermannia*), and two species of Brachypylida (genus *Scheloribates*) were analysed in detail. Secretions in these species generally exhibit multi-component profiles, being composed of hydrocarbons, terpenes, aromatics, and alkaloids. Secretion patterns, in all relying on about 30 different components, appear to be species- or at least group-specific, showing distinct evolutionary trends in different lineages. Preliminary data exist on some additional species, especially on Oribotritiidae and many brachypylines such as Liacaridae and Hermanniellidae (G Raspotnig, unpubl.). In contrast, much more is known about the chemistry of oil gland secretions of Astigmata where profiles from 61 species were already elucidated (Kuwahara, 2004).

With regard to the chemistry of secretions, hydrocarbons (mainly tri- and pentadecane, but also heptadecadiene and heptadecene) are probably plesiomorphic secretion components, being present in all oribatid secretions hitherto analyzed, as well as in oil glands of Astigmata. On the other hand, a set of terpenes and aromatics – the so-called 'astigmatic compounds' (sensu Sakata & Norton, 2001) – probably evolved stepwise in mixonomatan ancestors and thus characterize all further descendents: these are all oribatids higher than middle-derived Mixonomata (i.e., Collohmannioidea up to Brachypylida), including Astigmata. Especially for mixonomatans such as *Collohmannia*, for desmonomatan Trhypochthoniidae, and for Astigmata, rich development of astigmatic compounds has been reported (Sakata & Norton, 2001, 2003; Sakata et al., 1995, 2003; Raspotnig et al., 2001, 2004). In certain subgroups of the assumed monophyletic unit of 'astigmatic compounds-bearing Oribatida', trends in reduction and replacement of astigmatic compounds become obvious (e.g., Raspotnig et al., 2008, 2009): these trends occur in different phyletic lineages such as in Euphthiracaroidea, camisiid Crotonioidea, Hermannioidea, and Brachypylida. In these groups, only remnants of astigmatic compounds are present, mainly in near-basal representatives of groups under concern, or astigmatic compounds can be found in juveniles only ('adult-juve-

nile dimorphism'; so far reported from representatives of *Nothrus*, *Hermannia*, and *Scheloribates*). Furthermore, these groups are characterised by the development of novel chemical compounds, such as iridoid monoterpenes (e.g., chrysomelidial) and diterpenes (e.g. ß-springene) in euphthiracarid Oribotritiidae (Raspotnig et al., 2008) or alkaloids in Brachypylida (Takada et al., 2005; Saporito et al., 2007). The evolution of oil gland secretion profiles is illustrated in Figure 1.

Biological significance of oribatid oil glands

In spite of interpretations of oil gland functions as lubricating, osmo- or themoregulatory organs (Zachvatkin, 1941; Riha, 1951; Smrz, 1992), there is strong and corroborative evidence for a major role in chemical communication. In bioassays using synthetic oil gland secretion constituents, semiochemical roles have not only been proven for many astigmatic species (Kuwahara, 1991, 2004), but meanwhile also for two species of Oribatida, namely for nymphs of the desmonomatan *Nothrus palustris* (Shimano et al., 2002) and for adults of mixonomatan *C. gigantea* (Raspotnig, 2006) (Table 2). From the latter two species, alarm pheromonal communication was reported. Active spaces of alarm pheromonal components, as shown for *C. gigantea*, appear to be limited to a few millimetres, but correspond well to the narrow interstitial dimensions in the natural environment of soil.

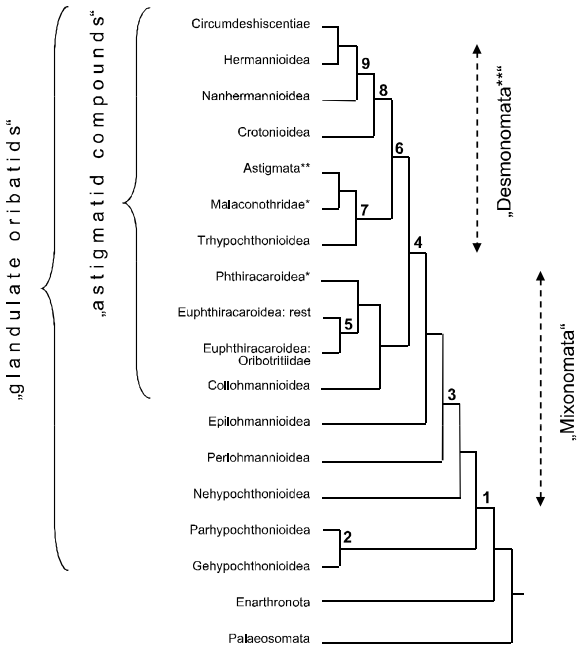

Figure 1 Evolution of oil gland secretion profiles in Oribatida. The tree presented is based on Maraun et al. (2004), but modified with respect to the status of Eulohmanniidae, Lohmanniidae, etc. (considered as families of Enarthronota). Mixonomata and Desmonomata are paraphyletic groups but retained with reference to the classical division of Oribatida into cohorts (**Astigmata do not belong to Desmonomata in the classical sense: according to Norton (1998), however, Astigmata are the sister group of trhypochthoniid Malaconothridae). *Oil glands are convergently reduced in Phthiracaroidea and Malaconothridae. Synapomorphies are: (1) oil glands in glandulate oribatids; hydrocarbons in secretions; (2) phenolic and naphtholic compounds in Parhyposomata; (3) small set of astigmatic compounds (neral, geranial, γ-acaridial) in Perlohmannioidea, Epilohmannioidea, and 'astigmatic compounds-bearing oribatids'; (4) full set of astigmatic compounds developed (neral, geranial, γ-acaridial, neryl formate, 2-hydroxy-6-methyl-benzaldehyde); (5) trend to reduction of astigmatic compounds in Euphthiracaroidea and development of novel components such as iridoid monoterpenes (e.g., chrysomelidial) and diterpenes (e.g., ß-springene) in Oribotritiidae; reduction of astigmatic compounds and sets of unknowns in the remaining Euphthiracaroidea; (6) novel components such as rhizoglyphinyl formate (e.g., in genus *Platynothrus*); (7) novel components such as farnesals (e.g., in *Trhypochthonius*); (8) increasing trend to juvenile-adult-'dimorphism' of secretion profiles (juveniles: astigmatic compounds; adults: reduced set of astigmatic compounds, novel sets of compounds: e.g., in *Nothrus*, *Hermannia*); (9) complete reduction of astigmatic compounds in adults; novel compounds such as alkaloids (e.g., in *Scheloribates*) and sets of unknowns (e.g., in *Hermanniella*).

Table 2 Pheromonal activity of oil gland secretions in Oribatida.

Species	Alarm pheromone	Ref
Mixonomata		
Collohmannia gigantea	2,6-HMBD[1], neral[1], geranial, neryl formate	[1]
Desmonomata		
Nothrus palustris	geranial[2]	[2]

[1]Main components of the pheromone. [2]Active in nymphal stages only. References: [1] Shimano et al. 2002, [2] Raspotnig 2006.

According to current ideas, pheromonal functions evolved early in glandulate oribatid ancestors from purely defensive secretions; allomonal defense, e.g., against oribatid predators such as scydmaenid beetles, is still realized in extant species such as *C. gigantea* (Raspotnig, 2006). Consistently, oil glands generally display the structural organisation of typical alarm pheromone and defence glands as well known from a variety of arthropods (e.g., Blum, 1985). Alarm pheromonal functions may have been added soon in the course of evolution and may be a much more widespread function of oil glands in Oribatida as hitherto expected. Different pheromonal roles, with regard to alarm, aggregation, and sex, all associated with oil gland secretions, finally have radiated in the Astigmata.

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How astigmatic mites control the emission of two or even three types of pheromones from the same gland

Yasumasa Kuwahara
Faculty of Bioenvironmental Science, Kyoto Gakuen University, Kameoka-shi, Kyoto 621-8555 Japan.
E-mail: kuwahara@kyotogakuen.ac.jp

Astigmatic mites are known to achieve intraspecific communication on alarm, sex, and aggregation by pheromones released from one pair of opisthonotal glands. So far, species have been found to use a single active compound, or different compounds, to achieve two of the three communication functions. Each communication function emerges from the conditions the mites are facing, from functional characteristics such as dose-response relationships and active dose-range, and from the relative abundance of the active compounds in the gland. Here, findings on combinations of two communication functions from products of opisthonotal glands are summarized for species of Astigmata and new evidence is presented for a species, *Rhizoglyphus setosus*, that is capable of achieving all three communication functions – alarm, sex, and aggregation – from products of opisthonotal glands. The possible mechanisms that lead to multiple communication functions from products of one paired gland are discussed.

Key words: Astigmata, *Rhizoglyphus setosus*, communication, sex pheromone, alarm, aggregation

Astigmatic mites possess one pair of opisthonotal glands as the sole source of pheromones. Whereas insects have a different gland for each pheromone, astigmatic mites can communicate by three types of pheromones (alarm, aggregation, and female sex pheromones) that are produced by a single gland. The opening of the gland possesses a trap-door structure, and mites seem to be capable of controlling the volume of secretion from this gland (Howard et al., 1988) (Fig. 1).

Among the 61 species of Astigmata examined to date, alarm pheromones are known from 20 species as a major or a minor component of the opisthonotal gland (Kuwahara, 2004). Moreover, female sex pheromones are known from 16 species (as a major component with a convex dose-response relationship), and aggregation pheromones from five species (as a major or a minor component, with sigmoid or convex dose-response relationships) (Kuwahara, 2004 and recent data). Moreover, 10 of the examined species achieve two of the three communication functions, either via one active compound with two functions, or via two active compounds each with a different function (Kuwahara, 2004, and recent data). The mite species, their pheromone compounds (and other gland components), and each communication function are summarized in Table 1. A total of 91 compounds, including various pheromone components, have been obtained from 61 species (Fig. 2).

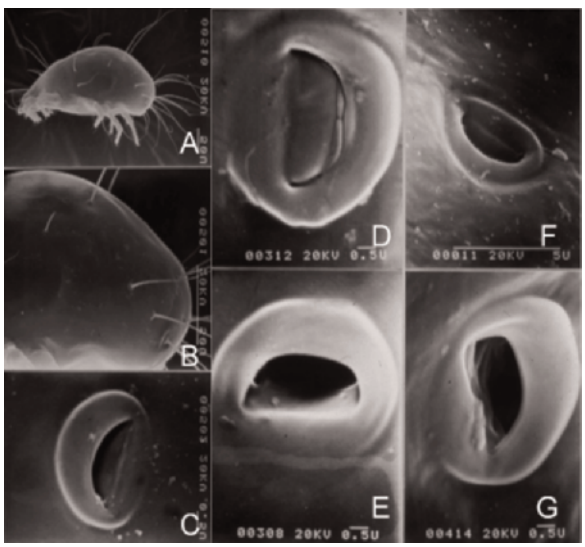

Figure 1 Opening of the opisthonotal gland (scanning electron micrograph). A-C: *Tyrophagus putrescentiae*, D-G: *Aleuroglyphus ovatus*.

This review shows that astigmatic mites can achieve two of three communication functions by one or two compounds released from opisthonotal glands. In addition, a new recent finding is presented showing that the opisthonotal glands of *Rhizoglyphus setosus*, known to release the alarm pheromone neryl formate (Akiyama et al., 1997) and the sex pheromone S-(+)-isorobinal (Mizoguchi et al., 2005), also produces a third, i.e., aggregation, pheromone.

GENERAL ASPECTS OF COMMUNICATION PHEROMONES IN ASTIGMATA

Alarm pheromones of Astigmata are mite-produced chemicals, that are active at a dose equivalent to a single-mite extract (or squash by a needle) in a rearing container, and that elicit alarm behavior (Kuwahara et al., 1980). Compounds eliciting alarm behaviors, such as geranial, neral, α-acaridial, and hydrocarbons, represent a major component in the gland, but other compounds are minor components, such as neryl formate in all species and dehydrogeranial (one example, Hiraoka et al., 2003). Most of these alarm pheromones generate a sigmoid dose-response relationship, except

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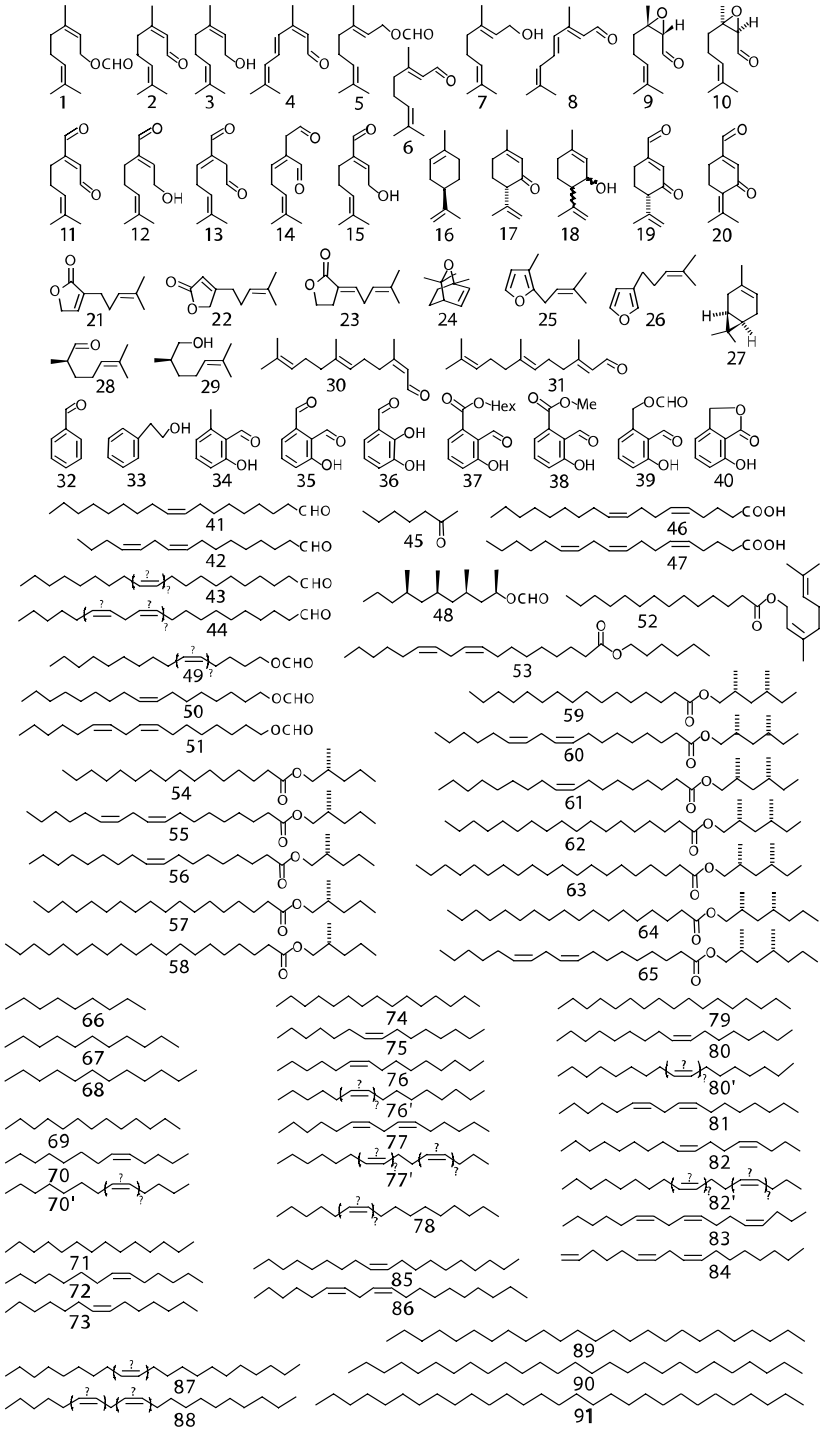

Figure 2 Compounds identified among 61 species of Astigmata: 1, Neryl formate [3,7-Dimethyl-Z-2,6-octadienyl formate]; 2, Neral [3,7-Dimethyl-Z-2,6-octadienal]; 3, Nerol [3,7-Dimethyl-Z-2,6-octadienol]; 4, Dehydroneral [3,7-Dimethyl-(2Z,4E)-2,4,6,-octatrienal]; 5, Geranyl formate [3,7-Dimethyl-E-2,6-octadienyl formate]; 6, Geranial [3,7-Dimethyl-E-2,6-octadienal]; 7, Geraniol [3,7-Dimethyl-E-2,6-octadienol]; 8, Dehydrogeranial [3,7-Dimethyl-(2E,4E)-2,4,6,-octatrienal]; 9, 2S,3S-Epoxyneral [2S,3S-Epoxy-3,7-dimethyl-6-octenal]; 10, 2R,3R-Epoxyneral [2R,3R-Epoxy-3,7-dimethyl-6-octenal]; 11, α-Acaridial [2(E)-(4-Methyl-3-pentyl)-butenedial]; 12, α-Acariolal [2(E)-(4-Methyl-3-pentenyl)-4-hydroxybutenal]; 13, β(E)-Acaridial [2(E)-(4-Methyl-3-pentenylidene)-butanedial]; 14, β(Z)-Acaridial [2(Z)-(4-Methyl-3-pentenylidene)-butanedial]; 15, [2(E)-4-Methyl-3-pentenylidene]-4-hydroxybutanal]; 16, Limonene; 17, S(+)-Isopiperitenone [3-Methyl-6-(S)-isopropenyl-2-cyclohexen-1-one]; 18, Isopiperitenol [3-Methyl-6-isopropenyl-2-cyclohexen-1-ol]; 19, S(+)-Isorobinal [S(+)-4-Isopropenyl-3-oxo-1-cyclohexene-1-carbaldehyde]; 20, Robinal [3-Oxo-4-isopropylidene-1-cyclohexene-1-carbaldehyde]; 21, α,α-Acariolide [3-(4'-methyl-3'-pentenyl)-2(5H)-furanone]; 22, α,β-Acariolide [4-(4'-Methyl-3'-pentenyl)-2(5H)-furanone]; 23, β-Acariolide [(E)-2-(4'-Methyl-3'-pentenylidene)-4-butanolide]; 24, Dehydrocineole; 25, Rosefuran [3-Methyl-2-(3-methyl-2-butenyl)-furan]; 26, Perillene [3-(4-methyl-3-pentenyl)-furan]; 27, 3-Carene; 28, 2,6-Dimethyl-5-heptenal; 29, 2,6-Dimethyl-5-hepten-1-ol; 30, Z,E-Farnesal; 31, E,E-Farnesal; 32, Benzaldehyde; 33, β-phenylethanol; 34, 2-Hydroxy-6-methylbenzaldehyde; 35, 3-Hydroxy-1,2-dicarbaldehyde; 36, 2,3-dihydroxybenzaldehyde; 37, Hexyl rhizoglyphinate [Hexyl 2-formyl-3-hydroxybenzoate]; 38, Methyl rhizoglyphinate [Methyl 2-formyl-3-hydroxybenzoate]; 39, Rhizoglyphinyl formate [2-Formyl-3-hydroxybenzyl formate]; 40, 7-Hydroxyphthalide (7-Hydroxy Isobenzofuranone); 41, Octadecenal; 42, Octadecadienal; 43, Eicosenal; 44, Eicosadienal; 45, 2-Heptanone; 46, Z,Z-5,9-Octadecadienoic acid; 47, Z,Z,Z-5,9,12-Octadecatrienoic acid; 48, R,R,R,R-Lardolure [1R,3R,5R,7R-1,3,5,7-Tetramethyldecyl formate]; 49, Pentadecenyl formate; 50, Z-8-Heptadecenyl formate; 51, Z,Z-8,11-Heptadecadienyl formate; 52, Neryl myristate; 53, Hexyl linolate; 54, (S)-2-Methylpentyl palmitate; 55, (S)-2-Methylpentyl linolanate; 56, (S)-2-Methylpentyl linolate; 57, (S)-2-Methylpentyl palmitate; 58, (S)-2-Methylpentyl stearate; 59, 2S,4S-2,4-Dimethylhexyl palmitate; 60, 2S,4S-2,4-Dimethylhexyl linolanate; 61, 2S,4S-2,4-Dimethylhexyl oleate; 62, 2S,4S-2,4-Dimethylhexyl stearate; 63, 2S,4S-2,4-Dimethylhexyl arachidinate; 64, 2S,4S-2,4-Dimethylheptyl stearate; 65, 2S,4S-2,4-Dimethylheptyl linolate; 66, Nonane; 67, Undecane; 68, Dodecane; 69, Tridecane; 70, Z-5-Tridecene; 70', Tridecene; 71, Tetradecane; 72, Z-6-Tetradecene; 73, Z-7-Tetradecene; 74, Pentadecane; 75, Z-6-Pentadecene; 76, Z-7-Pentadecene; 77, Z,Z-6,9-Pentadecadiene; 77', Pentadecadiene; 78, Hexadecene; 79, Heptadecane; 80, Z-8-Heptadecene; 80', Heptadecene; 81, Z,Z-6,9-Heptadecadiene; 82, Z,Z-4,8-Heptadecadiene; 82', Heptadecadiene; 83, Z,Z,Z-4,8,11-Heptadecatriene; 84, Z,Z-1,6,9-Heptadecatriene; 85, Z-9-Nonadecene; 86, Z,Z-6,9-Nonadecadiene; 87, Heneicosene; 88, Heneicosadiene; 89, Pentacosane; 90, Heptacosane; 91, Nonacosane.

Table 1 Astigmatic species with more than one pheromone known. Compound numbers correspond to structures shown in Figure 1. References indicate studies where biological activity (function) has been tested in bioassays.

Superfamily	Family	Species	Pheromones and compounds detected in extracts	References
Pyroglyphoidea	Pyroglyphidae	*Dermatophagoides farinae*	74, 2, 1, 2-Hydroxy-6-methylbenzaldehyde (34, female sex)*, 35, 6, 69, 71, 75	*Tatami et al., 2001
		D. pteronyssinus	Geranial (6, aggregation)*, 1, 81, 80, 35, 5, 39, 74, 2, 79, 75	*Unpubl.
Histiostomatoidea	Histiostomatidae	*Histiostoma laboratorium*	Geranial (alarm)*, 80', 76', 82', 74, 69, 2	*Kuwahara et al., 1991
Hemisarcoptoidea	Carpoglyphidae	*Carpoglyphus lactis*	69, Neral (2, alarm)*, 81, 35, 6, 40, 74, 76, 91, 90, 89	*Kuwahara et al., 1980
	Winterschmidtiidae	*Oulenzia* sp.	69, Neral (2, alarm)*, 68, 74, 6, 76', 35, 40, 3	*Shimizu et al., 2004
Glycyphagoidea	Glycyphagidae	*Glycyphagus domesticus*	69, Neral (2, alarm)*, 1, 35, 6, 89, 90, 91	*Kuwahara et al., 1991a
Acaroidea	Suidasiidae	*Suidasia medanensis*	86, Neral (2, alarm)*, 85, 35, 30, 6, 31, 1, 88, 87, 39	*Leal et al., 1989a
		Tortonia sp.	Z,Z-6,9-Heptadecadiene (81, alarm)*, 82, 80, 2, 1, 83, 75, 6, 35, 69, 46, 47	*Kuwahara et al., 1995
	Lardoglyphidae	*Lardoglyphus konoi*	Neral (2, alarm)*, 69, 6, *R*,*R*,*R*,*R*-Lardolure (48, aggregation)†	*†Kuwahara et al., 1980, †1982; †My-Yen et al., 1980a; †Mori & Kuwahara, 1986a,b
	Acaridae, Acarinae	*Aleuroglyphus ovatus*	2-hydroxy-6-methyl benzaldehyde (34, female sex)*, 52, 69, 13, 2, 35, 9, 6	*Kuwahara et al., 1992
		Acarus immobilis	69, 2-hydroxy-6-methyl benzaldehyde (34, female sex)*, 25, 35, Hydrocarbon mix. (71, 74, 79-81, 89-91, male sex)*	*Sato et al., 1993
		Tyrophagus putrescentiae	β-Acaridial (13, female sex)†, 76, 75, 69, 53, 74, 77, 72, 73, Neryl formate (1, alarm)*, 25, 70, 71, Neral (2, alarm)*, 34, 22, 11	*Kuwahara et al., 1975, 1979; *My-Yen,1980b; †Maruno et al., 2006
		T. neiswanderi	Hydrocarbon mix. (76, 75, 70, 72, 73, alarm)*, 13, 69, 25, 77, 74, 53, 26, 11, 1, 35, 71	*Kuwahara et al., 1989
		T. similis	*S*(+)-Isopiperitenone (17, alarm* & female sex†), 69, 53, 2, 35, 71, 20, 19, 6, 68	*Kuwahara et al., 1987; †Unpubl.
		T. perniciosus	69, 2-hydroxy-6-methyl benzaldehyde (34, alarm* & female sex†), 9, 53, 11, 1, 2, 13, 6	*Leal et al., 1988; †Unpubl.
		T. longior	69, β-Acaridial (13, alarm)*, 53, 11, 76', 71, 68	*Noguchi et al., 1998
		Tyroborus lini	76', 17, 53, 69, Neryl formate (1, alarm)*, 2, 74, 19, 77', 20	*Tomita et al., 2003
		Histiogaster sp.	Neral (2, female sex)*, 13, 69, 6, 55, 3, 11	*Hiraoka et al., 2002
		H. sp. 'A096'	69, 32, 45, 35, 4, Dehydrogeranial (8, alarm)*, 25	*Hiraoka et al., 2003
		H. rotundus	82', 69, 39, Neryl formate (1, alarm)*, 11, 13, 2, 25, 80', 74, 3, 26, 71, 40, 6	*Hiraoka et al., 2003a
		Rhizoglyphus robini	69, α-Acaridial (11, female sex)†, Neryl formate (1, alarm)*, 13, 20, 37, 35, 19, 2(alarm)‡, 25	*Kuwahara et al., 1988; ‡Baker & Krantz, 1984; †Mizoguchi et al., 2003
		R. setosus	11, 69, 35, S-(+)-Isorobinal (19, female sex) †, 13, 20, Neryl formate (1, alarm* & aggregation‡)	*Akiyama et al., 1997; †Mizoguchi et al., 2005; ‡Unpubl.
		R. sp. 'oki'	44, 69, 11, Neryl formate (1, alarm)*, 35, 43, 19, 20, 13, 35, 6, 7, 3, 21, 22, 26	*Akiyama et al., 1997
		R. sp. 'mori'	3-hydroxybenzene-1,2-dicarbaldehyde (γ-Acaridial) (35, female sex)†, 19, 13, 37, Neral (2, alarm)*, 25, 20, 26	*Akiyama et al., 1997; †Murakami et al., 2006

Table 1 Continued

Superfamily	Family	Species	Pheromones and compounds detected in extracts	References
[Pyroglyphoidea	Acaridae, Acarinae]	*Schwiebea elongata*	69, 1, Neral (2, aggregation† & alarm*), 35, 6, 34	*Kuwahara et al., 2001; †Nishimura et al., 2002
		S. similis	69, α-Acaridial (11, female sex)*, 1, 35, 13, 2, 40	*Nishimura et al., 2004
		S. sp. 'okabe'	11, 69, 1, 35, 2, 13, 40, 6, 34, 26	
		S. sp. 'chiba'	80', Neryl formate (1, male sex)*, 35, 82', 16, 6, 79	*Unpubl.
		Cosmoglyphus hughesi	2-hydroxy-6-methyl benzaldehyde (34, female sex)†, 69, 25, 35, 40	†Ryono et al., 2001
		Caloglyphus rodriguezi	34, Undecane (67, female sex)*, 69, 24, 35	*Mori et al., 1995
		C. polyphyllae	81, 80, β-Acaridial (13 female sex*, aggregation†), 84, 76', 11, 12, 25, 69, 74, 79, 15	*Leal et al., 1989; †Shimizu et al., 2001
		C. sp. 'MJ'	81, 80, (2R,3R)-epoxyneral (10, female sex)*, 13, 79, 11, 76'	*Mori et al., 1996
		C. sp. 'sasagawa'	80, 50, 51, Rosefuran (25, female sex)*, 38, 81, 66, 35, 76', 49, β-Phenylethanol (33, aggregation)†	*Unpubl.; †Kuwahara, 1990
		C. sp. 'HP'	Rosefuran (25, female sex)*, 56, 57, 80', 35, 24	*Mori et al., 1998

Compounds are listed in decreasing order as determined by gas chromatography (RIC traces), obtained with an HP 5989B mass spectrometer coupled to an HP 5890 series II+ gas chromatograph, using an HP-5 capillary column (0.39 mm × 30 m, 0.33 μm in film thickness), with a temperature program of 60 °C/2 min, 10 °C/min to 290 °C, hold for 5 min, using He carrier gas at a flow rate of 1.23 ml/min. Unidentified species are listed by the genus name with 'isolate names', if neccesary.

for neral in *Schwiebea elongata* that elicits a convex dose-response relationship (Nishimura et al., 2002).

Female sex pheromones of Astigmata elicit a male response at relatively high concentration. Different from Insecta, the active compound is contained not only in females but also in males and even in nymphal stages. Females, however, tend to contain a several times larger amount than males, and only males respond to the compounds when provided at sufficiently high concentration (response only at a dose of 1-10 ng). In the early stages of my research program, this pheromone was exclusively obtained from species that had no alarm pheromone. In the case of alarm-pheromone-emitting mites, crude extracts are usually inactive in the sex-pheromone bioassay. After removal of the alarm pheromone by column chromatography, the sex-pheromone activity in the extract becomes detectable. Most female sex pheromones, such as 2-hydroxy-6-methylbenzaldehyde (2,6-HMB), neral, α-acaridial, undecane, (2R,3R)-epoxyneral, and rosefuran, are present as one of the major components in the opstihonotal gland, but S-(+)-isorobinal of *R. setosus* and 3-hydroxybenzene-1,2-dicarbaldehyde (γ-acaridial) of *Rhizoglyphus* sp. 'mori' are the fourth major component out of 7 or 11 components, respectively. In all cases, their dose-response relationships are convex. Apart from the female sex pheromone, male sex pheromone is known, but only in two *Acarus* species (Levinson et al., 1989; Sato et al., 1993) and one *Schwiebea* sp.

Aggregation pheromones are known to be emitted by five species only, including *R. setosus*. Lardolure (Mori & Kuwahara, 1986a,b), neral, neryl formate, β-acaridial, and 2-phenylethanol are the active components. These can be major or minor components, and their dose-response relationships vary from sigmoid to convex. Response to these pheromones is usually within several minutes (which is relatively fast). It should be noted that Astigmata are known to be gregarious under natural conditions (Reka et al, 1992; Levinson et al., 1991). Therefore, many more species may possess an aggregation pheromone. Perhaps, the response time in other mite species is much greater (e.g., due to learning and conditioning), which may cause the bioassay (designed to detect short-time responses) to be insufficient. For example, in the case of *R. setosus*, it takes 1 h to obtain assay results (Fig. 3), whereas the active compounds are volatile and thus reach the mites in a very short time. The conventional assay method should thus be modified to allow mites to adjust their behavior on a longer time scale. This may explain why there are not many examples of aggregation pheromones despite the gregariousness of the Astigmata. A device should be developed to assay mites by exposing them to a very low concentration of the pheromone vapor but for a longer period.

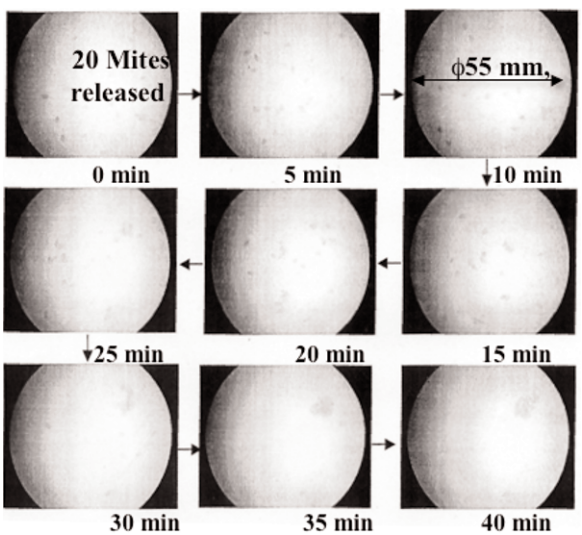

Figure 3 Aggregation behavior of *Rhizoglyphus setosus.*

ONE ACTIVE COMPOUND WITH TWO COMMUNICATION FUNCTIONS

Sex-aggregation pheromone combination

β-Acaridial is known as sex pheromone in *Caloglyphus polyphyllae*, active at 1-10 ng. It has a convex dose-response relationship (Leal et al., 1989). On the other hand the species indicates clear aggregation behavior when mites are transferred to a new rearing medium or a clean container without food. The active compound triggering aggregation is determined to be the same compound β-acaridial. Not only females but also males and nymphal stages respond to this compound at a similar dose of 1-10 ng and also this dose-response relationship is convex (Shimizu et al., 2001). The only difference with the sex-pheromone bioassay is that the arena to test for aggregation is not conditioned. Thus, the sex pheromone elicits an effect under a conditioned situation, whereas the aggregation pheromone does this in absence of conditioning.

Aggregation-alarm pheromone combination

The parthenogenetic species *S. elongata* exhibits an evasive reaction to neral, and neral is known as an alarm pheromone of this species (Kuwahara et al., 2001). Using a disposable pipette to offer the compound, neral apeared to be active at a dose of 30 ng and the dose-response relationship was convex. However, if neral was offered at a dose range of 1-3 ng, the same compound acted as an attractant and gave rise to a dose response relationship that was also of the convex type (Nishimura et al., 2002). This attraction is interpreted as being triggered by an aggregation pheromone. Sexual attraction can be excluded because there were no males present in the test

Alarm-sex pheromone combination

S-(+)-Isopiperitenone in *Tyrophagus similis* (Kuwahara et al., 1987) and 2,6-HMB in *T. perniciosus* (Leal et al., 1988) are among the major components in these two species and have been shown to act as alarm pheromones active at a dose of 100 ng, with sigmoid dose-response relationships. When testing for a role in sexual attraction, each of the two compounds acted as a sex pheromone at a dose of 10 ng with a convex dose-response relationship. Thus, a small discharge of secretion from the gland may function as a sex pheromone under undisturbed conditions, whereas a large discharge upon disturbance of the mite triggers alarm behavior in each of the two species.

TWO COMPOUNDS EACH WITH A DIFFERENT COMMUNICATION FUNCTION

Combination of a sex pheromone and an aggregation pheromone

Rosefuran is a major volatile component in the gland of an unidentified *Caloglyphus* sp., and it functions as a sex pheromone with a convex dose-response relationship. However, the species also clearly displays aggregation behavior, when a group of mites are introduced to an unconditioned container. The active compound 2-phenylethanol acts as an aggregation pheromone. It is a minor component in the gland (Kuwahara, 1990), and possibly the dose-response relationship is sigmoid.

Combination of an aggregation pheromone and an alarm pheromone

When the soma of *Lardoglyphus konoi* is squashed, mites nearby escape in response to the alarm pheromone neral (one of the major volatile components in the gland), whereas mites come together again after the alarm-activity has faded out (Kuwahara et al., 1980). Observation of this behavior has led to the discovery of lardolure as an aggregation pheromone. Lardolure is a minor component in the opisthonotal glands, and it is less volatile (hence, more durable) than neral. Dose-response curves of both compounds are sigmoid.

Combination of an alarm pheromone and a sex pheromone

To date, the following four species possess neryl formate as one of the minor components in the opisthonotal gland secretion: *Rhizoglyphus robini, R. setosus, Rhizoglyphus* sp. 'mori', and *T. putrescentiae*. The alarm pheromone activity is manifest in each species at 10, 100, 100 and 10-100 ng, respectively, and the dose-response relationship is sigmoid. (though the gland secretion content seems to be around 10 ng). The presence of a sex pheromone in the opisthonotal gland has also been shown, but this required first the removal of alarm pheromones from each solvent extract by SiO_2 column chromatography. α-Acaridial, S-(+)-isorobinal, γ-acaridial, and β-acaridial have been identified as the sex pheromones of these four species, respectively. Their dose-response relationships were convex, for α-acaridial around 10 ng, for S-(+)-isorobinal around 1 ng, for γ-acaridial in the range of 1-10 ng, and for β-acaridial in the range of 1-10 ng.

In the case of *R. robini* (Mizoguchi et al., 2003), ca. 10 ng of neryl formate is present in both sexes, whereas the female contains 388 ng of α-acaridial (thus, a ca. 40× smaller amount is required to trigger its communication function). The following scenarios are now considered: when a small discharge of secretion takes place under undisturbed conditions, the secretion comprises a mixure of 10 ng α-acaridial and 0.25 ng neryl formate, and acts as a sex pheromone. When, however, a large discharge of secretion is produced upon disturbance, the dose of the sex pheromone secreted is so high that it loses its activity in sexual attraction, and then only neryl formate at a dose of 10 ng may trigger evasive behavior of mites. The same interpretation may apply to the other three species. Why and how the sex pheromone at high doses loses its biological activity remains to be elucidated.

Test to detect all three types of pheromones in a single species

Rhizoglyphus setosus is known to have two different pheromones, one acting as an alarm and another acting in sexual attraction. Hence, this species was chosen to test for the presence of an aggregation pheromone. To avoid contamination with other pheromones released by the mite, the sample to be tested was applied in a circle (4 mm diameter) and covered by a glass cover. The sample was exposed to test mites at a distance of 3 mm (Fig. 4); aggregation occurs >30 min after introduction of test mites in the arena (55 mm diameter) (Fig. 3). Using this bioassay method, neryl formate 'contaminated with a keeper (diethylhexyl phthalate)' appeared to elicit aggregation in the mite. This is a serendipitous result, because a direct smear of pure neryl formate does not stay very long on the glass surface and the contam-

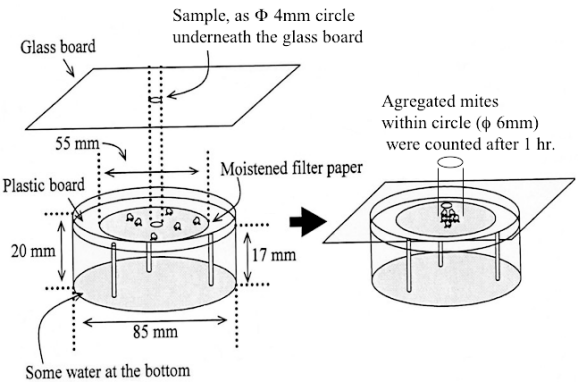

Figure 4 Bioassay for a pheromone eliciting aggregation.

ination with diethylhexyl phthalate caused slow release of neryl formate. Hence, to identify the aggregation pheromone in other species, it seems advisable to develop an experimental design in which the active compound is volatilized continuously at a low rate. Such a slow release of an otherwise volatile compound may be achieved by the mite by controlling the opening and closing of the gland orifice (Fig. 1).

CONCLUSION

Astigmata, using a single secretory gland, have developed a pheromone system enabling three communication functions: alarm, sex, and aggregation. These three functions emerge from (1) the conditions the mites are facing, (2) functional characteristics such as dose-response relationships and active dose-range, and (3) the relative abundance of the active compounds in the gland. How these three functions, sometimes mediated by a single gland component, are regulated by the mites is as yet unclear, and remains a fascinating area for future research.

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The role of infochemicals in the interaction between cassava green mite and its fungal pathogen *Neozygites tanajoae*

F.C.C. Hountondji[1,2,3], M.W. Sabelis[2] & R. Hanna[1]

[1]International Institute of Tropical Agriculture, 08 BP 0932, Cotonou, Republic of Benin, [2]Institute for Biodiversity and Ecosystem Dynamics, Population Biology Section, University of Amsterdam, 94084, 1090 GB Amsterdam, The Netherlands, and [3]Expertise, Action and Research for Development (EAR-Development), PO Box 2015 Abomey-Calavi, Republic of Benin. E-mail: fabienho@yahoo.com

The role of infochemicals in mediating interactions between herbivores and their foraging natural enemies, mainly predators and parasitoids, is well established, but very little is known about infochemical use in interactions between herbivores and their sit-and-wait pathogens. This paper reviews the role of infochemicals in interactions between the cassava green mite (CGM), *Mononychellus tanajoa*, and its fungal pathogen, *Neozygites tanajoae*. In a closed-dish test, herbivore-induced plant volatiles (HIPV) from cassava were found to influence conidia and capilliconida production of the fungus but the effect of HIPV varied between isolates. HIPV consistently promoted conidia production of one isolate and capilliconidia production of another. Olfactory trials with one of the HIPV, methyl salicylate (MeSA), also promoted conidia production of the same isolate, but no effect was found on capilliconidia production. In contrast to the effect of HIPV, green leaf volatiles inhibited spore production, suggesting that the fungus uses HIPV to signal the presence of hosts. The behaviour of the mite towards infective spores was investigated in a two-choice unit (discs with vs. without spores) and on detached leaves. Mites avoided the discs with spores, in particular for one isolate. Similar observations were made on detached leaves where more mites were found on leaf lobes without spores than on those with spores. However, mites did not avoid mummified infected mites that did not yet produce spores, suggesting that the fungus may profit from going unnoticed inside the live infected mite to reach densely infested patches.

Key words: Acaropathogen, avoidance, green leaf volatiles, herbivore-induced plant volatiles, methyl salycilate, *Mononychellus tanajoa*

Much evidence has been established regarding the role of infochemicals in mediating interactions between herbivores and their predators or parasitoids. These interactions can be mediated by volatiles emitted by the host plant as well as by cues produced by the herbivore and its natural enemies. Since the suggestion by Price et al. (1980) that plants can influence the third trophic level, many studies have been conducted on interactions within trophic systems involving predators and parasitoids in the laboratory and in the greenhouse. These studies have shown that herbivore-induced plant volatiles (HIPV) play a role in the attraction of predators and parasitoids to plants under attack by herbivores (Dicke & Sabelis, 1988; Dicke et al., 1990; Turlings et al., 1990; Gnanvossou et al., 2001; James, 2005).

Apart from volatiles emitted by plants, cues released by herbivores or their natural enemies may also influence the behaviour of the natural enemy or the herbivorous victim. Evidence has been established where prey and host hide and escape from their predators and parasitoids (e.g., Janssen et al., 1998; Pallini et al., 1999; Magalhães et al., 2002; Grostal & Dicke, 2000). It is also evidenced that predators or parasitoids can be influenced by cues of their prey/host or by their conspecific or heterospecifics (Janssen et al., 1997, 1998; Outreman et al. 2001; Nakeshima et al., 2004)

Although the use of infochemicals is well established for predators and parasitoids, very little is known about the use of infochemicals by arthropod pathogens, particularly fungi. Earlier reports include the observation of avoidance of *Beauveria bassiana*-infected conspecifics by the ant *Solenopsis invicta* (see review by Oi and Perera, 1993) and studies by Brown et al. (1995) on the aphid pathogen *Pandora neoaphidis* and Klingen et al. (2002) on the common arthropod pathogens *Metarhizium anisopliae* and *Tolypocladium cylindrosporium*. Brown et al. (1995) reported delay in germination of conidia of *P. neoaphidis* until after the pathogen comes into contact with its aphid host, whereas Klingen et al. (2002) reported an in-vitro inhibitory effect of isothyocyanates on *M. anisopliae* and *T. cylindrosporium*. Only recently, specific studies on the effect of HIPV on entomopathogenic fungi have been conducted with the entomophthorales *Neozygites tanajoae* (Hountondji et al., 2005) and *P. Neoaphidis* (Baverstock et al., 2005). Moreover, a series of interactions studies have been conducted on the *N. tanajoae* system which involves the cassava green mite (CGM), *Mononychellus tanajoa* (Bondar), a pest of cassava.

Neozygites tanajoae is pathogenic to CGM (Delalibera & Hajek, 2004; Delalibera et al., 2004), which is a major pest of cassava, a staple food crop in Africa (Yaninek & Herren, 1988). *Neozygites tanajoae* causes severe epizootics in Northeastern Brazil (Delalibera et al., 1992), whereas in Africa very low infections have been reported (Yaninek et al., 1996; Dara et al., 2001). On the cassava leaf the acaropathogenic fungus completes its cycle by attaching to mobile CGM stages by means of spores produced by sporulating infected mite cadavers. The attached spores germinate, invade the body of the mite, and cause its death within ca. 3-4 days at 28 °C (Oduor et al., 1995). Infected mites dry out and become mummified after death. These so-called mummies sporulate and spread spores over the leaf when relative humidity is near saturation and temperature around 18-23 °C, in the dark (Oduor et al., 1996).

In this paper we present the results of studies conducted on the *N. tanajoae-M. tanajoa*-cassava system towards understanding the role infochemicals can play in the interactions within such a system. In particular, the effect of plant volatiles on the development of the acaropathogen and the role of cues from the acaropathogen and from the mite are investigated.

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MATERIALS AND METHODS

Two main studies were conducted from 2002-2006 to understand the role of infochemicals in mediating interactions within the *N. tanajoae-M. tanajoa*-cassava system. One study evaluated the effect of green leaf volatiles (GLV) and HIPV on the development of the acaropathogen. A second study assessed the behaviour of the herbivorous mite (avoidance) imposed by cues released by *N. tanajoae* associated or not with its host.

Two isolates of *N. tanajoae* were used in the experiments. One Brazilian isolate collected from Colas das Almas in the state of Bahia in 1995 (Colal.brz), and one Beninese isolate collected from Cotonou in 1997 (Coton.ben). The isolates were maintained at 4 °C inside tightly closed photographic film canisters on top of dry cotton wool with glycerol at the bottom to keep humidity low. The stored specimens were renewed at approximately 6-month intervals through host-to-host multiplication to minimize loss of viability.

Effect of plant volatiles on *Neozygites tanajoae*

In this study, the influence of cassava GLV and *M. tanajoa*-induced cassava volatiles (HIPV) on *N. tanajaoe* was evaluated. As described above, *N. tanajoae* displays a sit-and-wait strategy to infect its host. Therefore, unlike predators and parasitoids that are shown to respond behaviourally to HIPV, we hypothesized that HIPV affect spore production. Two types of experiments were conducted. One experiment was carried out in a closed-dish environment in which CGM mummies infected by *N. tanajoae* were placed for sporulation, either exposed to GLV from clean, excised leaf discs, or to HIPV from excised leaf discs fed upon by CGM, or to dead *N. tanajoae*-infected mites or to clean air (control). The second experiment consisted of an airflow experiment where mummies were allowed to sporulate under HIPV environment provided by highly infested cassava leaves against clean air. For details about the design of these two experiments, see Hountondji et al. (2005).

Following a gas chromatography-mass spectrometry (GC-MS) analysis conducted to identify volatiles produced by cassava following herbivory by *M. tanajoa*, a few volatiles were identified as HIPV, including methyl salicylate (MeSA), a compound known to attract predators and parasitoids in many herbivorous arthropod-plant systems. As part of the HIPV tests, the effect of synthetic MeSA on the sporulation of *N. tanajoae* was also tested in an additional experiment. Mummified mites infected by *N. tanajoae* were allowed to sporulate in a closed plastic box in an environment with or without MeSA (Hountondji et al., 2006).

Avoidance study

To test whether CGM can avoid contact with the fungal spores, its behaviour in the presence of spores of *N. tanajoae* was assessed. In a two-choice unit female spider mites are allowed to make a choice between two opposite leaf discs, one with spores of *N. tanajoae* and the other without spores. The leaf discs were placed on top of moist cotton to prevent escape of CGM, and connected by a thin wooden bridge on the middle of which were placed the test mites. After 24 h the position of the mites was scored. Spores were obtained by incubating mummified mites infected by *N. tanajoae* for sporulation on the leaf disc to be treated. Since mummified infected mites may not sporulate immediately after formation depending on the prevailing conditions, we also tested the behaviour of the mite towards the fungus inside the mite using the same unit.

To mimic realistic conditions, the behaviour of the mite towards spores of *N. tanajoae* was also tested on cassava leaves. The leaves were placed upside down over an arena of moist cotton pad to prevent *M. tanajoa* from escaping. Test mites were placed on top of the petiole (partially cut off to allow only ca. 3 cm walk to the leaf blade) from where they are allowed to choose between lobes with and without spores in alternate positions.

Statistical analysis

Analyses of variance (ANOVA) were conducted using SPSS 12.0 for Windows to analyze spore production in the closed-dish experiment and to study preference of CGM between treated and control lobes of cassava leaves in the avoidance study. The Student t-test was used to compare spore production between treatment and control in the airflow trials and to analyze CGM preference between leaf discs with and without spores in the avoidance test.

RESULTS

GLV and HIPV studies

The results of the experiment conducted on leaf discs showed that odours can influence conidiation of the acaropathogen, and that the effect of these odours depends on pathogen isolate. There was a significant difference between the odour treatments for the isolate Colal.brz (F_3 = 6.80, P = 0.001), whereas no consistent difference was observed for the isolate Coton.ben (F_3 = 0.20, P = 0.89). Mean separation using Tukey's Studentized range test at the 5% level showed that conidia production by Colal.brz under clean air and environ-

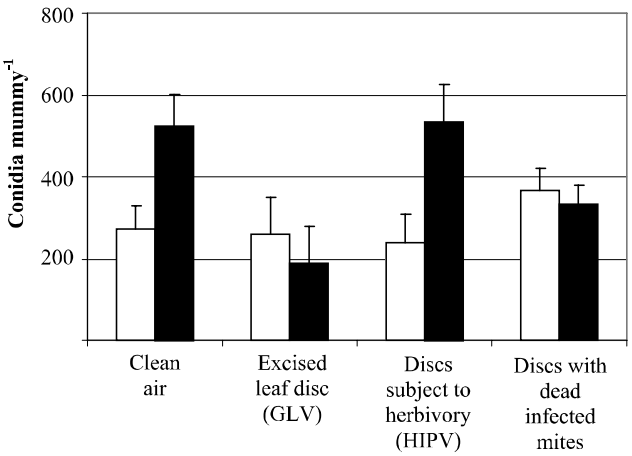

Figure 1 Conidia production of a Beninese isolate (Coton.ben; white bars) and a Brazilian isolate (Colal.brz; black bars) of *Neozygites tanajoae* in closed dish environment in the presence of green leaf volatiles (GLV), green mite-induced cassava volatiles and infected mites against the control (clean air).

ment with leaf discs fed upon by CGM was higher than in an environment with odours from a clean excised leaf disc (Fig. 1). No consistent difference was found between an environment with infected CGM on the leaf discs and any of the other environments (clean air, GLV, HIPV). Also, no effect of the odours was observed on the production of capilliconidia for any of the isolates (P>0.05; results not shown).

The airflow experiment, where cues from cassava leaves highly infested by CGM were compared with clean air, also demonstrated that volatiles have an effect on sporulation of *N. tanajoae* and that the effect varied with isolate (Fig. 2). For Coton.ben, in presence of HIPV more conidia were made than under clean air (251.0±33.6 vs. 183.8±28.7 conidia/mummy, respectively; P = 0.07). No consistent difference was found for isolate Colal.brz, although more conidia were produced in presence of HIPV than in clean air (404.7±66.2 vs. 354.0±48.9 conidia/mummy; P = 0.27). The effect of volatiles was also seen for capilliconidia production, but only for Colal.brz. The proportion of capilliconidia formed from conidia was significantly higher with HIPV than with clean air for Colal.brz (55.9±8.9 vs. 31.9±6.9 conidia/mummy; P = 0.02), but they were similar for Coton.ben (58.1±8.2 vs. 65.2±5.8 conidia/mummy; P = 0.27).

Similar findings were obtained for conidia production when MeSA was tested (Fig. 3). Higher conidia production was observed in the presence of MeSA than under clean air for Coton.ben (265.2±43.5 vs. 184.1±36.3 conidia/ mummy, respectively; P = 0.08) and no consistent difference for Colal.brz (360.5±46.0 vs. 384.6±50.2 conidia/mummy; P = 0.36). In contrast, no effect was observed in capilliconidia production for any of the isolates.

Avoidance study

Evidence for *M. tanajoa* avoiding the acaropathogen was obtained but this depended on the pathogen state and the isolate (Fig. 4). When the pathogen was outside the mite as spores, avoidance was observed for both isolates, with a stronger avoidance for the Colal.brz isolate (P = 0.009) compared with the Coton.ben isolate (P = 0.09). Fewer mites

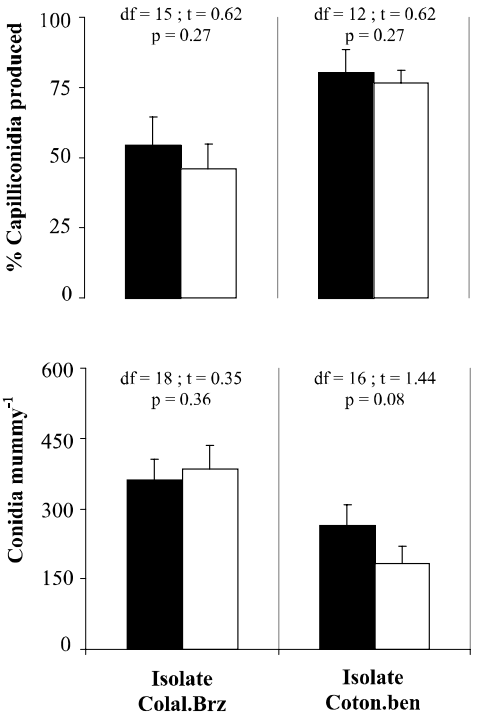

Figure 3 Conidia and capilliconidia production of a Beninese isolate (Coton.ben) and a Brazilian isolate (Colal.brz) of *Neozygites tanajoae* in an environment with (black bars) and without methyl salycilate (MeSA; white bars).

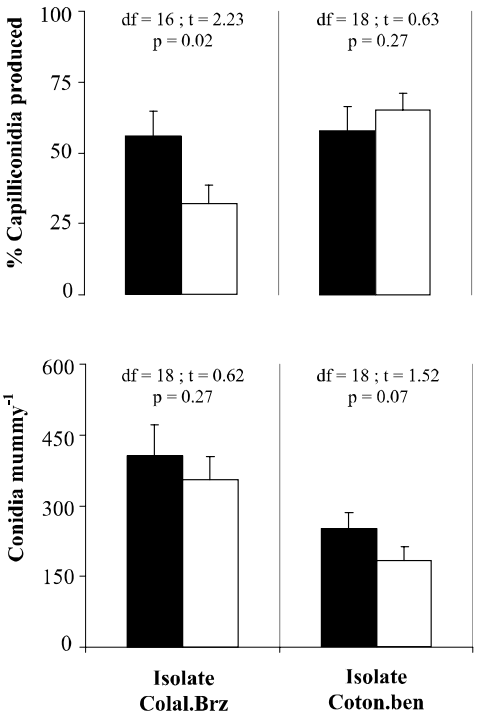

Figure 2 Conidia production and capilliconidia production of a Beninese isolate (Coton.ben) and a Brazilian isolate (Colal.brz) of *Neozygites tanajoae* in a two-arm airflow experiment with cassava leaves infested by the green mite *Mononychellus tanajoa* one side (black bars) and clean air the other side (white bars).

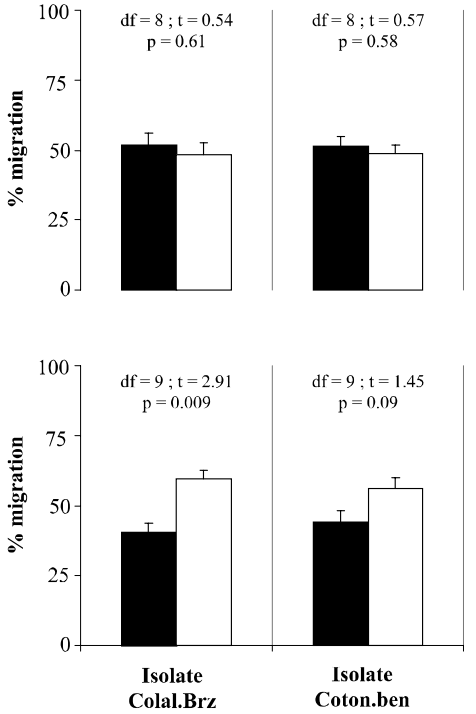

Figure 4 Migration of cassava green mite, *Mononychellus tanajoa*, in a two-choice unit between a clean cassava leaf disc (white bars) and a disc either treated (black bars) with a Beninese (Coton.ben) or a Brazilian (Colal.brz) isolate of its fungal pathogen *Neozygites tanajoae*. Top: treatment disc with mummified mite infected by *N. tanajoae*; bottom: treatment disc with spores.

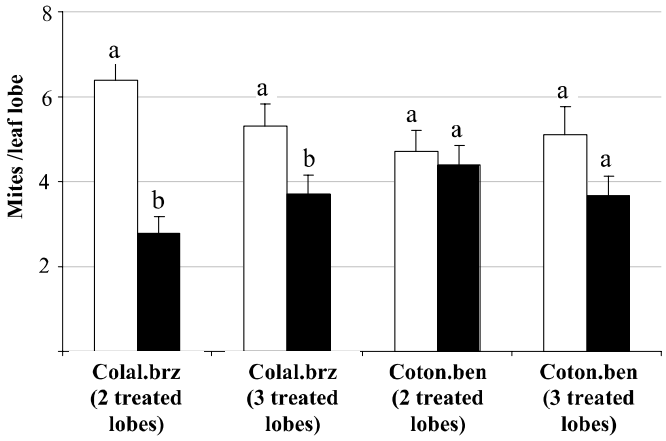

Figure 5 Migration of the cassava green mite *Mononychellus tanajoa* between clean cassava leaf lobes (white bars) and cassava leaf lobes either treated (black bar) with a Beninese (Coton.ben) or a Brazilian (Colal.brz) isolate of its fungal pathogen *Neozygites tanajoae*.

went to the side of the two-choice unit with spores: 40.7±3.1% for Colal.brz and 44.2±3.9% for Coton.ben. In contrast, when the pathogen was inside the mite (mummified CGM infected by the pathogen), CGM did not avoid the mummies. Nearly half of the migrating mites went to the side of the two-choice unit with mummies [51.8±4.2% for Colal.brz (P = 0.61) and 51.5±3.2% for Coton.ben (P = 0.58)].

Avoidance of *N. tanajoae* spores was also evident in the trial using cassava leaves, particularly for the Colal.brz isolate (Fig. 5). Although fewer migrating mites were generally observed on the lobes with than on those without spores for Coton.ben, the difference was not significant. Pathogen distribution among leaf lobes appears to influence the importance of avoidance depending on the isolate. Avoidance was more pronounced when spores were displayed on two lobes than on three lobes for Colal.brz, whereas it was the opposite for the Coton.ben isolate.

DISCUSSION

These experiments demonstrate that infochemicals influence interactions between CGM and its pathogen *N. tanajoae*. Cassava GLV inhibited spore production of *N. tanajoae*, whereas cassava HIPV produced following herbivory by CGM promoted spore production of *N. tanajoae*. Cues from *N. tanajoae* were found to influence mite behaviour.

Evidence of GLV influence on *N. tanajoae* results from observations on the Colal.brz isolate in the closed-dish experiment, where production of conidia was consistently higher in clean air than in an environment with damaged (excised) leaf discs. Evidence of an impact of HIPV influence is based on three arguments: (1) the highest production of conidia in the infested-disc treatment despite the inhibition effect of GLV in the closed-dish experiment; (2) the significantly higher production of conidia and capilliconidia for Coton.ben and Colal.brz isolates, respectively, in the HIPV compared with clean air treatment of the airflow experiment; and (3) the significantly higher production of conidia in an environment with MeSA than in an environment without MeSA. The increase in spore production in an environment with HIPV suggests a defense strategy of the cassava plant as it would promote host-to-host transmission on the leaves. Surprisingly, improved conidia production due to HIPV does not always result in improved infection (Baverstock et al., 2005). Inhibition of spore production by high production of GLV may reduce the action of HIPV as observed in the closed-dish experiment with excised leaves. However, temporary GLV-inhibition of spore germination was observed for another arthropod pathogen as a strategy for awaiting the host (Brown et al., 1995) and inhibition of growth was also observed for *M. anisopliae* and *T. cylindrosporum* in the laboratory, but not under field conditions (Klingen et al., 2002). Cassava volatiles, especially HIPV, may thus well help indirectly to protect the cassava plant from CGM attack.

The influence of cues from the acaropathogen is evident in the avoidance behaviour of CGM towards spores of *N. tanajoae* in both the two-choice and the leaf-lobe experiments, and the indifferent behaviour of *M. tanajoa* towards the acaropathogen when inside the mite, a result obtained for both isolates. It is realistic that avoidance takes place on infested cassava leaves given the spatial distribution of the acaropathogen. Spores are displayed within 8 mm around the sporulating mites hither and thither on the leaf surface, allowing pathogen-free spaces for avoidance. It is not clear why *N. tanajoae* produces cues that betray its presence. Production of cues may be the consequence of unavoidable physiological processes. However, the inability of the host mite to recognize the acaropathogen when inside the host would profit the pathogen. Infected mites mummify on the leaf and wait for favorable conditions to produce spores. It is thus profitable for the mummies to stay unnoticed in mite patches until they sporulate. The non-attraction of mummified infected mites may be the result of the confinement of the pathogen cues in the mite body and may have evolved as a strategy of the pathogen to increase its transmissibility. In this case it is expected from infected mites as long as they are still alive to transport the fungus to dense mite patches. We hypothesize that infected mites act as a Trojan horse for *N. tanajoa* to infect *M. tanajoa* populations.

As seen throughout these studies, *N. tanajoae*'s performance depends on its management of the chemical information present in the tritrophic system (Hountondji, 2008). In nature, chemical signaling is subject to variability due to the environment sensu lato and its impact on the various levels of the system (plant, herbivore, and pathogen). This could explain variability observed in the results of these studies, particularly in the responses of *N. tanajoae* isolates. The isolates of *N. tanajoae* demonstrated specificity in the treatment of the chemical information and infochemical dosage may play an important role in this as exemplified by the closed-dish vs. airflow HIPV experiments. Variation in emission of volatiles is found in many plant-herbivore systems (Dicke, 1999, 2000; Turlings et al., 1990); dosage might

thus interfere with the strength of interaction. On a different scale, our experiments on sporulation were conducted under supposedly optimal conditions whereas in the field, the pathogen is mostly subject to suboptimal conditions. Further studies should consider testing the effect of HIPV and GLV on sporulation of *N. tanajoae* under suboptimal conditions and the effect of dosage, density of host and pathogen in the different interaction studies. Further experiments should also be conducted to test the Trojan horse behaviour, as this may have profound consequences for evaluation of the transmission rate of arthropod pathogens.

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Herbivore-induced plant volatiles prime two indirect defences in lima bean

Yasuyuki Choh & Junji Takabayashi
Center for Ecological Research, Kyoto University, Otsuka 509-3, Hirano, Kamitanakami, Otsu, 520-2113, Japan.
E-mail: junji@ecology.kyoto-u.ac.jp

Herbivore-induced plant volatiles (HIPV) are emitted by plants in response to herbivory and attract natural enemies of herbivores, thereby inducing an important indirect defence against herbivores. Evidence supports the hypothesis that plants become more defensive against herbivores after exposure to HIPV and that this is a type of priming, or preparation by the plant perceiving an HIPV signal to respond to herbivory. We report the priming of two induced indirect defences: HIPV-mediated induction of predator attraction and the secretion of extrafloral nectar (EFN), known as an alternative food source for natural enemies of herbivores. When uninfested lima bean plants (*Phaseolus lunatus*) were exposed to HIPV, the plants attracted more predatory mites (*Phytoseiulus persimilis*) and secreted larger amounts of EFN than unexposed plants. Further, when HIPV-exposed plants were infested by spider mites (*Tetranychus urticae*) for 2 days, the plants attracted more predators and secreted larger amounts of EFN than plants that were infested for 2 days after exposure to uninfested plant volatiles. However, there were no differences in the attraction and the EFN secretion when they were infested for 4 days. Predatory mites survived longer when supplied with EFN and stayed longer on uninfested plants that had been supplemented with additional extrafloral nectar. From these results, we conclude that the priming of HIPV-exposed plants recruits predators and induces the secretion of EFN that functions to protect the plants before and after herbivory.

Key words: Extrafloral nectar, herbivore-induced plant volatiles, indirect defence, induced response, *Phytoseiulus persimilis*, plant-plant interactions, priming, *Tetranychus urticae*

Plants are known to induce direct defences by becoming a less suitable resource for herbivores (Bruin et al., 1992; Arimura et al., 2000; Dolch et al., 2000; Karban et al., 2000) as well as indirect defences by attracting carnivorous natural enemies of herbivores (Dicke et al., 1990; Horiuchi et al., 2003; Choh & Takabayshi, 2006a) in response to volatiles from neighbouring herbivore-infested plants (herbivore-induced plant volatiles: HIPV). HIPV are signals between infested plants and uninfested plants that communicate the presence of herbivores to both natural enemies and neighbouring plants (Bruin et al., 1995; Baldwin et al., 2006). A well-studied example of this communication is a tritrophic system consisting of lima bean plants (*Phaseolus lunatus*), herbivorous two-spotted spider mites (*Tetranychus urticae* Koch), and predatory mites (*Phytoseiulus persimilis* Athias-Henriot) (Dicke et al., 1990; Horiuchi et al., 2003; Choh et al., 2004; Choh & Takabayashi, 2006a). Lima bean plants infested by *T. urticae* emit specific blends of HIPV that attract *P. persimilis* (Dicke et al., 1999). When uninfested plants are exposed to HIPV from conspecific plants, they attract more *P. persimilis* than unexposed plants (Dicke et al., 1990; Bruin et al., 1992).

The attraction of predators to HIPV-exposed uninfested plants is proposed to be a plant defensive mechanism against herbivores (Dicke et al., 1990; Bruin et al., 1995). However, uninfested plants that attract predators as a consequence of HIPV induction do not offer any prey, therefore it is unlikely that the predators will remain to protect the exposed plants unless prey are present. Nevertheless, HIPV-exposed plants may recruit predators and supply them with extrafloral nectar (EFN) as an alternative food to herbivorous prey, which would guarantee that the predators stay on the plants. Several studies have reported that some predatory mite species feed on EFN as an alternative food (Van Rijn & Tanigoshi, 1999; Nomikou et al., 2003). To test this possibility, we focused on EFN that lima bean plants secrete as a possible alternative food source for predatory mites. We first tested the effects of EFN on the survival of *P. persimilis*. We then compared the secretion of EFN between uninfested plants and uninfested plants exposed to HIPV. Further, we determined whether the increased EFN secretion correlated with the movement of *P. persimilis* from plant to plant.

Uninfested plants are likely to become infested by herbivores that migrate from infested neighbours. Uninfested plants may prime defences against future herbivore attack by exposure to HIPV (Bruin et al., 1995). Priming for defence through volatile emission and perception in tritrophic systems has been shown to be an important mechanism for resistance to herbivorous insects (Choh et al., 2004; Engelberth et al., 2004; Choh & Takabayashi, 2006b; Heil & Kost, 2006; Kessler et al., 2006). In order to directly test this possibility, we examined the attraction of *P. persimilis* and EFN secretion by HIPV-exposed plants when infested by the herbivorous mite *T. urticae*. From these results, we discuss induced indirect defence strategies of HIPV-exposed plants against *T. urticae* before and after herbivory.

Experimental set-up to test plan-plant interactions

For the exposure of lima bean plants to HIPV, we used six acrylic 60 × 60 × 60 cm cages equipped with two 30 × 30 cm windows on opposite sides of the cage and a 30 × 30 cm sliding door at the front. Three cages were used for the treatment, and three were used for the control. The windows were covered with 225 mm nylon gauze. Airflow inside the cages was below detection levels. As an odour source, we used eight plants that had been infested for 1 day with 60 adult *T. urticae* females per plant. Preliminary experiments had established that eight plants would provide sufficient amounts of volatiles emitted. Eight uninfested plants were used as a control odour source. Four uninfested plants were

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put into the box with eight odour source plants (for details of the setup, see Choh et al., 2006), and exposed to HIPV or uninfested plant volatiles (UPV) for 10 days. All plants were placed into plastic containers (12 cm diameter, 9 cm height) filled with water, which prevented crossing over of *T. urticae* from infested to uninfested plants. The distance between the exposed plants and the odour source plants was 25 cm. For every replicate, we used a newly cleaned cage. Plants were visually inspected for signs of *T. urticae* infestation at the end of the exposure to HIPV and none were found on uninfested plants in any of the experiments.

Predator attraction to HIPV

More *P. persimilis* were attracted to HIPV-exposed plants than to UPV-exposed plants in a Y-tube olfactometer (Choh & Takabayashi, 2006a) (Fig. 1a). This finding corroborated previous studies (Dicke et al., 1990; Bruin et al., 1992). This attraction could be explained by the fact that uninfested lima bean leaves exposed to HIPV adsorb the volatiles and re-emit them (Choh et al., 2004). Volatile emissions by the exposed leaves decreased 1 day and 3 days after exposure (Choh et al., 2004), thus attraction may not be sustainable after exposure to HIPV has stopped.

To test whether the exposure to HIPV primes the attraction of *P. persimilis* by the exposed plants in response to *T. urticae*-infestation, we placed 60 adult *T. urticae* females on a HIPV-exposed plant and an UPV-exposed plant immediately after exposure. Olfactory responses of predators to HIPV were examined 2 and 4 days after the introduction of *T. urticae* using a Y-tube olfactometer. When plants were infested by *T. urticae* for 2 days, HIPV-exposed plants attracted significantly more *P. persimilis* than UPV-exposed plants (Choh & Takabayashi, 2007) (Fig. 1b). In contrast, when plants were infested for 4 days, there was no significant difference in the attraction of *P. persimilis* between HIPV-exposed and UPV-exposed plants (Choh & Takabayashi, 2007) (Fig. 1c). We have previously reported that detached lima bean leaves exposed to HIPV emit larger amounts of HIPV and attract more *P. persimilis* when infested by *T. urticae* (Choh et al., 2004). The attraction of *P. persimilis* by whole plants in response to the infestation was also enhanced by previous exposure to HIPV as detached leaves (Choh et al., 2004). Thus, we can conclude that the enhanced attraction of *P. persimilis* was due to the priming effect of HIPV exposure.

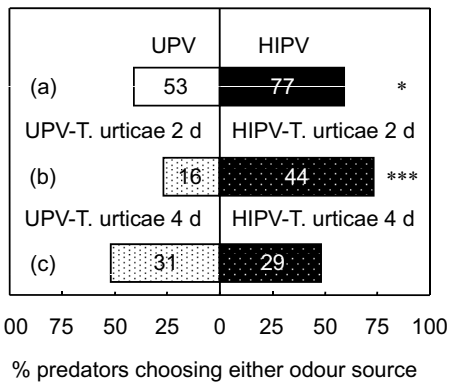

Figure 1 The olfactory response of *Phytoseiulus persimilis* females to volatiles from odour-exposed plants, as determined in a Y-tube olfactometer. (a) UPV-exposed vs. HIPV-exposed plants, (b) plants infested by *Tetranychus urticae* for 2 days after exposure to UPV (UPV-*T. urticae* 2d) vs. plants infested by *T. urticae* for 2 days after exposure to HIPV (HIPV-*T. urticae* 2d), and (c) plants infested by *T. urticae* for 4 days after exposure to UPV (UPV-*T. urticae* 4d) vs. plants infested by *T. urticae* for 4 days after exposure to HIPV (HIPV-*T. urticae* 4d). Asterisks beside each bar indicate significant differences between the first trifoliate leaves and the primary leaves. Asterisks between bars indicate a significant difference in preference between *P. persimilis* (binomial test; *, P<0.05, ***, P<0.001). Numbers in the bars indicate the number of predators.

Extrafloral nectar secretion

Phytoseiulus persimilis survived longer when they were supplied with water and EFN than when supplied with water alone (Choh et al., 2006) (Fig. 2). This result indicates that

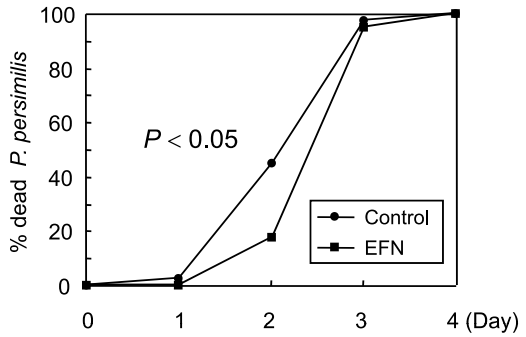

Figure 2 Proportion of dead *Phytoseiulus persimilis* when water and water + additional EFN were supplied to *P. persimilis* (n = 40, Log-rank test, P<0.05).

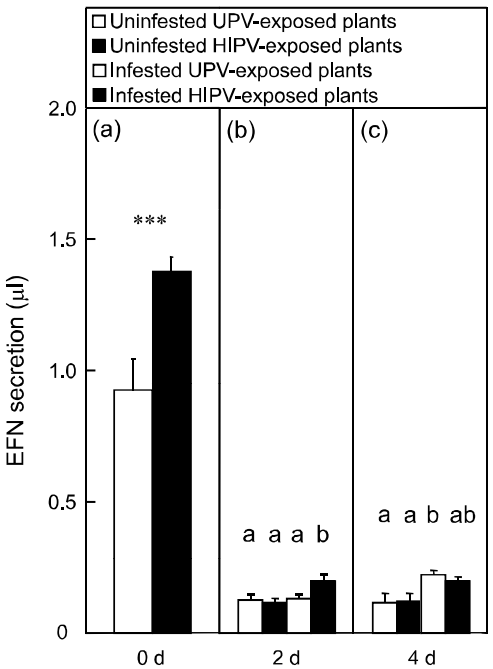

Figure 3 Quantities of extrafloral nectar (EFN) (mean ± SE) secreted by lima bean plants obtained from the following treatments: uninfested UPV-exposed plants, uninfested VIP-exposed plants, infested UPV-exposed plants, and infested VIP-exposed plants. The duration of plant volatile exposure was 10 days. Immediately after exposure, test plants were infested with *Tetranychus urticae*, controls were kept uninfested. Quantities of EFN were measured 0, 2, and 4 days after cessation of plant volatile exposure. Most EFN was removed after every test day. Twenty plants per treatment were used. Asterisks between bars indicate a significant difference (t test; ***, P<0.001). Different letters capping bars indicate significant differences among treatments within each time period by Tukey-Kramer's test (P<0.05).

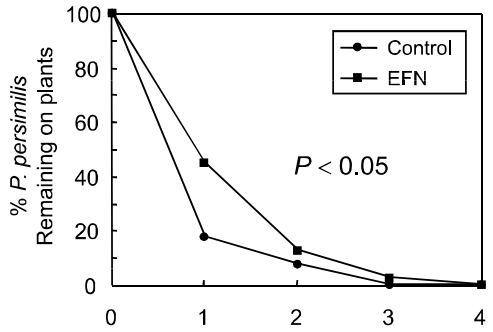

Figure 4 The proportion of *Phytoseiulus persimilis* that remained on the plant when placed on either uninfested plants or plants that were artificially manipulated with additional EFN (n = 40, Logrank test, P<0.05).

EFN is a viable food source for *P. persimilis*. Lima bean plants increased EFN secretion when they were exposed to HIPV (Choh et al., 2006; Choh & Takabayashi, 2006b) (Fig. 3a). However, the increase of EFN secretion was not observed 2 and 4 days after the exposure to HIPV (Choh & Takabayashi, 2006b) (Fig. 3bc). To examine the effect of EFN on the behaviour of *P. persimilis* on lima bean plants, we placed one female *P. persimilis* on an uninfested plant that was treated with additional EFN and monitored its behaviour (Choh et al., 2006). The residence time of *P. persimilis* on the treated plant was significantly longer than on an untreated control plant (Choh et al., 2006) (Fig. 4).

Priming of predator attraction was different depending on how long the herbivores were present on the plants. When plants were infested by *T. urticae* for 2 days, UPV-exposed plants did not increase EFN secretion. In contrast, HIPV-exposed plants secreted larger amounts of EFN in response to a 2-day *T. urticae*-infestation, than uninfested and UPV-exposed plants (Choh & Takabayashi, 2006b) (Fig. 3b). UPV-exposed plants increased EFN secretion when they were infested for 4 days (Choh & Takabayashi, 2006b) (Fig. 3c) but there was no significant difference in EFN secretion between HIPV- and UPV-exposed plants when they were infested for 4 days (Choh & Takabayashi, 2006b) (Fig. 3c).

DISCUSSION

When lima bean plants were exposed to HIPV emitted from *T. urticae*-infested conspecific neighbours, they attracted more *P. persimilis* (Choh & Takabayashi, 2006a) (Fig. 1a) and secreted larger amounts of EFN than UPV-exposed plants (Choh et al., 2006) (Fig. 3a). Emission of carnivore attractants and EFN secretion by HIPV-exposed plants would not be durable because the emission and the secretion decreased after exposure to HIPV was terminated (Choh et al., 2004; Choh & Takabayashi, 2006b). *Phytoseiulus persimilis* used EFN as an alternative food source when *T. urticae* were not present (Choh et al., 2006) (Fig. 2), and stayed longer on uninfested plants treated with additional EFN than on untreated unifested plants (Choh et al., 2006) (Fig. 4). HIPV-exposed plants attracted more predators that remained longer on the plants. Therefore, HIPV-exposed plants would be protected by the attracted predators, even in the absence of herbivorous prey (Choh et al., 2006). EFN functions as an indirect plant defence against herbivores by itself. The role of EFN has been well studied in ant-plant interactions (Agrawal & Rutter, 1998; Gaume & McKey, 1999; Raine et al., 2004; Rudgers, 2004), wherein EFN is a food resource that employs ant species as a constitutive indirect defense against herbivorous insects. It has already been reported that increased amounts of EFN benefit lima bean plants by attracting parasitoids and predators under natural conditions (Kost & Heil 2005).

When lima bean plants were infested by *T. urticae*, the attraction of *P. persimilis* by HIPV-exposed plants was stronger than that by UPV-exposed plants 2 days after the infestation (Choh & Takabayashi, 2007) (Fig. 1b). Further, EFN secretion by HIPV-exposed plants was larger than that by UPV-exposed plants when infested for 2 days (Choh & Takabayashi, 2006b) (Fig. 3b). In contrast, there were no differences in attraction of *P. persimilis* (Choh & Takabayashi, 2007) (Fig. 1c) and EFN secretion (Choh & Takabayashi, 2006b) (Fig. 3c) between HIPV- and UPV-exposed plants when they were infested for 4 days. These results indicate that previous exposure to HIPV at the beginning of herbivore infestation enhances both the attraction of *P. persimilis* and EFN secretion in response to herbivory .

Accumulating evidence suggests that HIPV prime plant defences against herbivores (Choh et al., 2004; Engelberth et al., 2004; Choh & Takabayashi, 2006b; Heil & Kost, 2006; Kessler et al., 2006; Choh & Takabayashi, 2007). However, the extent of the priming effect may change due to the period of exposure to HIPV, the conditions of infested neighbouring plants (i.e., the number of infested plants, the period of infestation, and the developmental stage of the plants), and the number of herbivores that invade to HIPV-exposed plants. Further studies are needed to clarify whether plants can gain higher fitness from enhanced induced defences resulting from priming, as compared to directly induced defences against herbivores.

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Differences in foraging strategies between populations of the predatory mite *Neoseiulus womersleyi*: correlation between olfactory response and dispersal tendency

Taro Maeda

Insect Interaction Research Unit, National Institute of Agrobiological Sciences, Ohwashi 1-2, Tsukuba, Ibaraki 305-0851, Japan. E-mail: tarom@affrc.go.jp

I investigated the relationship between olfactory response, patch-leaving tendency, predation rate, fecundity, and developmental time of 11 geographical populations and 13 isofemale strains of the predatory mite *Neoseiulus womersleyi*. Significant differences were found in all these traits and behaviors among the geographical populations and among the isofemale strains. A significant positive correlation was found only between the olfactory response and the patch-leaving tendency of geographical populations. There was no such relationship among the isofemale strains. These results suggest that the positive correlation between the behavioral traits among geographical populations was not caused by genetic factors. The difference in the behavioral traits and the correlation among geographical populations is discussed from an ecological point of view where foraging strategy is determined by both the olfactory response and the patch-leaving tendency.

Key words: dispersal tendency, genetic correlation, herbivore-induced plant volatiles, life-history traits, olfactory response, *Tetranychus urticae*

In optimal foraging theory, the decisions on when to leave a prey patch and how to search for another are central problems (MacArthur & Pianka, 1966; Krebs et al., 1974; Charnov, 1976). When searching for prey, predators that are attracted to herbivore-induced plant volatiles (HIPVs) would benefit if the volatiles inform the predators of the presence of prey patches (Vet & Dicke, 1992; Takabayashi & Dicke, 1996; Dicke & Vet, 1999). Various factors have been shown to influence when to leave a prey patch (or patch-residence time). For example, the residence times of predatory mites are prolonged by contact chemicals associated with prey inside a patch (Schmidt, 1976, 1977; Hislop & Prokopy, 1981; Sabelis & Dicke, 1985). On the other hand, HIPVs, indicating resource levels outside the patch, and previous foraging experiences influence the residence time of predatory mites (Maeda et al., 1998; Mayland et al., 2000; Maeda & Takabayashi, 2001, 2005). Predatory mites might be able to make better decisions by using information from various sources.

The predatory mite *Neoseiulus womersleyi* is one of the most important predators of spider mites of the genus *Tetranychus* in Japan. The predatory mites in different local populations experience different foraging conditions, including different combinations of herbivore-plant complexes, because spider mites such as *Tetranychus urticae* are highly polyphagous (Jeppson et al., 1975). As natural selection should favor the predator genotypes that are best able to use information about foraging conditions (Price et al., 1980; Dicke & Sabelis, 1988), it follows that the predatory mites of different local populations may have been subject to different local selection regimes with respect to their response to various types of information. The evolution of foraging behavior requires the existence of a genetic component in the predator's response to information. However, only a few studies have investigated the genetic background of foraging behavior of predatory mites (Margolies et al., 1997; Jia et al., 2002; Maeda, 2006).

The objective of the present study was to compare the intra- (Maeda, 2005) and inter-population (Maeda, 2006) genetic variation in foraging behaviors, notably the responses to HIPVs and the patch-leaving decisions of *N. womersleyi*. Eleven geographical strains were established from mites collected from different locations in Japan. Each strain was reared so as not to lose its genetic variation (Maeda & Hinomoto, 2006a,b). Thirteen isofemale strains were established from one of the geographical strains. Isofemale lines offer a useful way to assess the nature of phenotypic variation in natural populations, because each line is built up from a single female; this offers the major advantage that genetic information becomes measurable in species for which details of the genome are unclear (Parsons, 1980). Knowledge of the genetic basis for behavioral traits will improve our understanding of the evolution of prey-searching behavior based on HIPVs, and of the role of these plant volatiles in predator-prey dynamics (Dicke et al., 1990a,b; Vet & Dicke, 1992). In addition, increased knowledge of the genetic relationship between behavioral and other traits would have practical implications in the use of predatory mites as biological control agents.

MATERIALS AND METHODS

Plants and mites

Kidney bean plants (*Phaseolus vulgaris* L.) were used as a food source for the prey spider mite, *T. urticae*. The plants were grown in vermiculite in plastic pots (10 cm in diameter, 11 cm deep) in a climate-controlled room (19 ± 1 °C, 50-70% r.h., L16:D8). When the two primary leaves had fully unfolded (ca. 2-3 weeks after germination), the plants were used in the experiments. *Tetranychus urticae* were obtained from a laboratory culture that had been maintained on kid-

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ney bean plants in the laboratory for more than 3 years at 25±2 °C, 60-80% r.h., and L16:D8D.

Geographical strains of *Neoseiulus womersleyi*

Specimens of the phytoseiid mite *N. womersleyi* were collected from 11 sites in Japan. All populations were maintained separately on acrylic resin plates (20 × 30 × 0.5 cm) with four rubber feet (5 cm diameter, 4 cm height) placed in plastic boxes (30 × 47 × 12 cm deep), the lid of which had a rectangular opening (24 × 34 cm) covered with nylon mesh. To prevent the predators from escaping, the box was filled to a depth of 2 cm with water containing 0.4% detergent and 0.005% crystal violet as a fungicide. Five bean leaves infested with *T. urticae* (30-50 adult females plus other prey stages per leaf) were offered to the predatory mites three times per week. This rearing method can conserve the original genetic diversity for at least 1 year (Maeda & Hinomoto, 2006a).

Isofemale strains of *Neoseiulus womersleyi*

Adult females collected from one of the collection sites were individually reared on *T. urticae*-infested kidney bean leaves placed on moist cotton wool for 5 days. The females were removed before their offspring became adult. One of the mated females on each leaf was randomly selected for use as the mother of the next generation. This procedure was repeated for more than 20 generations.

Response to HIPV in Y-tube olfactometer

The olfactory responses of *N. womersleyi* were tested in a Y-tube olfactometer (Takabayashi & Dicke, 1992) with 10-cm arm length, 12-cm stem length, and 4-cm internal diameter. To standardize the quality and quantity of the volatiles, 30 adult female *T. urticae* were introduced onto each leaf of five bean plants with two primary leaves (i.e., a total of 300 mites per five plants) and reared in a climate-controlled incubator for 2 weeks (20±1 °C, 70±5% r.h.,L16:D8, ca. 10,000 lx). Five intact, uninfested plants grown under the same conditions were used as the control odor source. Air that had been cleaned by passing through granular activated charcoal was then passed through the odor-source chambers into both arms of the Y-tube olfactometer (3 l/min).

Females of *N. womersleyi* were placed individually in 0.5-ml plastic tubes without prey and water at least 100 min before each bioassay, and each was then individually introduced at the starting point (the downwind end) of a Y-shaped steel wire in the center of the olfactometer. The behavior of each predator was observed for 5 min. If the predator did not make a final choice within 5 min, the behavior was classified as 'no choice.'

The HIPV preference of each strain was calculated as the percentage of predatory mites that chose the HIPV arm divided by the total number of mites that made final choices. To account for any unexpected bias caused by the experimental setup, such as the shapes of the Y-shaped glass tube and the steel wire, the arms of the olfactometer were reversed between the prey-infested and control plants after every five mites, and the steel wire was replaced. In preliminary tests, predatory mites were equally attracted to the airflow from both arms of the olfactometer (50% chose each arm) when no odor source was present. The experiment was performed in a climate-controlled room (25±2 °C, 50-70% r.h., ca. 1,500 lx).

Dicke et al. (2000) and Schütte et al. (1998, 2006) reported that *Phytoseiulus persimilis* infected by a bacterial disease (*Acaricomes phytoseiuli*) (Pukall et al., 2006) showed decreased olfactory response, increased mortality, and low fecundity. However, as my mites exhibited no signs of disease (based on visual observations and a lack of any change in mortality or fecundity), and their olfactory preferences for prey-infested plants over intact plants did not change over a 1-year period under my laboratory conditions (Maeda & Hinomoto, 2006b), I assumed that any differences in response to plant volatiles in this study were not related to disease.

Patch-leaving tendency

The patch-leaving tendency of *N. womersleyi* was assessed using a leaf disc (30 mm diameter) infested with *T. urticae*. The leaf disc was used to mimic a prey patch. The leaf disc was placed on water-saturated cotton wool in a plastic Petri dish (90 mm diameter, 14 mm high). Five adult female *T. urticae* were allowed to oviposit on the leaf disc for 24 h (25±2 °C, 50-70% r.h., L16-D8) and then removed. A gravid female *N. womersleyi* was then introduced onto the leaf disc. After 30 min of acclimatization, the leaf disc was connected to a leaf rectangle (15 × 30 mm) using a bridge made of Parafilm (30 × 3 mm). The rectangular piece of leaf was heavily infested with *T. urticae* (the surface fully covered with spider mite webs) to trap predatory mites that dispersed from the leaf disc.

The Petri dish was placed in a wind tunnel (70 × 65 × 210 cm) in a climate-controlled room (25±2 °C, 50-70% r.h.). The air entering the wind tunnel (wind speed = 10-15 cm/s) was cleaned through a granular activated charcoal filter before it flowed over intact bean plants at the upwind end of the wind tunnel. To prevent the predators from perceiving the HIPVs released from the infested rectangular leaf, the leaf fragments were oriented with the rectangular leaf positioned upwind such that the Parafilm bridge was at a 45° angle to the wind direction (Maeda & Takabayashi, 2001, 2005; Maeda, 2005). After 6 h, the location of the predator was noted. Any individual that moved from the leaf disc to the leaf rectangle was classified as 'dispersed.' The percentage of mites that dispersed was calculated for each strain.

Prey-consumption rate, fecundity, and developmental time

To investigate the prey-consumption rate and fecundity of each strain, I placed squares (3 × 3 cm) of intact kidney bean leaf on water-saturated cotton wool in plastic Petri dishes (90 mm diameter, 14 mm high). Five adult *T. urticae* females were introduced onto each leaf square and allowed to oviposit for 24 h (25±1 °C, 70±5% r.h., L16-D8). After the *T. urticae* females were removed, the prey eggs that had been produced were counted. Then, individual *N. womersleyi* were introduced at 2-6 days after emergence onto each test leaf and allowed to oviposit. The prey-consumption rate was calculated as the decrease in the number of *T. urticae* eggs.

The development time of *N. womersleyi* was tested using kidney bean leaves infested with a sufficient number of *T. urticae* (i.e., so many that the predator never eliminated the prey during the observation period). Individual leaves were placed on water-saturated cotton wool in a plastic Petri dish (90 mm diameter, 14 mm high), then five adult females of *N. womersleyi* were allowed to oviposit for 24 h on each leaf, and the development of the offspring was monitored daily for 10 days (25±1 °C, 70±5% r.h., L16-D8).

Table 1 Comparison of five parameters among isofemale strains and geographical strains.

	Isofemale strains* (n = 13)		Geographical strains** (n = 11)	
Parameters	range	P	range	P
Olfactory response (%)	35.4-76.2	<0.001	51.1-72.5	<0.001
Patch-leaving tendency (%)	4.5-55.0	<0.001	0.0-62.5	<0.001
Prey consumption (eggs/day)	15.95-22.43	<0.05	15.51-25.13	<0.001
Fecundity (eggs/day)	3.12-3.95	<0.05	2.76-3.71	<0.001
Developmental time (days)	5.94-7.2	<0.001	5.97-7.05	<0.001

*Data from Maeda (2006). **Some data from Maeda (2005).

Statistical analysis

Logistic regression analysis was used to investigate the differences among the strains of *N. womersleyi* in their HIPV preferences and in their patch-leaving tendencies (% of mites that dispersed from a prey patch). To test whether there was variation among strains in prey consumption rate (number of *T. urticae* eggs consumed/female/day), in fecundities (number of eggs laid/female/day), and in developmental times (days from egg to adult), I used one-way ANOVA. Pearson's product-moment correlation coefficient (r) was calculated to test the relationship between each pair of parameters (HIPV preference, patch-leaving tendency, prey consumption rate, fecundity, and developmental time). For the estimation of correlation coefficients, the data on the HIPV preference and patch-leaving tendency were arcsine-square-root transformed. All analyses were performed in JMP 5.12 software (SAS, 2002; Sall et al., 2004).

RESULTS

Olfactory response and patch-leaving tendency

The olfactory responses and patch-leaving tendencies differed significantly among isofemale strains and among geographical strains (logistic regression: P<0.001). The preference for HIPV ranged from 35.4 to 76.2% for isofemale strains and from 51.1 to 72.5% for geographical strains (Table 1). The HIPV preferences differed significantly among isofemale strains and among geographical strains (logistic regression: P<0.001). The difference in the patch-leaving tendencies also was significant among isofemale strains (logistic regression: 4.5-55.0%, P<0.001) and among geographical strains (0-62.5%, P<0.001, Table 1).

Analysis of isofemale strains showed that there was no relationship between the HIPV preference in the Y-tube olfactometer and the patch-leaving tendency in the wind tunnel (P = 0.39, n = 13; Fig. 1A). On the other hand, a significant correlation was found in the dataset of geographical populations (y = 2.948x – 2.206; r^2 = 0.70, n = 11, P = 0.0013; Fig. 1B).

Prey-consumption rate, fecundity, and developmental time

The mean number of eggs consumed by an adult female of *N. womersleyi* per day differed significantly among the isofemale strains (one-way ANOVA: $F_{12,265}$ = 1.828, P = 0.044) and among the geographical strains (one-way ANOVA: $F_{10,355}$ = 3.382, P<0.001). The range of prey-consumption rates of the isofemale strains was almost the same as that of the geographical strains. Fecundity (eggs/day) differed significantly among isofemale strains (one-way ANOVA: $F_{12,265}$ = 1.893, P = 0.035) and among geographical strains (one-way ANOVA: $F_{10,355}$ = 3.209, P<0.001). The range of fecundities of isofemale strains was almost the same as that of the geographical strains.

The mean developmental time (days from egg to adult) also differed significantly among isofemale strains (one-way ANOVA: $F_{12,322}$ = 6.920, P<0.0001) and among geographical strains (one-way ANOVA: $F_{10,482}$ = 4.692, P<0.0001). The range of developmental times of the isofemale strains was almost the same as that of the geographical strains.

The three traits (prey-consumption rate, fecundity, and developmental time) were not significantly correlated with each other. In addition, no combination of these three traits and the two behavioral traits (preference for HIPVs and patch-leaving tendency) showed a significant relationship.

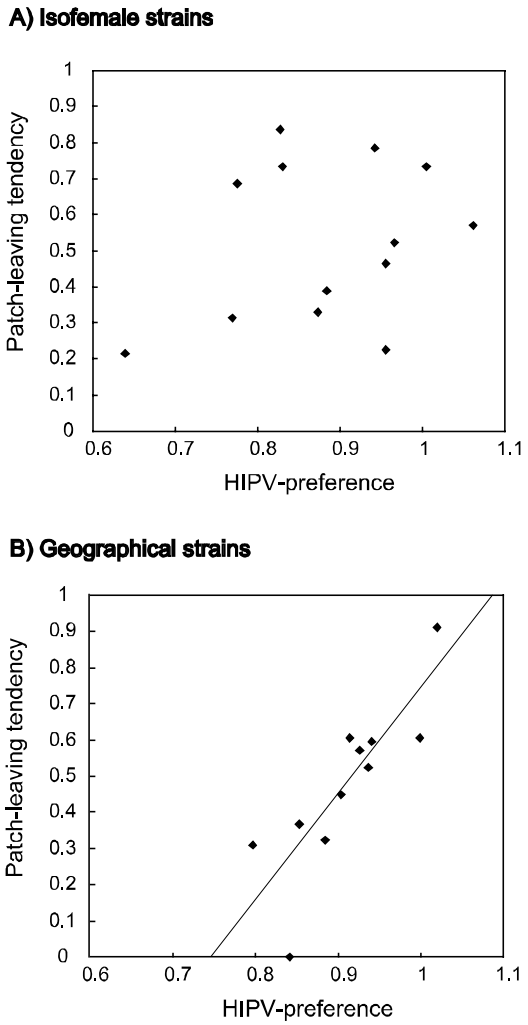

Figure 1 The correlation between HIPV preference and patch-leaving tendency among (A) isofemale strains, and (B) geographical strains. Each dot indicates the result for one strain. The data for both were arcsine-square-root transformed. The HIPV preference and the patch-leaving tendency were significantly correlated among geographical strains (y = 2.948x – 2.206, r^2 = 0.6999, P<0.005), but not among isofemale lines.

DISCUSSION

Because inbreeding reduces the total genetic variation within a population to the sum of the variation represented by the isofemale lines within that population (Falconer, 1989), the results of the present study suggest that much of the observed differences among isofemale strains can be attributed to genetic differences, and that the olfactory response, patch-leaving tendency, prey consumption rate, fecundity, and developmental time of *N. womersleyi* have genetic components.

In tritrophic systems in which HIPVs attract predatory mites, natural selection might be expected to favor predatory mites that are sensitive to volatiles (Price et al., 1980; Dicke & Sabelis, 1988). In the absence of other selection forces, genetic variation in the olfactory response is expected to decrease over time (Jia et al., 2002). However, the observed substantial and significant genetic variation in the olfactory response suggests that selection based on this response may be neither directional nor consistent. In theory, the existence of genetic variation in a trait can be caused by antagonistic pleiotropy that is manifested as negative genetic correlations with other traits (Rose, 1982), by disruptive selection in a variable environment through genotype*environment interactions, or by a combination of both (Ewing, 1979; Gillespie & Turelli, 1989). The genetic variation in the olfactory response of *P. persimilis* has been explained as the result of a trade-off between olfactory response and prey-exploiting ability, such as fecundity and prey consumption rate (Jia et al., 2002). In the present study, however, I found no genetic relationship between the olfactory response and the prey-consumption rate, fecundity, or developmental time. These results suggest that the habitat heterogeneity of *N. womersleyi* might play a major role in the maintenance of genetic variation in its olfactory response, as was suggested in studies of odor-guided behavior of *Cotesia glomerata* (Wang et al., 2004) and *Drosophila melanogaster* (Mackay et al., 1996).

A fascinating theory to explain the variation in the patch-leaving tendency is the milker-killer hypothesis suggested by van Baalen & Sabelis (1995). This hypothesis assumes two types of predator, the milker and the killer. Milker-type predators disperse at a constant rate from prey patches, and killer-type predators remain in a patch as long as there is prey. When there are few founders in a local population of predators, milker types will tend to have more offspring than killer types. In contrast, when a territory is invaded by multiple unrelated founders, the killer type is expected to be favored. The patch-leaving tendencies of the geographical strains of *N. womersleyi* appear to range from milker-type to killer-type behavior.

Analysis of geographical strains showed positive correlation between the HIPV preference and the patch-leaving tendency. On the other hand, no relationship was found between these two behavioral traits among the isofemale strains. If the base population used to establish isofemale strains had less genetic variation than the variation among geographical strains, correlation between the two behavioral traits might not have been detected. However, the variation among isofemale strains was as large as that among the geographical strains (Table 1). Therefore, the lack of correlation between the two behavioral traits in the isofemale strains cannot be explained by a lack of genetic variation within the base population.

An alternative interpretation is that the positive relationship observed in natural populations was not caused by genetic factors, but rather by ecological factors. If the olfactory response and the patch-leaving tendency are genetically unrelated, the predatory mites may be able to adopt different foraging strategies (e.g., high olfactory sensitivity and low patch-leaving tendency) as an adaptation to different foraging conditions. However, no natural population of *N. womersleyi* had a high olfactory sensitivity combined with a low patch-leaving tendency, or a low olfactory sensitivity combined with a high patch-leaving tendency. The absence of such combinations of the two behavioral traits in natural populations suggests that natural selection would favor specific combinations of the two behavioral traits (i.e., a foraging strategy comprising both olfactory response and patch-leaving tendency).

A statistical model of the effects of travel costs on the patch-leaving tendency of predators predicted that it is adaptive to disperse early to maximize foraging efficiency when the travel cost is small (Bernstein et al., 1991). Because a sensitive olfactory response to HIPVs would decrease the travel cost (Vet & Dicke, 1992; Dicke, 1994; Takabayashi & Dicke, 1996), predators with strong HIPV-preference should also exhibit high patch-leaving tendency (Bernstein et al., 1991). But when the travel cost is high, predators are expected to stay put. Therefore, if predators had a low olfactory sensitivity, a low patch-leaving tendency would also be expected.

Genetic variation in prey-consumption rate, fecundity, developmental time, and behavioral traits related to foraging efficiency has clear practical, ecological, and evolutionary implications. To utilize natural enemies as biological control agents, it is important to increase their ability to find pest species (Lewis & Nordlund, 1985). The study about the genetic background of *N. womersleyi* (Maeda, 2005, 2006) suggests that it would be possible to select and breed predatory mites that have a strong olfactory response, a high fecundity, a short developmental time, and a high prey consumption rate. However, the results of Maeda (2005) suggest that intensifying the olfactory sensitivity of the predatory mite might not always heighten its efficiency in controlling pest species.

It remains unclear how genetic variation in the olfactory response and correlated foraging traits influences the predator's search efficiency and predator-prey population dynamics (Jia et al., 2002). In biological control programs, increasing the efficiency of the natural enemies of a pest species is of key concern (Lewis & Nordlund, 1985). Although no study has proposed to select phytoseiid mites for biological control, analysis of whether or not there is a relationship between foraging behaviors and life-history traits provides important information for the rearing of any natural enemy as an agent for biological control. For example, if there is no relationship between life-history traits and foraging behavior, as shown in this study, it may be possible to select a predatory mite genotype that has not only high foraging efficiency but also high fecundity.

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Evolutionary and Ecological Acarology: Intraspecific Variation

Species or morphological variation? A multivariate morphometric analysis of *Afroleius simplex* (Acari, Oribatida, Haplozetidae)

Louise Coetzee
Acarology Department, National Museum, PO Box 266, Bloemfontein 9300, South Africa.
E-mail: louise.coetzee@nasmus.co.za

Population differences of *Afroleius simplex* Mahunka have been studied by means of multivariate morphometric analyses based on nine variables measured from 87 specimens. Principal component (PCA), discriminant function, and cluster analyses were performed. There is no separation of specimens into clusters and therefore the specimens are regarded as a single species.

Key words: *Afroleius simplex*, principal component analysis, discriminant function analysis, cluster analysis

The oribatid genus *Afroleius* Mahunka (Haplozetidae) comprises three known species, *A. deformis*, *A. minor*, and *A. simplex*, all described by Mahunka (1984), and they have thus far been recorded only from the type localities in the Western Cape Province of South Africa. The Acarology collection of the National Museum (Bloemfontein, South Africa) contains many more specimens of this genus, including species new to science, collected in other parts of South Africa.

The genus *Afroleius* is diagnosed by the presence of sacculi, movable pteromorphs, absence of translamella, moderately long rostral and lamellar setae, minute or very short interlamellar, notogastral, epimeral and ventral setae, presence of foveolae on dorsal and ventral surfaces (to a certain extent), six pairs of genital setae, and all adanal setae (three pairs) located posterior to the adanal lyrifissure. The species are mainly distinguished by the shape, size, and orientation of the sensillus, shape, size, and distribution of the foveolae, and the shape of the notogaster, especially in lateral view.

Certain specimens collected from various localities in South Africa are very similar to *A. simplex*, but differ from the type material in the presence of foveolae in the epimeral region (Fig. 1A,B), the length of the lamellar setae, and body size, including individuals with intermediate character states. Multivariate morphometric analyses were carried out to try to resolve the uncertainty as to whether these specimens belong to *A. simplex* or to a different (new) species.

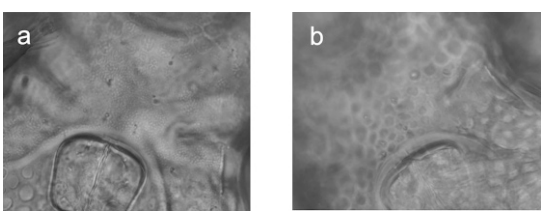

Figure 1 Epimeral region (A) specimen without foveolae (locality 1632), (B) specimen with foveolae (locality 1769).

MATERIALS AND METHODS

Eighty-seven specimens from 39 samples were used for the morphometric analyses. The samples were pooled to form eight groups, clustering the samples based on proximity of collection site and habitat (Fig. 2). Group A: 58 Tzitzikama forest (33°S, 23°E) litter in indigenous forest; 66, 68 Knysna (34°S, 23°E) litter in indigenous forest; 97 Knysna (33°S, 22°E) bark; 99, 3288, 3290, 3291 George (33°S, 22°E) litter in indigenous forest. Group B: 653, 790 Mtunzini (28°S, 31°E) soil and plant litter in natural forest; 3302 Vernon Crookes Nature Reserve (30°S, 30°E) litter and soil; 3730, 3740, 3741, 3745, 3749 Cape Vidal (28°S, 32°E) litter in indigenous forest; 3751 St Lucia (28°S, 32°E) litter in riverine forest. Group C: 3 King Williamstown (32°S, 27°E) moss and bark; 6, 2061 Queenstown (31°S, 26°E) litter and soil. Group D: 249, 3878 Fouriesburg (28°S, 28°E) litter and compost; 1632, 1635, 1638 Leliehoek (29°S, 27°E) litter and soil. Group E: 1774 Clarens (28°S,

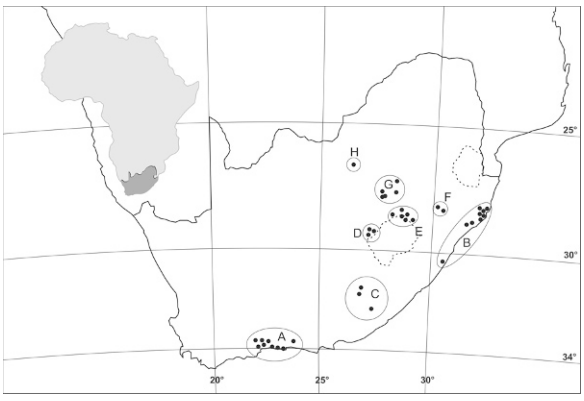

Figure 2 Map of South Africa with collection sites illustrated.

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28°E) litter; 1776, 3460 Golden Gate (28°S, 28°E) litter and soil; 1783 Witsieshoek (28°S, 28°E) litter and soil; 2905 Royal National Park (28°S, 29°E) litter in indigenous forest. Group F: 1878, 1880 Dundee (28°S, 30°E) litter and soil. Group G: 335 Lindley (27°S, 27°E) litter; 1728, 1743 Frankfort (27°S, 28°E) litter and soil; 1768, 1769 Edenville (27°S, 27°E) litter and soil. Group H: 3241 Coligny (26°S, 26°E) litter and soil.

Temporary preparations were made from which camera-lucida drawings were produced. Landmark points (homologous points) (Houck & OConnor, 1998) were selected so that measurements can be taken with both points sharply focused simultaneously. Distances from the left and right side of the body were averaged for each specimen.

Data

Measurements (Fig. 3A,B): Dorsal side: 1) total length, 2) length of lamellar seta *le*, 3) distance between insertion points of lamellar setae *le*, 4) distance between insertion points of interlamellar setae *in*, 5) distance between insertion points of notogastral setae *la*, 6) distance between insertion points of notogastral setae *lp* and h_3. Ventral side: 7) diagonal distance between insertion points of epimeral seta *1a* left and *3a* right, and vice versa, 8) distance between posterior border of genital opening *gen* and anterior border of anal opening *an*. Ordinal data: 9) presence or absence of foveolae in the epimeral region (1, present; 2, vague; 3, absent).

Statistical procedures

Data were standardized [(measurement – sample mean)/standard deviation] to compensate for the use of continuous data as well as ordinal data (Quinn & Keough, 2002), and also to eliminate the effect of non-normal distribution. A principal component analysis (PCA) and discriminant function analysis (DA, also referred to as canonical analysis) were performed. The use of standardized data is the standard transformation used in PCA and DA when variables are considered equally important (Fowler et al., 1998). A cluster analysis was also performed on the standardized data using Euclidian distances and unweighted pair-group averages (UPGMA) as linkage method (Quinn & Keough, 2002). All analyses were performed using STATISTICA v.6.

RESULTS

Principal component analysis

The first four components contribute 77.9% of the total variance (Table 1). Component 1 is a general component (all coefficients of the same sign) and is related to size, whereas components 2, 3, and 4 are bipolar (containing positive and negative coefficients) and are related to shape (Pimentel, 1979) (Table 2). A scatterplot of component 1 against component 2 (Fig. 4) of all cases show no clustering of the cases (specimens).

Discriminant function analysis

A summary of the results of the DA is given in Table 3, with the variables listed in order of significance of contribution to the model. The last 3 variables (distance between the insertion points of the interlamellar setae, distance between genital and anal plates, and distance between the insertion points of *1a* on the left and *3a* on the right, and vice versa) are statistically not significant. The length of the lamellar setae, presence or absence of foveolae on the epimeres, and total length are the variables that contribute most to the dis-

Table 1 Eigenvalues and percentage of variance.

Component	Eigenvalue	% total variance	Cumulative %
1	4.026	44.7	44.7
2	1.188	13.2	57.9
3	0.976	10.9	68.8
4	0.817	9.1	77.9

Table 2 Eigenvectors based on the correlation matrix.

	Component			
Variable	1	2	3	4
Total length	0.428	-0.022	-0.189	-0.095
Length lamellar seta	0.189	-0.196	0.846	0.055
Distance *le-le*	0.378	0.218	0.104	0.065
Distance *in-in*	0.283	0.296	0.158	0.673
Distance *la-la*	0.436	0.053	-0.161	0.015
Distance *lp-*h_3	0.265	-0.461	-0.337	0.160
Distance *1a-3a*	0.302	-0.201	0.220	-0.620
Distance *gen-an*	0.441	-0.071	-0.151	-0.032
Epimeral foveolae	0.095	0.751	-0.028	-0.344

Table 3 Discriminant function analysis: summary (n = 87).

Variable	Wilks' λ	Partial λ	F	P
Length lamellar seta	0.070	0.442	12.827	<0.001
Epimeral foveolae	0.053	0.584	7.219	<0.001
Length	0.048	0.645	5.581	<0.001
Distance *lp-*h_3	0.045	0.687	4.631	<0.001
Distance *le-le*	0.043	0.721	3.926	0.001
Distance *la-la*	0.040	0.780	2.853	0.011
Distance *in-in*	0.037	0.830	2.079	0.057
Distance *gen-an*	0.036	0.849	1.801	0.100
Distance *1a-3a*	0.035	0.877	1.421	0.210

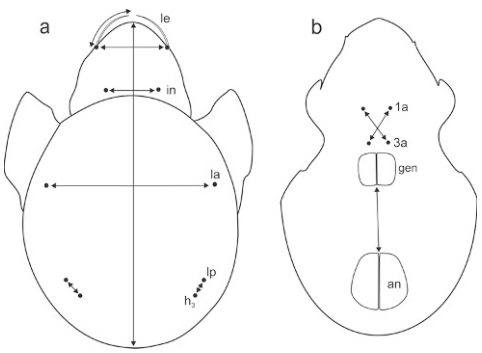

Figure 3 Diagram of *Afroleius simplex* indicating landmark points; (A) dorsal view, (B) ventral view.

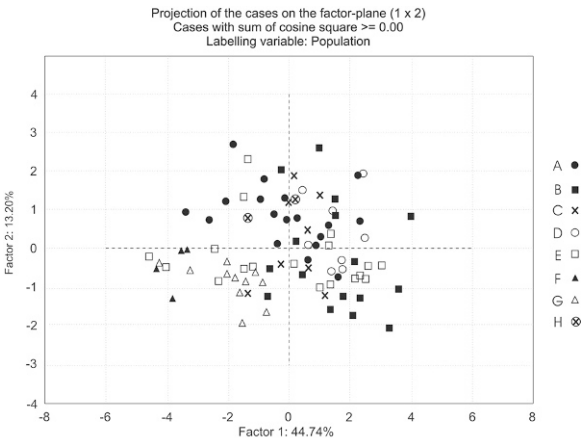

Figure 4 Principal component analysis: projection of cases, components 1 × 2.

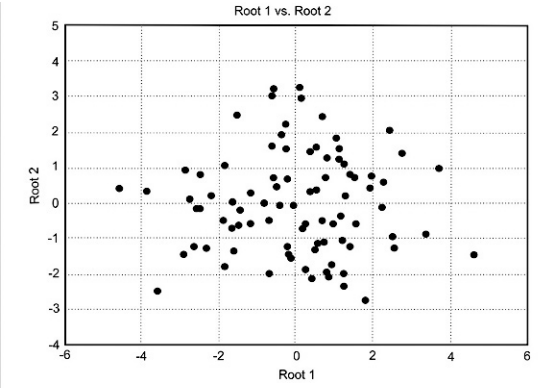

Figure 5 Discriminant function analysis: projection of cases, root 1 × root 2.

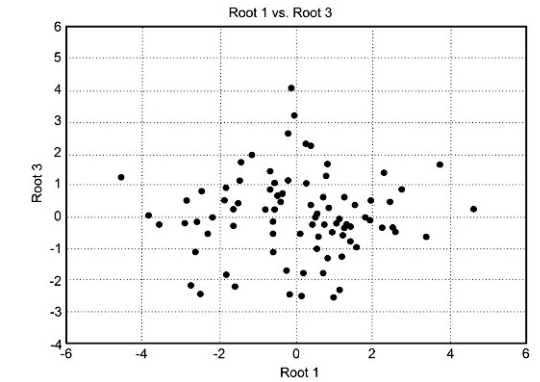

Figure 6 Discriminant function analysis: projection of cases, root 1 × root 3.

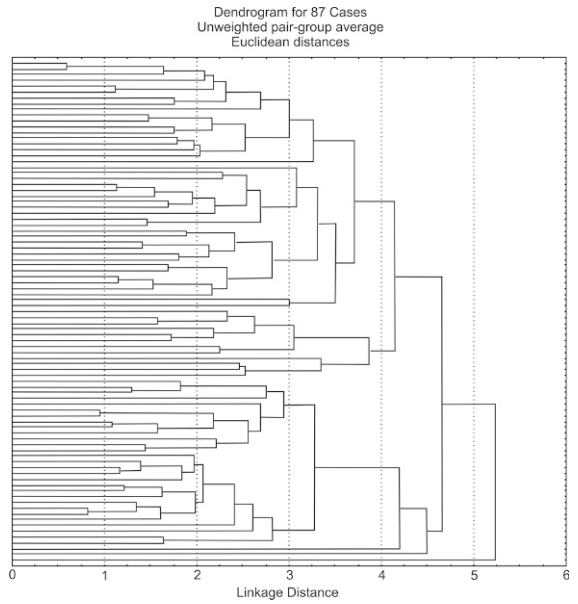

Figure 7 Dendrogram of cases (case names omitted).

crimination of cases (low partial lambda and high F-value). Scatter plots of the canonical scores of all cases show no clustering (root 1 vs. 2, Fig. 5; root 1 vs. 3, Fig. 6).

Cluster analysis

A dendrogram of the Euclidian distances of all 87 cases (Fig. 7) indicates no distinct clustering of any of the cases.

DISCUSSION

The multivariate analyses show no clustering of specimens on the basis of the selected morphometric measurements and the degree of expression of foveolae in the epimeral region. Therefore all the specimens investigated may be identified as *A. simplex*. The diagnosis of the species should be broadened to include the variation displayed. The foveolae on the epimeral region can be present, absent, or vaguely expressed, the apices of the left and right lamellar setae may or may not meet anteriorly, and the total length varies between 262 and 357 µm, with an average of 309 µm. Measurements taken on the ventral side of the body are remarkably constant, irrespective of the size of the specimen, and show the lowest discriminatory power in these analyses.

Acknowledgments

I am grateful for the help of Dr Lizel Hugo, National Museum, Bloemfontein.

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Assessment of morphological and molecular variation among strains of *Neoseiulus californicus* (Acari: Phytoseiidae)

S. Guichou, M.-S. Tixier & S. Kreiter
Montpellier SupAgro / UMR CBGP 2 Place Pierre Viala, 34060 Montpellier cedex 01, France. E-mail: tixier@supagro.inra.fr

Neoseiulus californicus is distributed worldwide and well-known for its predatory effectiveness on mite pests, especially *Tetranychus* species. The objective of this study was to (1) assess the range of variation of morphological and morphometrical characters defining the species, and (2) find diagnostic tools to discriminate between strains. Thirty females of each of 10 strains were mounted on slides and 42 characters were analysed. There are significant differences among strains for 31 characters; the range of (intra-strain) variation for each parameter is low. The discriminant analysis indicated that all factors measured allowed the discrimination of the strains with less than 6% error, except for the Greek strain. Molecular experiments indicated very low variability for all five markers tested (two nuclear and three mitochondrial fragments). For the 18S fragment, no nucleotide divergence was observed between three populations. For the ITS-5.8S, the variation was 0-0.6% between two strains. For the 12S fragment, nucleotide divergence was observed between the 10 strains (0-0.4%). For mitochondrial markers, the variation rate was also surprisingly low: 0.3% between two strains for one fragment, 0-2.5% between five strains for the other. The highest diversity was observed between the Chile strain vs. the others (not differentiated). This result is very similar to that from morphological analysis. However, the Spanish strain that could be separated based on morphological markers appeared to be molecularly similar to the other strains tested. Worldwide dispersion of *N. californicus* and mixing may explain the low molecular variability. Up to now, morphological markers would be more helpful for discriminating between *N. californicus* strains than molecular ones. However, other molecular markers will be tested.

Key words: Phytoseiidae, *Neoseiulus californicus*, taxonomy, morphology, mt-DNA, nuclear DNA

Spider mites of the genus *Tetranychus* are important pests of many food and ornamental crops. Phytoseiid mites, particularly *Neoseiulus californicus* (McGregor), are known to be excellent biological control agents for suppressing these pest mite populations in a great variety of crops and for preventing yield losses (Helle & Sabelis, 1985; McMurtry & Croft, 1997). Commercial firms mass-rear phytoseiid species in order to sell them to distributors or growers for augmentative releases in crops (Hunter, 1997), but often the identity is not verified before sale, or contamination could occur during mass-rearing. This may lead to unintended release of unwanted species.

This study is a collaborative effort to identify and mass-rear a strain of *N. californicus* expected to be more effective under arid conditions than the standard commercial strain from California, USA. To achieve this goal, 10 strains have been collected in several arid areas worldwide and morphometric and genetic methods have been developed, to search for diagnostic tools that would allow the differentiation of these strains.

MATERIAL AND METHODS

Mite origin and rearing

Through exchange between collaborating research groups and by direct collections, 10 strains of *N. californicus* were obtained and reared in the laboratory. The strains chosen originated from arid regions:
-France: collected in Mauguio (Hérault) on egg plant in July 2004 and maintained in our lab on *Tetranychus urticae* Koch;
-Chile: collected in La Cruz on *Phaseolus vulgaris* in 2000 maintained in Chile on *T. urticae* and *Tetranychus turkestani* and in our lab since 2004 on *T. urticae*;
-Firenze (Italy): collected on strawberries and maintained in our lab on *T. urticae*;
-Spain: collected in Bolbaite (Valencia) on strawberries in 2000, maintained in Spain on *T. urticae*, and in our lab since 2004, also on *T. urticae*;
-Sicily (Italy): collected in Paternico (Palermo) on strawberries, reared in Sicily on *T. urticae* and pollen, and in our lab since 2004 on *T. urticae* only;
-California (Koppert): maintained in our lab since 2004 on *T. urticae*;
-Greece: maintained in our lab since May 2005 on *T. urticae*;
-Japan: maintained in our lab since May 2005 on *T. urticae*:
-Brazil: maintained in our lab since March 2005 on *T.urticae*;
-Tunisia: collected in Cap Bon in June 2005 and maintained in our lab on *T. urticae.*

Among these strains, 'Koppert' was considered as the control or reference strain. All strains were split in two and reared in two growth chambers in order to spread the risk of losses. They were all fed on *T. urticae*, which was mass-reared on bean in an isolated greenhouse. Leaves infested with tetranychid mites, double-checked in order to prevent contaminations with other predators, were provided two times a week.

Morphological parameters measured

Thirty females per strain were mounted on slides. Morphometrical measurements were made with a picture analysis station (a computer with picture analysis software coupled to a Leica microscope with a high resolution digital camera). In total, 42 characters were assessed and used for analysis. The length was measured of all idiosomal setae: j1, j3, z2, z4, s4, Z1, S2, S4, S5, Z5, J5, j4, j5, z5, j6, J2, Z4, r3 and R1 (Fig. 1), as well as the width and length of the ventrianal shield, the distance between the setae present on the sternal and genital shields, and the length of metasternal plates (Fig. 2). In addition, the spermatheca length and form, and the lengths of leg setae were included in the analysis. Not

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included in the analysis were chelicerae dentition and dimensions, ornamentation or pore absence/presence, nor sigilla, because they did not vary between the strains.

Analysis of morphological data

In order to characterise differences between strains, ANOVA was carried out for each of the 42 characters studied. In order to determine which character allows for the best discrimination of the strains, a discriminant analysis was performed with the 42 variables studied. In order to determine similarities between the 10 strains, a canonical multifactorial analysis was performed and euclidian distances among the strains were calculated, using Statistica® 2001, version 6.0 (Statsoft, Tulsa, OK, USA).

Molecular markers used

Numerous molecular markers have been used in entomology (Loxdale & Lushai, 1998), but only few in acarology (Navajas & Fenton, 2000; Cruickshank, 2002). Three mitochondrial (mt) and two nuclear genes were used in the present study; mtDNA has been widely used in taxonomic and population studies of insects (Simon et al., 1994; Roehrdanz & Degrugillier, 1998) and some mites (Navajas et al., 1996; Navajas & Fenton, 2000; Toda et al., 2000, 2001; Otto & Wilson, 2001; Cruickshank, 2002; Evans & Lopez, 2002). The sequence variability of mtDNA markers allows differentiating distinct genetic entities at the species or even subspecies level. Furthermore, the many copies of mtDNA facilitates molecular approaches (Harrison, 1989; Loxdale & Lushai, 1998). Regions of rDNA are particularly likely to yield informative data for almost any systematic question (Hillis & Dixon, 1991; Navajas et al., 1996; Gotoh et al., 1998; Navajas & Fenton, 2000).

DNA extraction

The DNA of five starved females of each population was extracted, to avoid contamination from ingested prey. The CTAB extraction method was used as described by Tixier et al. (2004).

DNA amplification and electrophoresis

The primers used to amplify and the thermal cycling conditions of PCR are presented in Table 1. PCR was performed in a total volume of 25 µl containing 2 µl mite DNA, 1 µl DNTP (2.5 Mm for each nucleotide), 2.5 µl Taq buffer, 1µl of each primer (100 µM), 0.5 µl Taq (Qiagen, 5 U/µl), and 18.9 µl water. Electrophoresis was carried out on 1.5% agarose gel in 0.5× TBE buffer during 30 min at 150 V.

DNA sequencing

PCR products were sequenced using the dynamic ET terminator cycle sequencing kit. Purification of DNA was carried out with Exosap-IT (Amersham). The sequencer used was the Megabase 1,000 apparatus. All DNA fragments were sequenced along both strands.

Sequence alignment and distances

Sequences were analysed, checked, and read manually using Mega3.1® (Kumar et al., 2004). They were aligned using ClustalW® (1997) (Higgins et al., 1994). The distance matrix was constructed using the Jukes & Kantor model for the five genes.

RESULTS

Morphological study

As the ranges of variation for the length ratios were small and did not permit accurate separation of any of the strains, these data were not included in the analysis. The ANOVA carried out with the 42 characters (Table 2) shows significant differences between the strains for almost all variables; the range of variation for each parameter is low (Table 2). For almost all the dorsal setae lengths, the strain from Firenze has the lowest values and the strain from Chile the highest. For some characters, such as the length of metapodal plate 1, the width of the ventrianal shield, the distance st4-st4, and the width of the spermatheca, the Brazilian strain shows

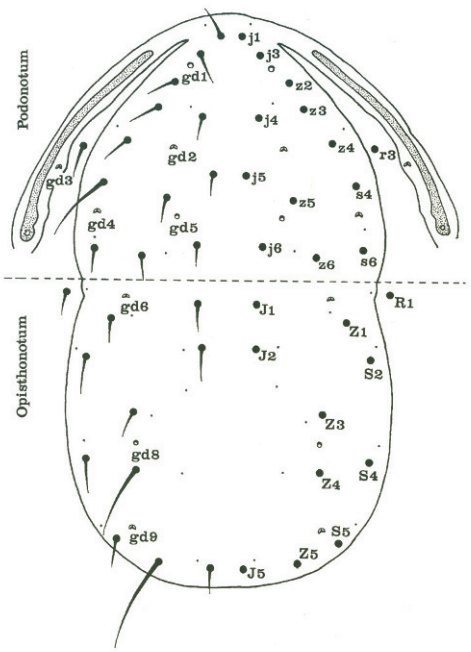

Figure 1 Chaetotaxy and adenotaxy of the dorsal shield of phytoseiid mites (reproduced from Swirski et al., 1998).

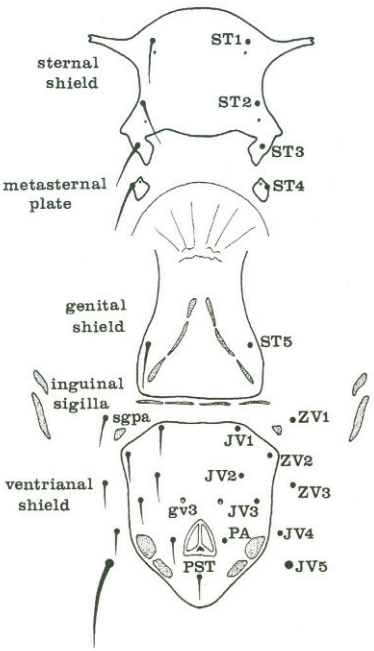

Figure 2 Chaetotaxy and adenotaxy of the ventral shields of phytoseiid mites (reproduced from Swirski et al., 1998).

Table 1 Primers used and PCR conditions for each fragment sequenced..

Fragments	Primers	PCR conditions
18S fragment 2	5'-3' CAAATCACTCCACCAACTAA	Step1: 92 °C, 1 min
R	5'-3' TCCGTAGGTGAACCTGCGGA	Step2: 92 °C, 15 s
		Step3: 54 °C, 45 s
		Step4: 72 °C, 1 min
		Go to step 2; 30 times
		Step 5: 72 °C, 7 min
COI fragment 1	5'-3' TGATTTTTTGGTCACCCAGAAG	Step1: 95 °C, 1 min
	5'-3' TACAGCTCCTATAGATAAAAC	Step2: 92 °C, 1 min
		Step3: 45 °C, 1 min
		Step4: 72 °C, 1 min
		Go to step 2; 40 times
		Step 5: 72 °C, 5 min
COI fragment 2	5'-3' GGTCAACAAATCATAAAGATATTGG	Step1: 95 °C, 1 min
	5'-3' TACAGCTCCTATAGATAAAAC	Step2: 92 °C, 1 min
		Step3: 50 °C, 1 min
		Step4: 72 °C, 1 min
		Go to step 2; 30 times
		Step 5: 72 °C, 5 min
ITS-5.8S	5'-3' AGAGGAAGTAAAAGTCGTAACAAG	Step1: 92 °C, 1 min
	5'-3' ATATGCTTAAATTCAGGGGG	Step2: 92 °C, 15 s
		Step3: 50 °C, 45 s
		Step4: 72 °C, 1 min
		Go to step 2; 30 times
12S	5'-3' TACTATGTTACGACTTAT	Step1: 95 °C, 1 min
	3'-5' AAACTAGGATTAGATACCC	Step2: 94 °C, 30 s
		Step3 :40 °C, 30 s
		Step4: 72 °C, 1 min
		Goto step 2; 35 times
		Step 5: 72 °C, 5 min

Table 2 ANOVA results, means of 42 characters of the 10 strains of *Neoseiulus californicus* studied. Means within a character followed by different letters differ significantly (based on Newman-Keuls test).

	DSL	**DSW**	**Lleg1**	**Lleg2**	**Lleg3**	**lleg4**	**j1**	**j3**
Tunisia	361.66b	164.69a	315.91a	260.90abcd	263.73ab	349.48	21.08de	30.83cd
Spain	364.83b	147.37c	312.37abc	259.21abcd	256.78b	348.37	20.86de	29.90d
Chile	372.48b	161.05a	311.27abc	259.11abcd	260.62ab	354.62	24.13a	35.05a
France	367.97ab	155.06b	316.09a	264.80a	264.92a	350.06	22.34bc	32.65b
Sicily	369.04ab	155.25b	314.87a	262.74ab	258.97ab	343.25	22.15bc	32.77b
Koppert	366.45ab	150.30bc	307.90bc	255.87bcd	256.73b	344.93	22.37bc	31.47bc
Florence	361.97ab	150.06bc	306.67c	253.13d	257.15b	349.20	20.44e	28.30e
Greece	369.74ab	162.10a	312.70abc	261.93abc	263.11ab	349.76	21.61cd	29.91d
Japan	362.74a	150.03bc	313.99ab	254.23c	259.92ab	351.38	22.61b	30.31cd
Brazil	371.68a	150.06bc	313.13ab	261.06abcd	261.58ab	342.20	22.78b	31.60bc
SD	2.12	1.56	1.57	1.93	1.75	3.23	0.25	0.36
$F_{9,290}$	3.5	15.31	4.1	3.9	2.9	1.4	17.86	27.54
P	0.00040	<0.001	0.00007	0.00012	0.00288	0.18845	<0.001	<0.001
	z2	**z4**	**s4**	**Z1**	**S2**	**S4**	**S5**	**Z5**
Tunisia	26.98d	28.19ef	35.58bc	33.05b	41.12bc	37.19a	31.25b	74.50a
Spain	26.73d	28.76def	33.51d	30.48cd	37.66e	34.74b	29.18c	69.01bc
Chile	33.50a	33.26a	40.16a	37.89a	44.04a	37.61a	32.45ab	67.34c
France	31.09b	31.20bc	36.38bc	32.61bc	41.03bc	37.97a	30.93b	70.89b
Sicily	29.74bc	31.80b	37.21b	33.48b	41.53b	37.04a	32.02ab	70.40b
Koppert	29.32bc	29.73cde	36.72bc	31.88bc	39.84cd	38.56a	33.04a	71.20b
Florence	26.10d	25.86g	31.49e	29.27d	36.09f	32.76c	31.45ab	67.04c
Greece	27.79cd	27.78f	33.47d	31.63bc	38.92d	37.71a	32.51ab	70.09b
Japan	27.62cd	28.28ef	33.89d	30.54cd	37.35e	37.03a	31.85ab	67.58c
Brazil	29.29bc	30.01cd	35.05cd	31.92bc	39.35d	37.71a	31.13b	70.70b
SD	0.56	0.45	0.48	0.59	0.48	0.49	0.41	0.63
$F_{9,290}$	16.23	23.69	25.57	15.57	31.49	12.70	6.96	12.9
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.00001	<0.001
	j4	**j5**	**z5**	**j6**	**J2**	**Z4**	**J5**	**r3**
Tunisia	22.23bcd	22.31bc	22.23bc	27.13	32.19c	50.68c	12.51bcd	22.97d
Spain	21.53cd	21.42c	21.44c	25.83	30.69d	49.93c	12.57bcd	24.08cd
Chile	24.31a	23.59a	23.76a	29.81	35.01a	52.11b	11.86d	28.27a
France	22.75bc	22.79ab	22.70b	27.69	32.28c	53.86a	12.48bcd	24.25bcd

Table 2 Continued

	j4	j5	z5	j6	J2	Z4	J5	r3
Sicily	22.70bc	23.01ab	22.47bc	27.55	33.65b	53.84a	12.21cd	25.42b
Koppert	21.23d	22.01bc	21.73bc	33.54	31.30cd	53.53ab	13.03ab	25.48b
Florence	19.26e	19.33d	19.59d	23.28	28.86e	47.47d	12.49bcd	23.41d
Greece	22.18bcd	22.02bc	22.22bc	27.04	31.28cd	52.32ab	12.40bcd	23.33d
Japan	21.85bcd	22.28bc	22.17bc	26.29	30.29d	48.50d	13.43a	25.22bc
Brazil	23.09b	22.90ab	22.60b	27.74	32.30c	50.35c	12.71bc	25.17bc
SD	0.31	0.32	0.27	2.5	0.37	0.43	0.18	0.33
$F_{9,290}$	18.10	13.72	16.02	1.169	22.30	27.4	5.66	21.12
P	<0.001	<0.001	<0.001	0.31	<0.001	<0.001	<0.00001	<0.001
	R1	**st1-st1**	**st2-st2**	**st3-st3**	**st1-st3**	**st4-st4**	**st5-st5**	**st2-st3**
Tunisia	22.12d	49.73c	60.95bc	70.97abcd	66.54ab	77.10c	69.83ab	27.39ab
Spain	21.82d	50.01c	57.24f	69.07cd	65.13bc	84.27bc	67.79bc	27.13ab
Chile	25.72a	53.24a	62.24a	71.85ab	67.04a	89.86ab	69.31ab	27.34ab
France	22.58d	50.19c	59.37de	71.04abcd	66.44ab	82.17bc	69.77ab	27.59ab
Sicily	23.50c	49.87c	62.01ab	72.17a	66.37ab	83.41bc	68.65ab	27.88a
Koppert	22.54d	49.91c	59.05de	69.63bcd	65.69abc	85.83abc	68.59ab	27.18ab
Florence	21.95d	49.03c	59.91cd	69.27cd	64.18c	90.91ab	65.76c	26.06d
Greece	22.00d	49.77c	60.36cd	71.36abc	65.29bc	79.55c	71.13a	27.58ab
Japan	23.93bc	49.53c	58.42e	68.92d	65.56abc	93.62a	65.75c	27.87a
Brazil	24.47b	51.35b	59.28de	71.28abc	64.40c	94.34a	69.79ab	26.78c
SD	0.27	0.29	0.38	0.56	0.38	2.14	0.65	0.23
$F_{9,290}$	23.54	16.9	16.5	4.7	6.2	6.93	7.2	5.7
P	<0.001	<0.001	<0.001	0.000008	<0.00001	<0.00001	<0.00001	<0.00001
	Lmetap1	**Wmetap1**	**Lmetap2**	**LPVA**	**WPVA1**	**WPVA2**	**JV5**	
Tunisia	29.43bc	5.45bc	12.22c	117.28b	107.97ab	74.56ab	51.45cd	
Spain	27.67e	5.61bc	12.53ab	112.75c	102.29cd	71.75bc	50.38d	
Chile	32.27a	5.68b	11.63c	121.94a	105.31abc	73.42ab	56.10a	
France	30.06b	5.13c	12.56ab	116.00bc	104.66bc	74.20ab	53.93ab	
Sicily	28.62cd	6.72a	11.57c	120.21a	100.68d	72.52abc	54.36ab	
Koppert	28.12cd	5.50bc	11.97c	116.64bc	106.39ab	73.97ab	55.92a	
Florence	28.44cd	5.54bc	11.52c	113.24bc	99.57d	75.19ab	49.62d	
Greece	29.99b	5.05c	11.96c	115.63bc	102.74cd	73.74ab	51.28cd	
Japan	28.46cd	5.12c	13.39a	116.33bc	99.79d	70.00c	50.27d	
Brazil	32.72a	5.06c	12.28c	116.01bc	108.45a	75.75a	53.32bc	
SD	0.39	0.13	0.28	0.98	0.91	0.86	0.69	
$F_{9,290}$	19.37	13.58	4.29	8.1	12.7	3.89	11.74	
P	<0.001	<0.001	0.00003	<0.00001	<0.001	0.00011	<0.00001	
	Lsper	**Wsper**	**StIV**					
Tunisia	10.93b	10.32bc	50.62a					
Spain	11.15ab	11.01ab	48.85ab					
Chile	11.44ab	9.66c	48.90ab					
France	11.61ab	9.75c	51.55a					
Sicily	11.60ab	9.73c	50.17a					
Koppert	11.97a	10.77b	45.39b					
Florence	11.78ab	10.88b	50.13a					
Greece	11.87a	10.83b	47.76ab					
Japan	11.89a	11.15ab	48.92ab					
Brazil	11.40ab	11.69a	48.99ab					
SD	0.21	0.21	0.94					
$F_{9,290}$	2.70	10.31	3.17					
P	0.00503	<0.00001	0.00116					

the highest values. A significant correlation was observed between body length and the other characters studied (r^2 = 0.67, $F_{41,214}$ = 13.94, P<0.00001). However, this correlation was only significant for the following characters: leg 1 length, st2-st2 distance, st5-st5 distance, and ventrianal shield length and width. No significant correlation was observed between body and setae lengths, except for setae Z5, but the correlation was negative. This suggests that the biggest specimens are not those that bear the longest setae.

The results of the discriminant analysis show that very few individuals are incorrectly classified in their original strain (Table 3). All the factors measured allow the discrimination of the 10 strains with 5% error. The Greek strain was relatively the worst classified (six specimens out of 30 studied were not included in their original group). Among the 42 characters studied, only 30 are significant: body length and width, lengths of leg 1 and 3, length of setae j1, z4, s4, S2, S4, Z5, j4, J2, Z4, J5, r3, R1, and JV5, distances st1-st1, st2-st2, st1-st3, and st2-st3, metapodal plate 1 length and width, metapodal plate 2 length, ventrianal shield widths and length, and the spermatheca width and length. Another discriminant analysis was thus carried out only with these 30

Table 3 Classification given by discriminant analysis with 42 characters on 10 strains of *Neoseiulus californicus.* The percentage of individuals that were correctly classified in their original strain is given in the first column of the table.

	% correct	Spain	Chile	France	Sicily	Koppert	Firenze	Greece	Japan	Brazil	Tunisia
Spain	93.3	**28**	0	1	0	0	1	0	0	0	0
Chile	100	0	**30**	0	0	0	0	0	0	0	0
France	96.7	0	0	**29**	0	1	0	0	0	0	0
Sicily	100	0	0	0	**30**	0	0	0	0	0	0
Koppert	93.3	1	0	0	0	**28**	0	0	0	1	0
Firenze	96.7	0	0	0	0	0	**29**	0	1	0	0
Greece	80	2	0	2	0	0	1	**24**	0	0	1
Japan	100	0	0	0	0	0	1	0	**30**	0	0
Brazil	96.7	0	0	0	0	0	0	0	0	**29**	1
Tunisia	93.3	0	0	0	0	1	0	1	0	0	**28**
Total	95	31	30	32	30	30	31	25	31	30	30

Table 4 Distances of Jukes and Kantor for mt-COI (fragment 1) for the 5 strains and 8 specimens of *Neoseiulus californicus.*

	Brazil	Brazil	Japan	Japan	Spain	Firenze	Firenze	Chile
Brazil	-							
Brazil	0.008	-						
Japan	0.008	0	-					
Japan	0.008	0	0	-				
Spain	0.008	0	0	0	-			
Firenze	0.008	0	0	0	0	-		
Firenze	0.008	0	0	0	0	0	-	
Chile	0.025	0.016	0.016	0.016	0.016	0.016	0.016	-

significant variables. Again, only few individuals are not well classified in their original strain. The 30 variables would permit a good classification of the individuals with less than 6% error. Therefore, focusing only on these 30 morphological characters seems sufficient to allow diagnosis of an individual of any of the 10 strains. The discriminant analysis thus established a model that allows classification of any new individual measured from these 10 strains. To assess the success of this classification, blind tests have been performed, randomly taking specimens from the strains and we obtained successful classification with less than 5% error. However, carrying out blind tests with other strains will only give the proximity of these individuals to the 10 strains.

A multifactorial canonical analysis, carried out with the 30 significant characters (Fig. 3), shows that the strain from Chile and, to a lesser extent, the strain from Spain are separated from the other ones and from each other. The other eight strains are also better separated from each other than with the 42 characters. However, this separation is very weak, as shown in Figure 3.

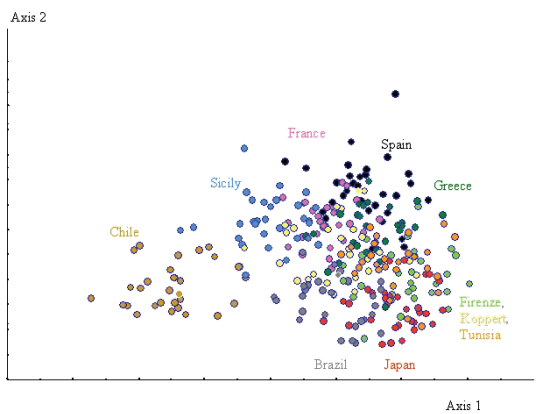

Figure 3 Canonical analysis representation of the individuals belonging to the 10 strains of *Neoseiulus californicus* with an analysis carried out with 30 characters.

The dendrogram based on euclidian distances between the strains is shown in Figure 4. The strain from Chile is clearly separated from the others, as well as the strains from Brazil and Sicily. Three other groups can be seen: (1) Firenze, Tunisia, Japan, (2) France, Koppert, Greece, and (3) Spain. No correlation between geographic and euclidian distances was noticed.

Molecular study

The 18S r-DNA fragment 1 (323 nucleotides aligned). No variability was observed between the three strains tested (Chile, Italy, and France).

The ITS-5.8S r-DNA fragment (333 bp aligned). The divergence rates between the strains from Sicily and Italy ranged between 0.3 and 0.6%.

The mt-DNA COI fragment 2 (339 bp aligned). Again, very low nucleotide divergence was observed between the strains from Chile and Italy (0.3%).

The mt-DNA COI fragment 1 (285 bp aligned). Five strains and eight specimens were tested. The results are presented in Table 4. The nucleotide divergence was very low both between and within the strains, without any differences between these two taxonomic levels. The strain from Chile was genetically the most distant from the others.

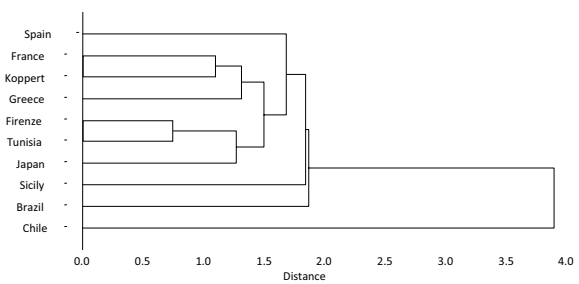

Figure 4 Dendrogram based on euclidian distances between the 10 strains of *Neoseiulus californicus.*

For some specimens of each strain, two DNA fragments were obtained after amplification. As the presence of one or two bands could be used as a diagnostic tool to separate *Typhlodromus exhilaratus* and *T. phialatus* (Tixier et al., 2006), this mtDNA fragment was amplified for 30 specimens of the 10 strains in order to determine the frequency of occurence of one/two bands for each strain. Some strains have similar frequency of occurrence of one band: Greece, Japan, Koppert, and Spain (low frequency: <33%). Another group, containing France, Firenze, Sicily, and Tunisia, showed a high frequency of one band (>33%) (Table 5). The strains from Chile and Brazil had intermediate values. These differences are, however, too tiny to base a diagnostic on.

The 12S mtDNA fragment (329 bp aligned). The genetic distances between the 50 specimens ranged between 0 and 1.7%. The range of variation is again very low. Only two specimens (one from Chile, one from Greece) are separated (with 1.4 and 1.7%, respectively) from the other individuals. The distances between all the other individuals ranged between 0 and 0.6%. The genetic distances within strains were 0-0.5%. The strains from Chile and Greece had a value of 0.5%, the strain of Brazil of 0.1%, and for the other strains no intra-strain variation was observed. The genetic distances between the 10 strains were 0-0.4%. The strains from Greece and Chile were the most separated from the other ones, even when the genetic distances were low (Table 6).

When a phylogenetic parsimony analysis was performed, the cladogram showed that the specimens from the 10 strains are mixed, and harboured no genetic structure. It is thus impossible with the 12S fragment to discriminate between the 10 strains studied.

DISCUSSION

This study is the first for mites in the family Phytoseiidae, based on so many morphological characters and on so many strains of the same species. In this sense, this study provides a more precise definition of the species *N. californicus*.

Very low intra-population variation was observed for all characters measured, indicating a great homogeneity within the strains. The ranges of variation between geographically very distant strains were also low. This homogeneity of morphological characters is quite surprising for such remote populations with low expected genetic exchange. However, even if the differences were small, morphological characters allowed a good separation between the 10 populations studied, especially due to the intra-strain homogeneity. Some characters seem to be more relevant for the separation of populations. No single character allows the separation between all strains and the combination of 30 morphological characters proved useful. This combination could thus be used as a basis for a diagnostic model construction.

Some strains are particularly well separated from others, especially the strains from Chile and Spain. The differences in setal length observed between the strains were independent of body lengths, showing that these differences are not correlated to mite size. No correlation between strain proximity and geographic distances was observed. Furthermore, it is hard to explain the similarities observed between the 10 strains, as no correlation between strain proximity and host plant or prey were noted, either.

The molecular experiments conducted are new for the species *N. californicus* and even for the whole Phytoseiidae family. The nucleotide divergences obtained between the strains with the five molecular markers tested were too small to distinguish between them. This study confirms that the region 18S is too conserved to assess species and population differences for mites in the Phytoseiidae, a conclusion which also applies to other organisms (Hillis & Dixon, 1991; Cruickshank, 2002; Cruickshank & Thomas, 1999). The ITS1-5.8S-ITS2 fragment has been used before to discriminate between two species of Phytoseiidae (Tixier et al., 2004; 2006). The ITS2 fragment was the most studied, especially for assessing intra- and interspecific differences, for example for tetranychid mites (Navajas et al., 1994, 1999; Gotoh et al., 1998) and for the genus *Chorioptes* (*C. bovis* and *C. texanus*) (Ochs et al., 1999). However, the present results show very low intraspecific variation for this fragment. The 12S rRNA gene has been poorly used for Acari. Jeyaprakash & Hoy (2002) showed a divergence of 10% between two morphologically close species of the genus *Neoseiulus*: *N. fallacis* (Garman) and *N. californicus*. We thus expected more intraspecific variation than presently observed. However, once again, this mtDNA marker does not allow for differentiation between the strains studied.

The more surprising result concerns the very low genetic variation observed with the COI mtDNA fragments commonly used for intraspecific studies (Navajas et al., 1994, 1998, 1999). Salomone et al. (2002) for instance showed a level of divergence of 7.8% beween populations of *Steganacarus carlosi* Niedbala. This intraspecific variation exceeded the divergence observed for *N. californicus*. The

Table 5 Frequencies of occurrence of one band after mtCOI fragment amplification of 30 individuals per strain of *Neoseiulus californicus*.

Greece	8	Brazil	12
Japan	9	Sicily	14
Koppert	9	Tunisia	14
Spain	9	France	15
Chile	11	Firenze	17

Table 6 Mean distances of Jukes and Kantor for the 12S fragment for the 10 strains (five specimens per strain) of *Neoseiulus californicus.*

	Tunisia	Sicily	Greece	Firenze	Brazil	Chile	Japan	Koppert	France	Spain
Tunisia	-									
Sicily	0.001	-								
Greece	0.004	0.003	-							
Firenze	0.001	0.001	0.003	-						
Brazil	0.001	0.001	0.004	0.001	-					
Chile	0.004	0.003	0.004	0.003	0.004	-				
Japan	0.001	0.001	0.003	0.000	0.001	0.003	-			
Koppert	0.001	0.001	0.003	0.000	0.001	0.003	0.000	-		
France	0.001	0.001	0.003	0.000	0.001	0.003	0.000	0.000	-	
Spain	0.001	0.001	0.003	0.000	0.001	0.003	0.000	0.000	0.000	-

absence of nucleotide divergence between the 10 strains could be due to great genetic exchange among strains and high dispersal of *N. californicus* or/and to a low rate of genetic change of the strains after their collection from the original site.

This lack of molecular differentiation is also surprising as morphological differentiation has been possible and as the strains were geographically distant. Differences between the levels of variation with morphological and molecular markers could be due to a high morphological plasticity linked to environmental adaptation. This adaption does not seem to involve genetic plasticity and is thus probably labile. In the future, it would be interesting to repeat the morphological measurements at different dates in order to see if the morphological differences are being conserved.

In conclusion, this study has shown that it is difficult to develop diagnostic tools to separate strains of *N. californicus*. However, the 30 morphological markers selected could be used to construct a diagnostic model. The amount of morphological data (1,260 measurements) on *N. californicus* could then constitute a reference for the description of this species, that will be useful for scientists but also for commercial firms involved in production, quality, and traceability of natural enemies in pest management. It is the first time that five genes were tested to assess the intraspecific variability in a phytoseiid mite. The sequences are kept in a database and can be used for taxonomic studies by acarologists all over the world. Moreover, the differences between the levels of variation observed for morphological and molecular markers raised questions about the plasticity of morphological characters and their validity for species identification for *N. californicus* and phytoseiid mites in general.

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Identification of a drought-adapted *Neoseiulus californicus* strain: egg hatchability, juvenile survival and oviposition at low humidities

A. Walzer, M. Castagnoli[1], S. Simoni[1], M. Liguori[1], E. Palevsky[2] & P. Schausberger

Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources & Applied Life Sciences, Peter Jordanstrasse 82, A-1190 Vienna, Austria. E-mail: andreas.walzer@boku.ac.at; [1]Istituto Sperimentale per la Zoologia Agraria, via di Lanciola 12/A, Firenze 50125, Italy; and [2]Department of Entomology, Agricultural Research Organization, Ministry of Agriculture, PO Box 1021, Ramat Yishay 30095, Israel

Biological control of spider mites (Acari: Tetranychidae) under dry conditions is a serious problem, which cannot be solved with the currently available strains of predatory mites (Acari: Phytoseiidae). However, there is considerable intraspecific variability in the degree of adaptation to low humidity, allowing the screening for a drought-resistant strain. Due to its plasticity in biological and ecological traits *Neoseiulus californicus* qualified as a suitable species for strain screening. Egg hatchability, juvenile survival and oviposition rate of eight *N. californicus* strains were evaluated at three or four humidity levels (ranging from 64.0 to 75.6% r.h. at 25 °C). Strains came from Hérault/France (FR), Tuscany/Italy (FL), Valencia/Spain (SP), La Cruz/Chile (CH), Sicily/Italy (SI), and California/USA (C-BOKU, C-ISZA, C-ARO). Pronounced intraspecific variation of the traits evaluated at 64.0% r.h. served as a basis to rank the strains and to determine three distinct groups. In the first group (SP, FL, C-ISZA) egg and juvenile survival probability was nearly zero. The second group (FR, CH, C-ARO) was characterized by low egg survival probability (0.14-0.18), but high survival probability of mobile juveniles (0.69-0.82). Strains of the third group had the highest egg and juvenile survival probabilities among the strains evaluated: 0.37 and 0.83 (SI), and 0.38 and 0.96 (C-BOKU), respectively. We conclude that the strains of the latter group are the most promising ones with respect to spider mite control under relatively dry conditions.

Key words: Biological control, humidity, intraspecific variation, life-history traits, *Neoseiulus californicus*, spider mites

Dry ambient conditions favour spider mite population growth (Acari: Tetranychidae) but not that of their main natural enemies, the phytoseiid mites (Acari: Phytoseiidae), a feature that may generate failures in biological control (Force, 1967; Stenseth, 1979; Ferragut et al., 1987). Currently, the phytoseiids *Phytoseiulus persimilis* Athias-Henriot, *P. longipes* Evans, *P. macropilis* (Banks), *Neoseiulus californicus* McGregor, *N. fallacis* (Garman) and *Galendromus occidentalis* (Nesbitt) are commercially available for biological control of spider mites in outdoor and protected crops (McMurtry & Croft, 1997). However, under dry ambient conditions the efficacy of the currently available phytoseiid species is often insufficient. Thus, there is an urgent need for a better-adapted species or strain of the above-mentioned species to solve this problem.

The sensitivity of phytoseiid mites to low humidity exhibits considerable inter- and intraspecific variation reflecting their adaptation to the climatic conditions in their natural habitat (McMurtry et al., 1976; Dinh et al., 1988; Perring & Lackey, 1989; Bakker et al., 1993; Schausberger, 1998). Apart from being tolerant to low humidities the ideal candidate should also meet other criteria, such as high predation capacity and rapid population increase enabling effective spider mite suppression. Furthermore, the desired species of predator should be able to establish on the plants and to provide long term spider mite control. These traits are typical for type-II diet generalists with a preference for spider mites (McMurtry & Croft, 1997) but exclude *Phytoseiulus* species, which are specialists with poor abilities to persist on plants with low spider mite densities (McMurtry & Croft, 1997; Schausberger & Walzer, 2001; Blümel & Walzer, 2002). Within the group of commercially available generalist phytoseiids, *N. californicus* seems the most suitable species for intraspecific comparisons among strains. *Neoseiulus californicus* exhibits an effective functional and numerical response to densities of *Tetranychus urticae* Koch (Tetranychidae) (Friese & Gilstrap, 1982; Castagnoli & Simoni, 1999). Furthermore, *N. californicus* is successfully used to control spider mites in outdoor and protected crops (Castagnoli & Simoni, 2003). It is able to persist on plants at low or negligible spider mite densities (Schausberger & Walzer, 2001). Additionally, this species naturally occurs in California, South America, North Africa and the Mediterranean basin (De Moraes et al., 2004), indicating its potential for adaptation to arid environments. Finally, some strains of *N. californicus* are highly resistant to pesticides (Croft, 1990; Castagnoli et al., 2005), allowing the use of *N. californicus* in integrated pest management systems.

This study was part of a multi-institutional project aiming at the identification of an *N. californicus* strain that is adapted to dry ambient conditions (Palevsky et al., 2006). It was based on the hypotheses that the humidity tolerance varies among *N. californicus* strains, and that the influence of humidity on life-history traits decreases during development from egg to adulthood. We conducted three separate experiments on egg hatchability, juvenile survival and oviposition of eight strains of *N. californicus* at three or four humidity levels.

MATERIAL AND METHODS

Strain origin and history, general methods

The experiments were conducted in three laboratories: in Austria (University of Natural Resources and Applied Life Sciences, BOKU), Israel (Agricultural Research Organisation, the Volcani Center, ARO), and Italy [CRA-Istituto Sperimentale per la Zoologia Agraria (ISZA)]. The rearing and experimental protocols were strictly standardized among laboratories. Eight strains of *N. californicus* were screened for their

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Table 1 Origin and rearing histories of the *Neoseiulus californicus* strains used for experiments.

Strain acronym	Origin (company)	Source	Host plant	Sampling year
C-BOKU	Koppert/ Biotactics[1]	Mass-rearing		2004
C-ISZA	Koppert/ Biotactics[1]	Mass-rearing		2004
C-ARO	Koppert/ Biotactics[1]	Mass-rearing		2004
FR	Hérault/ France	Field	*Solanum melongena*	2004
FL	Tuscany/ Italy	Field	*Fragaria ananassa*	2004
SP	Valencia/ Spain	Field	*Fragaria ananassa*	2000
CH	La Cruz/ Chile	Field	*Phaseolus vulgaris*	2000
SI	Sicily/ Italy	Field	*Fragaria ananassa*	2004

[1]Obtained from Koppert (NL), but produced by Biotactics (CA, USA).

humidity tolerance levels, five of which were field collected strains and the other three strains were obtained from Koppert (The Netherlands), but derived originally from Biotactics (California, USA) (Table 1). Despite coming from the same source the latter three strains were considered separate strains because they were withdrawn from the mass rearing at different dates, with intervals of more than several weeks. Presumably, repeated harvesting and additions of field-collected predators constantly alter the gene pool in the mass rearing. Colonies of the *N. californicus* strains were maintained on artificial rearing arenas consisting of a tile resting on water-saturated foam in a plastic box half-filled with water. The edges of the tile were covered with moist tissue paper to confine the predators to the arena. The predators were fed at 2–3-day intervals by adding bean leaves infested with *T. urticae* to the arena (McMurtry & Scriven, 1965) – at BOKU and ISZA the spider mites were green, but at ARO they were reddish, sometimes referred to as *T. cinnabarinus* (Boisduval), a junior synonym of *T. urticae*. To create an additional barrier for the mites the edge of the plastic box was lubricated with Tanglefoot™. Additionally, the smaller plastic box was placed in a larger box, containing water and dish detergent. Each strain was housed in a different room.

Closed cages were used as experimental units, consisting of circular acrylic cells of 15 mm diameter and 3 mm height each with a fine mesh screen at the bottom and closed at the upper side by a microscope slide (Schausberger, 1997). Stable humidity values were created by using salt solutions (saturated and unsaturated) in sealable boxes (Winston & Bates, 1960). The closed experimental cages were put on a grid above the salt solution in the boxes. The boxes were placed in a climatic chamber at constant environmental conditions (25 °C, L16:D8, 60 ± 5% r.h.). Humidity was continually monitored with calibrated data loggers placed inside the boxes during the experiments. To make the results comparable with studies published by others, humidity levels are presented both in relative humidity (r.h.) and absolute humidity values (saturation deficit, SD). SD was calculated by the equation SD = SVP * [1-(RH/100)], where SVP (saturation vapour pressure) is a constant related to temperature and atmospheric pressure. Under our experimental conditions (25 °C, at sea level) SVP = 3.17 kPa.

Table 2 Salt solutions with corresponding relative and absolute humidity values.

Solutions	Relative humidity (% ± SD)	Saturation deficit (kPa)
NaCl[1]	75.6 ± 0.7	0.8
NaCl + KCl[2]	72.4 ± 1.0	0.9
NaCl + sucrose[2,3]	68.6 ± 1.1	1.0
NaCl + sucrose[2]	64.0 ± 1.1	1.1

[1]used only in the egg hatch experiment; [2]used in all experiments; [3]unsaturated salt solution.

Egg hatch

Neoseiulus californicus eggs (<12 h) were placed in groups of 20 to 40, with each group in a different closed cage. Care was taken that the eggs did not touch each other. The state of the eggs (shrivelled, hatched, not hatched) was checked every 24 h at four humidity levels until the eggs hatched or died (Table 2). Per humidity level and strain 150-250 replicates were conducted.

Juvenile survival

Single *N. californicus* eggs (24-36 h old) were placed in closed cages and provided with a surplus of spider mites (*T. urticae*) at three humidity levels (Table 2). Survival and developmental progress were recorded at intervals of 8 and 16 h until the juveniles reached adulthood or died. Per strain and humidity level 20-37 replicates were conducted.

Oviposition

Single couples consisting of a female deutonymph and an adult male were placed in closed cages and provided with a surplus of spider mites (*T. urticae*) at each of three humidity levels (Table 2). The developmental progress of the females was observed every 24 h. After the first *N. californicus* egg was laid the males were removed and oviposition of the females was recorded every 24 h over a period of 10 days. The eggs deposited by the females in our experiments were removed daily. For every strain and humidity, 16-22 replicates were conducted.

Statistical analysis

Using SPSS 11.0 (Bühl & Zofel, 2002) the analysis of the life-history traits evaluated for the *N. californicus* strains was conducted in two steps. First, the effects of strain and humidity were assessed for each life history trait. Second, if humidity and strain had a significant influence, each trait was analysed within each strain among the humidity values. Additionally, we compared each trait among strains at 64.0% r.h. (1.1 kPa) and ranked the strains in relation to the analysed trait under dry conditions. If neither statistical analysis revealed an effect of strain affiliation, nor humidity, nor an interaction of both factors, the data were pooled over the non-significant factor.

In experiment 1, egg hatch probability was compared among strains and humidities using contingency tables. Pairwise comparisons among strains at 64.0% r.h. (1.1 kPa) followed (χ^2 tests). Probit analysis was used to calculate the r.h./SD values allowing 50% survival of the eggs. In experiment 2, juvenile survival functions (combination of survival time and cumulative survival) were analysed using the Kaplan-Meier procedure. Within this procedure the survival functions were compared among the strain-humidity combinations using Breslow tests. In experiment 3, the effects of strain and humidity on the oviposition rates were analysed by two-factor ANOVAs and post-hoc Bonferroni tests.

RESULTS

Egg hatch

Egg hatch probability was significantly influenced by strain (χ^2 = 24.1, d.f. = 7, P = 0.001) and humidity (χ^2 = 112, d.f. = 3, P<0.001). The RH_{50} (SD_{50}) values, i.e., humidities at which 50% die, ranged from 65.6% (1.09 kPa) to 70.3% (0.94 kPa) (Table 3). For strain ranking we analysed the egg hatch probabilities at 64.0% (1.1 kPa). The eggs of the SI (0.38) and C-BOKU (0.37) strains were much less sensitive to dry conditions than those of the other strains, followed by the eggs of the C-ISZA (0.22), C-ARO (0.18), and CH (0.18) strains. Eggs of the FR strain (0.14) had similar hatching probabilities as the eggs of the C-ARO, CH, and FL (0.07) strains. Egg hatch probability of the SP strain (0.01) was nearly zero and significantly lower than in all other strains (Fig. 1).

Juvenile survival

Both humidity and strain had an effect on the survival function (combination of survival time and cumulative survival) of juveniles (humidity: U = 58.08, d.f. = 2, P<0.001; strain: U = 57.88, d.f. = 7, P<0.001). Humidity did not influence juvenile survival of the strains C-BOKU, C-ARO, and SI, which had survival probabilities of 0.82-0.96. Survival functions differed among humidities within the strains CH, FR, C-ISZA, and SP and were the lowest at 64.0% (1.1 kPa). Similarly, the survival of the juveniles from the FL strain was lower at 64.0% (1.1 kPa) than at other humidity levels. However, at 72.4% (0.9 kPa) juvenile survival of this strain was lower than at 68.6% (1.0 kPa) (Table 4). Overall, the juvenile survival function was mainly determined by mortality in the larva and protonymph stage; mortality rate of deutonymphs was nearly zero in all strains and at all humidities tested. Larvae and protonymphs of the strains SI, C-BOKU, CH, C-ARO, and FR had high proportions of survivors (larvae: 0.72-1.00, protonymphs: 0.90-1.00) irrespective of humidity, whereas larvae and protonymphs of the strains C-ISZA, SP, and FL had rather low survival probabilities at 64.0% r.h. (1.1 kPa) (larvae: 0.39-0.46; protonymphs: 0.55-0.67).

Strain comparison at 64.0% r.h. (1.1 kPa) revealed high survival functions in juveniles of the strains C-BOKU, SI, C-ARO, and CH, with survival probabilities ranging from 0.81-0.95. Juvenile survival of the FR strain did not differ from the SI, C-ARO, and CH strains, but was lower than the corresponding data of the C-BOKU strain. Juveniles of the C-ISZA, SP, and FL strains had the lowest survival probabilities (0.21-0.31; see Table 4).

Oviposition

Oviposition rates were significantly affected by strain (F = 14.48, d.f. = 7, P<0.001), but not by humidity (F = 1.09, d.f. = 2, P = 0.34). The interaction between strain and humidity was not significant (F = 1.56, d.f. = 14, P = 0.087). The oviposition rate pooled over all humidities of the SP strain was significantly lower (1.56 eggs/female/day) than the corresponding rates of all other strains, followed by similar oviposition rates of the FL (2.12), SI (2.24), CH (2.39), and C-ISZA (2.48) strains. Females of the C-BOKU strain produced the highest number of eggs (3.12), followed by females of the C-ARO strain (2.81). However, the oviposition rate of the latter strain did not differ from those of FR (2.55), C-ISZA, CH, and SI.

Table 3 RH_{50} (SD_{50}) values, i.e. humidities at which 50% of the eggs of different *Neoseiulus californicus* strains die. See Table 1 for strain acronyms.

Strain	RH_{50} (%)	SD_{50} (kPa)
C-BOKU	65.55	1.09
SI	66.26	1.07
C-ISZA	66.95	1.05
FL	67.00	1.05
C-ARO	68.20	1.01
FR	68.52	0.99
CH	70.24	0.94
SP	70.28	0.94

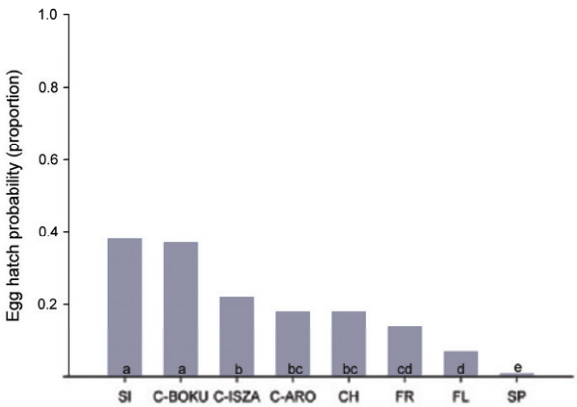

Figure 1 Egg hatch probabilities of *Neoseiulus californicus* strains at 64.0% r.h. (1.1 kPa). See Table 1 for strain acronyms. Different lower script letters in the bars indicate significant differences in the egg hatch probabilities among the strains (pairwise χ^2 tests, P<0.05).

Table 4 Survival functions [combination of survival time (days) and cumulative survival (proportion)] of single juvenile *Neoseiulus californicus* of different strains held in closed cages and provided with surplus spider mites for 4.5 days at 64.0% r.h. (1.1 kPa), 68.6% r.h. (1.0 kPa), and 72.4% r.h. (0.9 kPa). Means within each strain among humidities followed by the same lower case letter are not significantly different at P = 0.05. Means among strains at 64.0% r.h. (1.1 kPa) followed by the same capital letter are not significantly different at P = 0.05 (Kaplan-Meier analysis, pairwise Breslow tests). See Table 1 for strain acronyms.

	63.95% r.h. (1.14 kPa)			68.57% r.h. (1.00 kPa)			72.36% r.h. (0.88 kPa)		
Strain	Survival time	Survivors	Breslow test	Survival time	Survivors	Breslow test	Survival time	Survivors	Breslow test
C-BOKU	4.32	0.95	Aa	4.16	0.91	a	4.40	0.95	a
FR	3.45	0.70	ABa	3.85	0.80	ab	4.38	0.95	b
FL	2.34	0.21	ABa	4.04	0.82	b	3.15	0.52	c
SP	2.41	0.27	ABa	4.05	0.86	b	3.72	0.72	b
C-ISZA	2.59	0.31	Ba	4.38	0.94	b	4.37	0.94	b
C-ARO	4.05	0.86	Ca	4.28	0.90	a	3.91	0.82	a
CH	3.93	0.81	Ca	4.50	1.00	b	4.14	0.89	ab
SI	4.07	0.87	Ca	4.35	0.96	a	4.16	0.86	a

DISCUSSION

Our results on the sensitivity of *N. californicus* to low humidity can be summarized as follows. First, as we hypothesized, the influence of humidity on life-history traits decreased during ontogeny, with eggs being the most sensitive to dry conditions, followed by larvae and protonymphs. The performance of deutonymphs and adult females was strain-specific but not affected by humidity. Second, also in agreement with our hypothesis, there appears to be significant variability of the *N. californicus* strains in their performance at dry conditions.

Egg hatchability was influenced by both humidity and strain. At 64.0% r.h. (1.1 kPa) the egg survival probabilities ranged from 0.38 (SI) and 0.37 (C-BOKU) to 0.07 (FL) and 0.01 (SP). These huge differences in egg hatchability might have been mediated by different egg exposure times (= egg developmental time). Assuming that egg exposure time is the decisive factor influencing the egg hatch probability, embryonic developmental times should be negatively correlated with egg hatch probabilities. Alternatively, the key survival tactic for *N. californicus* eggs could be optimization of water conservation. An impervious nature of the egg chorion linked with low water content of the egg should lead to low respiration rates minimizing water loss. However, low respiration rates also lower the gas exchange of eggs, which should prolong egg developmental times (Walzer et al., 2007). The humidity values at which 50% of the *N. californicus* eggs failed to hatch (RH_{50}/SD_{50}) ranged from 65.6% (0.94 kPa) to 70.3% (1.09 kPa) at 25 °C. RH_{50} values of other phytoseiid species and *N. californicus* strains from the literature are not directly comparable with our data, because these experiments were partly carried out at other temperatures. We therefore converted the RH_{50} into SD_{50} values, which are directly related to temperature. Except for the very drought-resistant phytoseiid species *Neoseiulus idaeus* Denmark et Muma (SD_{50} = 1.77 kPa) and *G. occidentalis* (1.68), the SD_{50} values of other phytoseiids range from 1.20-0.39 kPa (Bakker et al., 1993; Croft et al., 1993; Castagnoli & Simoni, 1994; van Houten et al., 1995; Schausberger, 1998; De Courcy Williams et al., 2004). Within this range the SD_{50} values of all *N. californicus* strains tested here are in the upper third, indicating a species-specific adaptation to low humidities. The values for other *N. californicus* strains were 0.70 kPa (at 20 °C), 0.65 (at 21 °C), 0.98 (at 25 °C), and 1.02 (at 29 and 33 °C) (Bakker et al., 1993; Castagnoli & Simoni, 1994; De Courcy Williams et al., 2004), which are lower than the corresponding values of the SI (1.07 kPa) and C-BOKU (1.09 kPa) strains.

Regarding the influence of humidity on larval and protonymphal survival probabilities there are two distinct groups of strains. Larvae of the strains C-BOKU, SI, CH, and C-ARO had high survival probabilities irrespective of humidity, whereas larval survival probabilities of the strains FR, FL, C-ISZA, and SP decreased dramatically between 72.4% r.h. (0.9 kPa) (proportion of survivors: 0.87-1.00) and 64.0% r.h. (1.1 kPa) (0.39-0.73). Again, the exposure time (= larval developmental time) may be a crucial factor affecting the survival chances under dry conditions. Protonymphs of the strains C-ISZA, SP, and FL had low survival probabilities at 64.0% r.h. (1.1 kPa) (0.68, 0.60, and 0.55, respectively), whereas mortality of the protonymphs of the C-BOKU, C-ARO, FR, CH, and SI strains was nearly zero at all humidity values. A potential way to replenish water under dry conditions may be water intake via increased prey consumption.

Oviposition of the *N. californicus* strains was not influenced by humidity. The females may have compensated water loss through increased predation, as was previously shown for *P. persimilis* and *N. fallacis* females (Mori & Chant, 1966; Swift & Blaustein, 1980). Increased predation rates should lead to higher metabolic rates, which may shorten the longevity of the females. De Courcy Williams et al. (2004) assessed the longevity of *N. californicus* females supplied with a surplus of spider mites within a humidity range of 60-82% r.h. Female longevity was only 43.0 days at 60% but reached 69.2 days at 82% r.h. Statistical analysis, however, revealed no significant effect of humidity on female longevity, which may be attributed to the low number of replicates per treatment (n = 6).

Strain ranking

Comparison of the humidity-sensitive traits among strains at 64.0% r.h. (1.1 kPa) together with the oviposition rate as a humidity-insensitive trait creates a basis for identifying strains that are the most suitable for spider mite suppression under dry conditions. The eight *N. californicus* strains tested here can be classified into three groups. First, SP, FL, and C-ISZA are worst adapted to dry conditions due to high egg and juvenile mortality rates. Egg survival was nearly zero at 64.0% r.h. (1.1 kPa) in the strains SP and FL, whereas the egg survival probability of the C-ISZA strain (0.22) was the second highest among strains. However, the survival probability of mobile juveniles was only 0.31, which reduced the overall juvenile survival probability to ~0.07. Population growth of these strains should be negligible at dry conditions. The second group consists of the strains FR, CH, and C-ARO, which are characterized by low egg survival (0.14-0.18), but high survival rates of mobile juveniles and adult females. The capacity of population increase of these strains should be too low for effective spider-mite control under dry conditions mainly because of the high egg mortality rates. The third group consists of SI and C-BOKU , which had the highest egg survival probabilities at 64.0% r.h. (1.1 kPa) among all strains evaluated: 0.38 and 0.37, respectively. The considerably higher oviposition rate of the C-BOKU strain may give this strain an advantage over the SI strain with respect to efficacy in spider mite control. Overall, the two latter strains are the most promising candidates for biological control at dry conditions. It remains to be shown whether they are able to successfully suppress the spider mites in the field.

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Evolutionary and Ecological Acarology: Reproductive Behaviour and Sociality

Spider mites as study objects for evolutionary biology

Yutaka Saito
Laboratory of Animal Ecology, Research Faculty of Agriculture, Hokkaido University, Japan.
E-mail: yutsat@res.agr.hokudai.ac.jp

I consider spider mites, and other mite groups, to be excellent material for evolutionary ecology and genetic studies, because of their low mobility, rapid development, ease of rearing, diverse genetic systems, and variable ecology. Using spider mites, we have revealed various topics in evolutionary ecology: (1) spider mites have variable (communal social and sub-social) life types, thus variable ecologies on plant leaves; (2) cooperation and aggressiveness vary among spider mite species, making them promising material for the study of natural selection operating on behaviour; (3) despite their haplo-diploid genetic system, spider mites have retained female-limited recessive genes that cause inbreeding depression; (4) life-type diversity of spider mites has evolved partly through interactions with predators; and (5) speciation is thought to have occurred through a shift in host plants and the accompanying differences in predation pressure. Here, I review my 30 years of work to show the useful and unique aspects of spider mites and to point out problems that had – and still need – to be solved in order to learn how spider mites can become useful as an object for evolutionary study

Key words: Diversity, evolutionary ecology, sociobiology, bamboo mites, life types, *Stigmaeopsis*

Spider mites have become serious pests in agriculture and forestry, as a side-effect of the excessive use of artificial chemical compounds after World War II. Studies on this group have developed exponentially mainly because of their practical importance as pests. An important review by van de Vrie et al. (1972) and a seminal book by Jeppson et al. (1975) attracted much attention. However, most studies on spider mites were restricted to applied fields and only few studies on spider mite evolution had been published before 1980.

Evolutionary and behavioural ecology established new trends in the biological sciences during the 1970's, and have rapidly developed over the last two decades to use many different arthropods for testing hypotheses. However, genetics-related studies appear to be restricted to just a few model animals, such as *Drosophila*. Little attention has been given to the potential of spider mites as study objects. Mitchell's (1973) study of spider mite life history in connection with the r-K selection theory, Helle & Overmeer's (1973) work on genetics, as well as Potter et al.'s (1976) work on intrasexual competition among males were the first steps towards an evolutionary biology of spider mites.

After that, studies on spider mites have been reviewed in the book 'Spider mites, their biology, natural enemies and control' (Helle & Sabelis, 1985), published more than 20 years ago. In the same book, Crozier (1985), a non-mite researcher, stressed the availability of spider mites as model animals for studying evolutionary ecology and genetics. I also participated in this book, and addressed the diversity of spider mite life types in relation to their webbing and defecation habits. Krantz (1987) introduced the book in Trends in Ecology & Evolution, now one of the high impact journals in the field of animal ecology and evolution. By pure coincidence, Yamamura (1987) introduced Saito's (1986) first discovery of mite sociality in the same volume. This might have been the second time where spider mites were in the spotlight for evolutionary biologists. Since then, many important studies on sociobiology, evolutionary and behavioural ecology have been published.

Along with this brief history on the emergence of evolutionary biology of spider mites, I provide a review of my 30-years of work with the intention to show the unique and useful aspects of these mites and to point out the problems that had to be solved in order to learn how spider mites can become useful as an object for evolutionary study (more details appear in Saito, 2010).

Diversity of spider mites: descriptive and comparative studies

Saito (1979) and Saito & Ueno (1979) showed that there is much variation in life history patterns among spider mite species and discussed that such diversity might be explained by the spatio-temporal stability of host plants (Fig. 1). It was no more than an application of the well known r-K selection theory that governed the ecological sciences at that time, but its contribution was in showing the importance of diversity in spider mite life-types. At present, we know that life histories vary depending upon phylogeny, seasonality, anti-predator adaptations, and the persistence and quality of the host plant (Sabelis, 1985, 1991; Saito, 2010). By studying communities of spider mites on various plants, and especially on bamboo, in Japan, Saito (1982, 1985, 1995) showed that there is great interspecific variation in life styles expressed in the way they form colonies, exploit the plant surface, use silk, and deposit faeces (Fig. 2). These studies suggested that spider mites have evolved by selection pressures arising from physical features of the host plant, host-plant phenology, and the natural enemies that have been intensively studied with the aim to develop methods of biological control of spider mites (Chant, 1959; van de Vrie et al., 1972; Helle & Sabelis, 1985).

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Evolution of spider mite life types and behavioural traits: in search of the ultimate factors

Descriptions of diversity and global comparisons between species are necessary steps for the development of evolutionary ecological studies. However, as pointed out by Harvey & Pagel (1991), there is a great difficulty in studying evolutionary processes solely by such between-species comparisons without accurate phylogenic information. Several hypotheses on spider mite phylogeny have been proposed by Gutierrez & Helle (1985) from morphology and karyotypes, and by Navajas et al. (1996) and Sakagami (2002) from molecular information. These will partly support evolutionary studies, but they have not always been sufficient for this purpose, because they are not sufficiently comprehensive and suffer from inconsistencies.

This is why we had to restrict comparisons between sibling species to minimize effects of phylogenetic constraints. Because of such a restriction, we have focused mainly on *Stigmaeopsis* spp., previously known as the *Schizotetranychus celarius* species group (Saito et al., 2004). The genus *Stigmaeopsis* now includes seven species: *Stigmaeopsis longus* (Saito), *S. takahashii* Saito & Mori, and *S. saharai* Saito & Mori inhabit *Sasa* dwarf bamboo plants; *S. celarius* Banks inhabits bamboo plants (e.g., *Phyllostachys pubescens*) in Japan; *S. miscanthi* (Saito) (of which there are two forms; Saito & Sahara, 1999) inhabit Japanese pampas grass (= Chinese silver grass, *Miscanthus sinensis*; Gramineae) and occurs in Japan, Korea, China, and Thailand; and *S. nanjingensis* (Ma & Yuan) and *S. tenuinidus* (Zhang & Zhang), both inhabiting the Giant bamboo (*P. pubescens*) are known to be serious pests in bamboo plantations in China (Zhang & Zhang, 2000). These species commonly make woven nests on the undersurface of host plant leaves and live there more or less gregariously (Fig. 3).There is a great deal of nest size variation among these species (Fig. 4), which gives rise to the question as to why such variation in nest size evolved in these closely related species.

Nests made with solid and dense web undoubtedly function to protect the individuals within them against something. One of the hypotheses about the nest function was that the nests function as refuges from predators. Mori & Saito (2004) proved this by revealing that the nests of *Stigmaeopsis* spp. can more or less protect nest members from some predator species. However, a more important point of their discovery was variation in the protective effect of nests of different sizes: larger nests are vulnerable to the invasions by many predator species, whereas smaller nests can prevent more predator species from entering. If smaller nests are more effective as a refuge, the inevitable question is why there are species making larger nests. This is part of the more general question why nest size and structure diversified among species in evolutionary history.

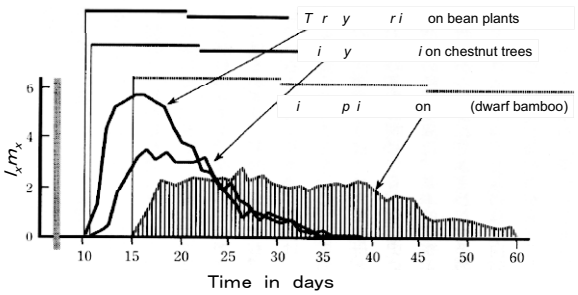

Figure 1 Various life histories known in Tetranychinae explained from the spatio-temporal persistence of host plants (25 °C, 50-60% r.h.; after Saito & Ueno, 1979).

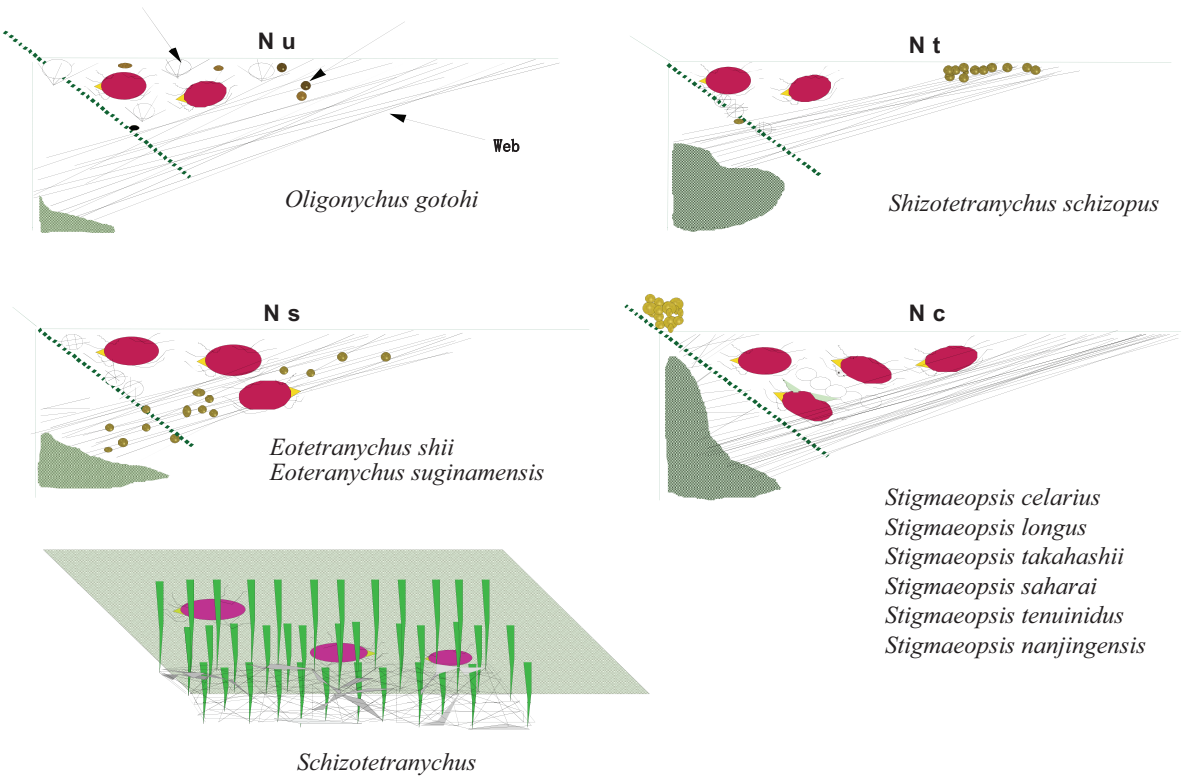

Figure 2 Several subtypes of WN life-type.

Table 1 Sociality of *Stigmaeopsis* species.

Stigmaeopsis	Nest size	Generation overlapping (= group size) for interaction	Counterattack efficiency	Waste management	Sociality level
longus	largest	3 generations	highest	outside nest by chemical cues	communal social
miscanthi HG	large	2-3 generations	high	inside nest by chemical cues	communal social
miscanthi LW	large	2-3 generations	high	inside nest by chemical cues	communal social
nanjingensis	medium-large	2-3 generations	high	inside nest, cues unknown	communal social?
celarius	medium	1-2 generations	medium	outside nest by tactile cues	subsocial
takahashii	small	very short	low	outside nest by tactile cues	subsocial
tenuinidus	small	very short	?	outside nest, cues unknown	primitive subsocial
saharai	smallest	very short	no effect	outside nest by tactile cues	primitive subsocial

Sociality development

Mite sociality

To understand the evolution of nest variation, sociality of *Stigmaeopsis* spp. needs to be addressed first. Mori & Saito (2005) showed that nest size is correlated with the development of sociality. Twenty years before their finding, Saito (1986a,b) reported counter-attack behavior in females and males of *S. longus*, a large-nest builder, against their specific predator, *Typhlodromus bambusae* Ehara. Based on the observation of bi-parental defence (Fig. 5) as well as on the cooperative nest building and utilization (nest sanitation) behaviours, he concluded that *S. longus* has a highly developed sub-sociality (termed 'communal sociality' by Mori & Saito, 2005). This was the first discovery of sociality in the Acari. After that, Saito (1990) and Saito (2010) observed that *S. miscanthi* also possesses a high ability to kill or drive away predators (Table 1). Mori & Saito (2005) indicated that as nests become smaller, the social behaviours are less pronounced. This means that there is a conflict between nest size and social development. Large nests harbour more individuals and can persist longer as a place where they live and feed. Saito (1986b) showed that as the defensive success increases with the density of adult mites in the nest, *S.*

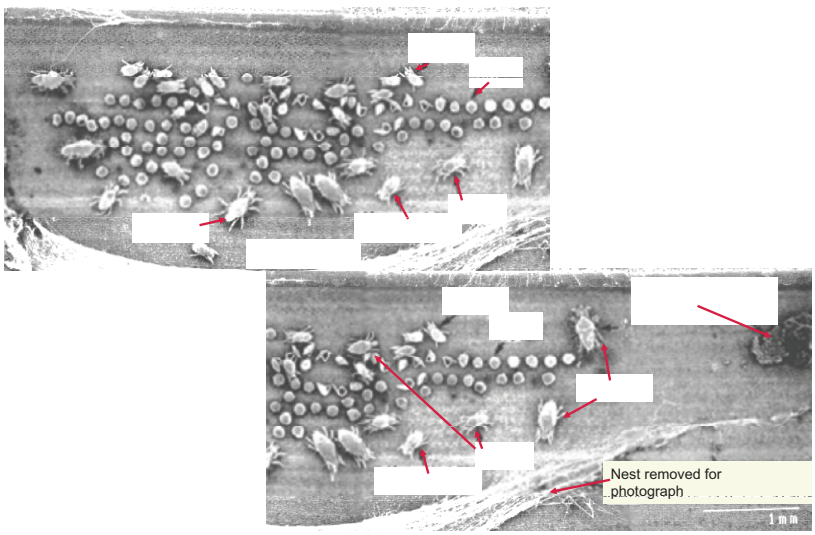

Figure 3 *Stigmaeopsis longus* inhabiting *Sasa* bamboo leaf (SEM photographs by Y Saito).

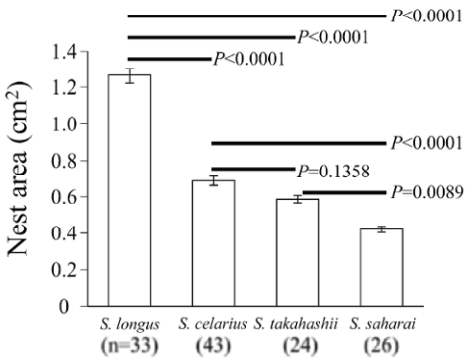

Figure 4 Variation in nest size (mean area ± SE) between *Stigmaeopsis* spp. (from Mori & Saito, 2004).

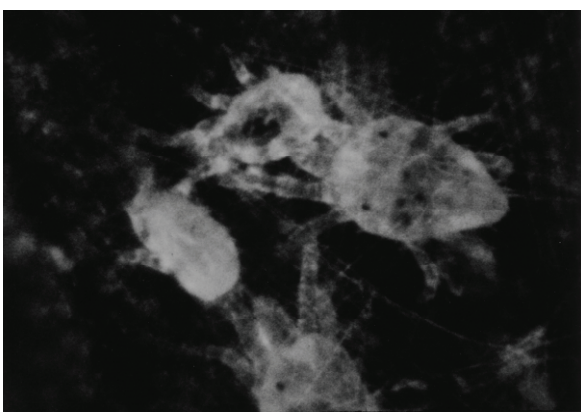

Figure 5 Defense by *Stigmaeopsis longus* males against *Typhlodromus bambusae*. The highest counterattack success was observed in the nests in which several males and females lived together.

longus and *S. miscanthi* enhance this efficiency by cooperative nest defence involving many nest members. Possibly, this nest defence enhanced by cooperation is sufficiently advantageous to overcome the fact that larger nests are more vulnerable to predator invasion.

Speciation through predation pressure and host plant shift

These two combinations of traits, namely large nests with cooperative sociality and small nests functioning to prevent predator invasion, might have advantages of their own and lead to divergent evolution of these mites. Therefore, Mori & Saito (2004, 2005) proposed the possibility of predator-mediated speciation. Figure 6 shows the outline of this process. If there is an ancient species with middle-sized nests and a mutation for making smaller nests occurs, the smaller-nest builder would have an advantage in lowering predation pressure. On the other hand, if another mutation to make larger nests occurs and it incorporates sociality, such a mutant also gets the advantage of lowered predation pressure. Therefore, two extreme strategies, one making the nest larger and another making it small, might be selected provided that all other things are equal. This scenario of predator-mediated speciation may apply to some *Stigmaeopsis* spp.

An alternative mode of speciation is hypothesized for *S. miscanthi* living on *Miscanthus* grass and *S. longus* on *Sasa* bamboo. Both are large-nest builders, and both are thought to have diverged by a host plant shift. Three kinds of evidence support this host shift hypothesis. Phylogenic analysis based on ribosomal DNA (28S) variation between *Stigmaeopsis* species suggests that *S. miscanthi* and *S. longus* might have originated from a common ancestor inhabiting *Sasa* bamboo and/or bamboo (Sakagami et al., 2009). Moreover, in the context of how these species deal with faeces, it was found that *S. miscanthi* can respond to the volatile chemicals in faeces from *Sasa* leaves, but *S. longus* cannot respond to those originating from *Miscanthus* leaves (Sato & Saito, 2008). That *S. miscanthi* retains an ability to use chemical cues from *Sasa* bamboo suggests that *Sasa* is its ancestral host plant. The third piece of evidence obtained quite recently is that *S. miscanthi* immatures can survive on *Sasa* bamboo for a long period, and some of them attain adulthood. However, *S. longus* immatures cannot use *M. sinensis* at all. Together, these three pieces of evidence strongly suggest that *S. miscanthi* evolved from a common ancestor of *S. longus* living on *Sasa* bamboo and that the host plant shift from *Sasa* to *Miscanthus* may have played a great role in this speciation event (Sakagami et al., 2009).

Male aggression – as material for studying sexual selection and kin selection

During observations on the behaviour of *S. longus* and *S. miscanthi*, Saito (1990) noticed that there was a big difference in male pugnacity between these two species. As shown by Potter et al. (1976), males of the two-spotted spider mite, *Tetranychus urticae*, are very aggressive against conspecific males, and sometimes kill each other in order to get mating priority. Such male aggressiveness has been empirically observed in many species of Tetranychinae, e.g., *Panonychus citri*, *Aponychus corpuzae*, *Schizotetranychus bambusicola* (Saito, unpubl.). Evolutionary theory tells us that such male aggressiveness evolved by intra-sexual selection (Wilson, 1975), and will frequently occur if available females are scarce (West et al., 2001). On the other hand, Hamilton (1979) hypothesized that the intensity of male-to-male aggression would vary with the relatedness between confronting males. These two hypotheses about the variation in male aggressiveness are still a matter of some controversy among evolutionary biologists, with the former known as the 'resource competition' and the latter as the 'relatedness' hypothesis (Saito & Mori, 2005).

Thus, we investigated the variation in male aggressiveness between *S. longus* and *S. miscanthi* (Saito, 1990, 1995; Saito & Sahara, 1999). Saito (1995) discovered that there is clinal variation in male pugnacity in *S. miscanthi*, namely male aggressiveness measured by a common-garden analysis greatly varies with minimum temperature of the regions where mite populations were collected (Saito, 1995). Furthermore, cluster analysis indicated there are two groups in Japan (called LW and HG) showing different levels of male aggression (Saito & Sahara, 1999).

The 'resource competition' hypothesis is not applicable to the case of *S. longus* and *S. miscanthi* (Saito & Mori, 2005), because there is no correlation between the intensity of male aggressiveness and the number of females available for males. On the other hand, the minimum temperature in winter would be closely related to the probability of male overwintering, because male spider mites may not have a diapause adaptation. The probability of male overwintering (or survival during winter) will affect the probability of inbreeding (mostly through mother-son mating) in spring

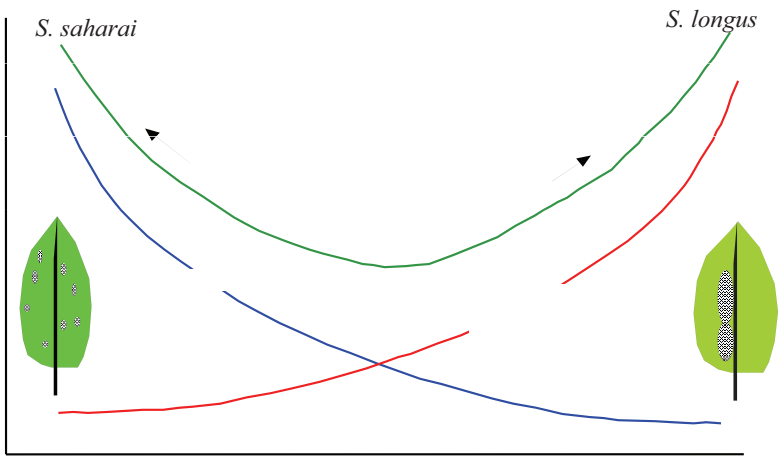

Figure 6 A hypothesis of predator-mediated speciation in *Stigmaeopsis* (Mori & Saito, 2005, unpubl.).

nests. Therefore, the minimum temperature in the winter period would determine the relatedness among nest members in spring. Our estimate of relatedness from the overwintering probability of males and unfertilized females and the consequent population mating structures in spring, provide a tenable explanation for the variation of male-aggressiveness, as evaluated by laboratory experiments (Saito, 2010). The prediction from a newly developed game-theorical model (Saito & Takada, 2009) explaining variation of aggression, cooperation, and altruism in animals, also fits well to the observed variation, showing that the 'relatedness hypothesis' is a better explanation for clinal variation in male-male aggressiveness of *S. miscanthi*, as well as for male-male cooperation in *S. longus* (Saito, 2010).

Haplo-diploidy – as material for genetic and evolutionary studies

Crozier (1985) mentioned that spider mites are very promising study objects for understanding the genetics of haplo-diploid organisms, especially because they lack special problems of bees and ants, which combine haplo-diploidy and eusociality. At that time, there were two contradictory views on the genetics of haplo-diploidy. One predicted that there are few deleterious genes in haplo-diploidy because such genes had been selected out through haploid (hemizygotic) males (Smith & Shaw, 1980; Atmer, 1991). The other predicted that there is a sufficient number of deleterious genes that only affect diploid females even in haplo-diploid organisms (Crozier, 1985). I considered the possibility that such features of haplo-diploidy might be related to the fact that there are so many eusocial species among haplo-diploid organisms. That is why we tried to find out whether the hypothesized genetic features also apply to spider mites.

Using *S. miscanthi*, we investigated how strong inbreeding, via repeated mother-son mating, causes inbreeding depression. We found significant inbreeding depression in female fertility, but no depression in immature survival (Saito et al., 2000b). Furthermore, we found that genes causing this depression are mildly deleterious and recessive (Saito et al., 2000b), yet present in small populations under natural conditions (Mori et al., 2004). The load of deleterious recessive genes varies among diploid females and causes variation in their reproductive success and inbreeding depression. This is hypothesized as one of the possible sources of eusocial evolution (West Eberhard, 1975; Saito, 1994, 1995) and I believe this finding might contribute to future hypotheses about the origin of eusociality in haplo-diploid organisms (Saito, 1997).

PROBLEMS AND FUTURE STUDIES

Evolutionary ecology has as its aim to study selection pressures operating in nature. From this point of view, there are some difficulties involved in using spider mites. A trait of a species that we are focusing on, e.g., nesting behaviour of *S. longus*, has to be checked against every kind of selection, i.e., natural selection operating on survival and reproduction, sexual selection on mating success, and synergistic and/or kin selection on social living, all of which may exist in natural habitats. The minute size of spider mites makes it hard to assess life-time fitness of an individual in the field. Furthermore, we are not entirely sure to which environmental conditions such minute organisms are exposed. As is well known for insects and some mites, chemical cues may be very important means for recognizing the external world. Therefore, we have to pay more attention to the chemical

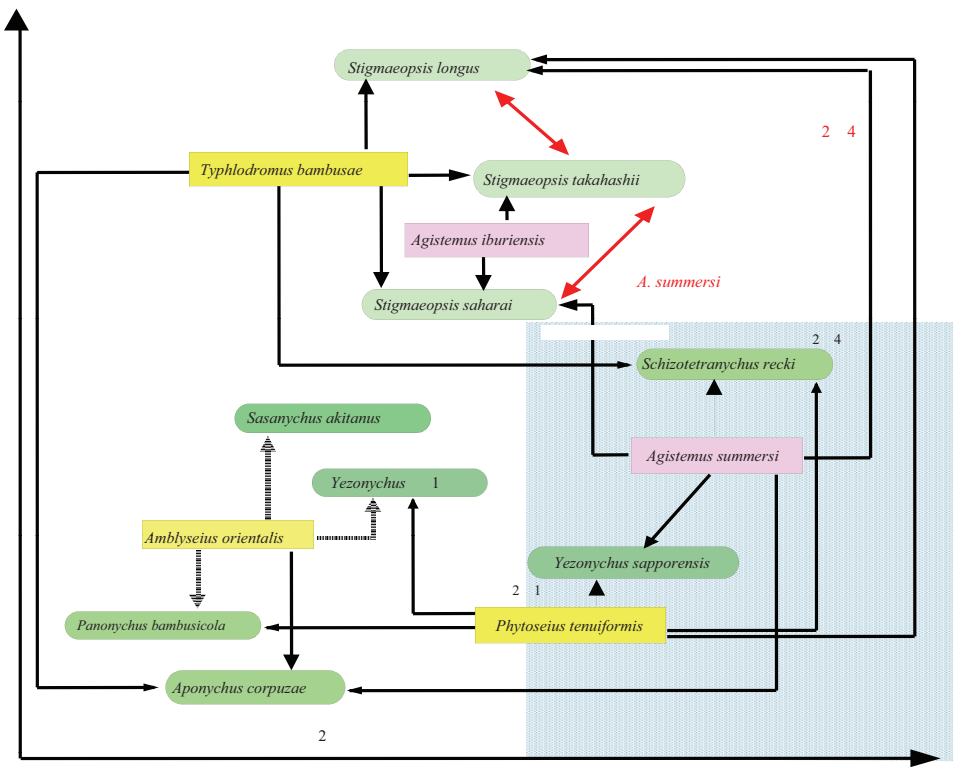

Figure 7 Expected and observed mite fauna network on *Sasa* bamboo.

and tactile world, in order to understand how mites interact with conspecifics and other organisms (Sabelis & Dicke, 1985; Janssen et al., 1998; Dicke, 1999; Sato & Saito, 2006).

The study of spider mite genetics is also fraught with problems. As mentioned earlier, Saito et al. (2000) revealed that there are recessive genes that govern the depression of female fecundity. However, they could not determine how many genes (alleles and loci) are responsible for this effect, nor how intense this effect is compared to the wild type. In order to assess this, we have to observe the fitness changes between generations under constant conditions without environmental variance. Because spider mites are phytophagous, it has been (and still is) very difficult to keep host plant conditions constant. Therefore, it is hard to gain insight in this. It will be necessary to develop some kind of artificial diet that can keep the food conditions of spider mites constant in order to overcome this difficulty.

Finally, our recent attempts to understand the structure and function of mite communities. *Sasa* bamboo is known to have a complex mite community (Mori & Saito, 2004). We have only focused on *Stigmaeopsis* spp. on *Sasa* bamboo so far, but at least nine species of spider mites (all host specific) and nine species of predacious mites occur as well. Some of them live syntopically, others allotopically in the same forests. I do not know whether mite diversity observed on *Sasa* bamboo is extraordinary or not, but the reasons why there are so many species should be explained from the perspective of evolutionary ecology. The diversity of organisms is widely recognized as being important, but do we really know the reasons why there are such complex communities? The relationships between spider mites and their natural enemies are very easy to observe under semi-natural experimental conditions. I believe that this should enable us to understand how the complex community on *Sasa* evolved. Fig. 7 shows the mite diversity on *Sasa* bamboo and what we know about their interactions. We hope to extend our insight in this interaction network through behavioural experiments and a community approach.

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The effect of a phosphogluconate dehydrogenase genotype on sperm competitiveness in the bulb mite, *Rhizoglyphus robini*

Piotr Łukasik, Małgorzata Zygadło & Jacek Radwan
Institute of Environmental Sciences, Jagiellonian University, ul. Gronostajowa 7, 30-387 Kraków, Poland.
E-mail: radwan@eko.uj.edu.pl

Previous work on the acarid mite *Rhizoglyphus robini* has shown that the male's success in sperm competition is significantly affected by the electrophoretic form of the enzyme phosphogluconate dehydrogenase (*Pgdh*): males homozygous for the slow (S) form were superior to males homozygous for the fast (F) form. However, in that study the paternity was measured when progeny reached adulthood, therefore sperm competitiveness might have been confounded with the effect of *Pgdh* on survival. Here, we avoided this problem by using a sterile-male technique to assess the effect of *Pgdh* genotype on the success of males in sperm competition. We also estimated the competitive ability of heterozygotes for the first time. We found that S-homozygous males sired a significantly higher proportion of offspring than F-homozygous males while competing against irradiated males from the background population, whereas sperm competitive ability of SF heterozygotes was intermediate between that of the two homozygotes.

Key words: Phosphogluconate dehydrogenase, *Rhizoglyphus robini*, sperm competition, enzyme polymorphism

In sexually reproducing species where females mate with more than one male, a significant part of an individual male's reproductive success is determined by factors manifested after insemination. Examples taken from across animal taxa include the structure of female reproductive organs, the mode of sperm penetration to the site of fertilization, and the post-insemination reproductive behaviour of either of the two sexes (Witaliński, 1999). Variation in fertilization efficiency between individual males competing against other males from the same population may depend on the quantity of sperm they produce, sperm quality (success in reaching and fertilizing ova), the males' ability to displace sperm stored during previous matings, or their ability to prevent other males from subsequently introducing sperm (Keller & Reeve, 1995; Simmons, 2001).

Some species exhibit large variation in sperm competitiveness between individual males (Lewis & Austad, 1990; Radwan, 1996). This variation has a significant genetic component (Radwan, 1998; Friberg et al., 2005; Konior et al., 2005, and references therein), most likely resulting from more than a single gene – although the genetic architecture underlying sperm competitiveness remains largely unknown. Only few studies succeeded in detecting genes affecting sperm competitiveness. Drnevich et al. (2004) identified 27 candidate genes whose expression levels were associated with intra-population variation in male competitive reproductive success in *Drosophila melanogaster*. In the same species, Clark et al. (1995) found a significant association between alleles at four accessory gland protein (*Acp*) genes and the males' ability to prevent sperm displacement. In the yellow dung fly, *Scatophaga stercoraria*, an interaction was found between both male's and female's genotype at the phosphoglucomutase (*Pgm*) locus and the fertilization success of the female's second mate (Ward, 2000). Finally, in the bulb mite, *Rhizoglyphus robini*, a strong association was found between the male's genotype at the phosphogluconate dehydrogenase (*Pgdh*) locus and the proportion of offspring he sires in competition with another male (Konior et al., 2006). However, Konior et al.'s (2006) study did not fully explain the *Pgdh* effect on male competitive reproductive success in *R. robini*, because paternity was assessed by genotyping adult progeny. Thus, the association between male *Pgdh* genotype and fertilization efficiency might have been confounded with differential survival of offspring genotypes at the *Pgdh* locus. Moreover, Konior et al. (2006) only assessed the competitiveness of homozygous males, whereas that of the heterozygous males is required for a complete picture of sperm competitiveness.

In the present study, we looked at the effect of all three genotypes at the phosphogluconate dehydrogenase locus on male sperm competitiveness using a sterile-male technique (Parker, 1970; Radwan, 1991; Radwan & Siva-Jothy, 1996), which allows assessing paternity at the egg stage, thus avoiding sperm competitiveness to be confounded with differential survival of offspring.

MATERIAL AND METHODS

The mites used in the experiment originated from two populations of approximately 100 individuals, collected from decomposing onions from gardens near Kraków, Poland. Throughout the study, the animals were kept at constant laboratory conditions [25 °C, 90% relative humidity buffered by 270 g dm^{-3} KOH solution, *Allinson*'s (Peterborough, UK) dried yeast as substratum], at a population size of several thousands of individuals.

The fast (F) and slow (S) electromorphs of *Pgdh* (EC 1.1.1.44) were separated with cellulose acetate electrophoresis, following the protocol described by Konior et al. (2006). From a culture isolated in autumn 2001, a set of 20

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families homozygous to the S form of *Pgdh* was developed, which were used to start the S-homozygous population (further referred to as the background population) in spring 2002 (see Konior et al., 2006, for details). In a culture isolated from a single onion in November 2003, the frequency of *Pgdh* alleles was measured in the first generation produced in the laboratory. Then, a population with initial frequency of the F form of *Pgdh* of 0.50 (further referred to as the experimental population) was established in January 2004, by transferring to a common culture 10 offspring produced by each of 22 females paired with single males, with both parents' genotypes known.

The proportion of eggs fertilized by males mated with virgin females was studied using a sterile-male technique (Parker, 1970; Radwan, 1991; Radwan & Siva-Jothy, 1996). Protonymphs isolated directly from both the experimental and background populations were transferred to glass vials of 0.6 cm diameter. Soon after maturation, the mites were separated by sex and male morph. From the experimental population, the two male morphs were used – heteromorphic fighters and homoeomorphic scramblers (Radwan, 1995; Woodring, 1969). Fighter males from the background population served as a reference; females used for the sperm competition experiment also came from the background population. All males used in the experiment were supplied with a female from a background population 24 h before the experiment, in order to mimic natural conditions where males have continual access to females, and to standardize the reserves of semen between males (Radwan, 1997). A few hours before the experiment, all fighter males from the background population were transferred to a common Petri dish and irradiated with 20 krad of gamma-ray from ^{60}Co, a dose high enough to prevent hatching of 99% of the eggs fertilized by these irradiated males (Radwan & Siva-Jothy, 1996). Then, virgin females from the background population were allowed to mate sequentially with three males, for 2 h with each: first with an irradiated fighter male from the background population, then with a male from the experimental population, and finally with another irradiated fighter from the background population. Thereafter, all males from the experimental population which were used in the study were genotyped, and the mated females were allowed to lay eggs for 3 days, and then for an additional 4 days in a fresh vial. Offspring and unhatched eggs were counted in each vial 7 days after the female was removed, which is long enough for all viable eggs to hatch. Paternity of experimental males was calculated as the proportion of eggs hatching.

The data was analyzed using Statistica v. 6.0. (Statsoft, 2001). Two-way ANOVA, with male morph and genotype as fixed effects, was used to compare the proportions of hatched eggs. Chi-squared tests were used to compare genotype distributions within populations with the Hardy-Weinberg distribution and Yates' correction was applied where necessary.

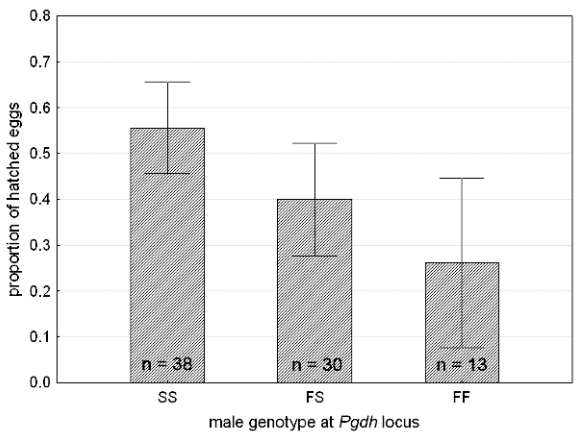

Figure 1 Mean (± 95% confidence intervals) proportion of eggs fertilized by males of *Rhizoglyphus robini* homozygous to the fast form of *Pgdh* (FF), homozygous to the slow form of the enzyme (SS), or heterozygous (FS), competed against irradiated SS males from the background population.

RESULTS

In a population of *R. robini* freshly isolated from a single decomposing onion in November 2003, the frequency of the F allele was 0.34 (n = 45), with the genotype frequencies not significantly deviating from the Hardy-Weinberg distribution (χ^2 = 2.38, d.f. = 2, P = 0.30). In the experimental population (initiated in January 2004, with 0.50 frequency of each of the two alleles) the frequency of the F allele dropped to 0.31 (n = 105) by April 2004, and the genotype frequencies were again not significantly different from the Hardy-Weinberg expectations (χ^2 = 2.15, d.f. = 2, P = 0.34).

Mean paternity (± SD) of a male from the experimental population was not affected by his morph: 38.4±6.6% (scramblers; n = 32) vs. 42.6±4.7% (fighters; n = 49) ($F_{1,75}$ = 0.25, P = 0.62), nor by a morph*genotype interaction ($F_{2,75}$ = 0.37, P = 0.69), but male genotype had a significant effect on the proportion of eggs hatching ($F_{2,75}$ = 4.59, P = 0.013; Fig. 1). Tukey's HSD test revealed significant differences between S- and F-homozygous males (P = 0.011), but no significant differences between heterozygous and either F- nor S-homozygous males were detected (P>0.20) (Fig. 1).

DISCUSSION

Our results are consistent with those of Konior et al. (2006), who found that males of *R. robini* homozygous for the S allele of *Pgdh* sire a significantly higher proportion of offspring when competed against males homozygous for the F form of the enzyme. Similarly, we detected a significant effect of *Pgdh* alleles on male fertilization efficiency, with the slow form of the enzyme conferring an advantage. We found S-homozygous males fertilizing a much higher proportion of eggs than F-homozygotes, with heterozygotes taking an intermediate position, not significantly different from fertilization efficiency of either group of homozygotes (Fig. 1). In the study of Konior et al. (2006), male competitive reproductive success was estimated from the proportion of the genotypes among the mature offspring, leaving room for confounding sperm competitiveness with differential juvenile survival. Such an effect of *Pgdh* was documented in a study on a tropical palm tree, *Euterpe edulis* (Conte et al., 2003). In contrast, we scored the reproductive success of males from the experimental population soon after the eggs hatched, with no delay required for the offspring to mature, and therefore reducing this potential confounding effect to differential embryo survival. Therefore, we have no reason to suspect that Konior et al.'s (2006) results were an artefact resulting from better survival of S homozygotes until adulthood. Also, our finding that male morph did not affect sperm-competitive success is consistent with previous reports (Radwan, 1997).

Phosphogluconate dehydrogenase (E.C. 1.1.1.44) is involved in the pentose phosphate pathway for the oxidation of carbohydrates. It converts 6-phospho-D-gluconate into D-ribulose 5-phosphate, an important intermediate product of the pathway, but also an essential substrate in nucleotide biosynthesis (Murray et al., 2003). It seems plausible that alternative forms of this important enzyme might have a strong effect on metabolic activity of the genotypes. Apart from affecting sperm competitiveness (Konior et al., 2006; this study), the enzyme was shown to affect fitness components such as the probability of survival in a tropical palm (Conte et al., 2003) or resistance to salinity in a pupfish (Stockwell & Mulvey, 1998). The phosphogluconate dehydrogenase effect on male competitive fertilization efficiency in *R. robini* could be explained in several ways. Obviously, alternative forms of the enzyme participating in oxidative processes might affect metabolism, and therefore fertilization efficiency of amoeboid sperm cells of *R. robini* (Radwan, 1996), whose competitive success may well depend on the speed of movement (LaMunyon, 1998). The effect could be associated with the genotype of individual sperm cells. However, given the generally low transcriptional activity of sperm cells (Erickson, 1990), individual male's sperm quality is more likely to depend on the efficiency of the enzymes coded by both copies of the gene in spermatocytes, before meiosis. The genotype at the *Pgdh* locus might also affect a male's general condition, thus influencing, for example, the quantity of sperm produced or the composition of seminal fluids. Furthermore, behavioural traits, affecting sperm utilisation by females, might be affected. Further work is necessary to elucidate the mechanism conferring an advantage in sperm competition to the bearers of the S alleles.

A detrimental effect of the F allele on male competitive reproductive success probably caused the rapid decline of this allele in laboratory-reared populations. In our experimental population of at least several hundreds individuals at any given time, the F frequency went down from 0.50 to 0.31 in 4 months, i.e., within approximately eight generations. Similarly, Konior et al. (2006) observed fixation of the S allele after 3 years (ca. 60 generations) in a large laboratory population with the initial frequency of the F allele of 0.22. Nevertheless, in wild populations of *R. robini* the F form of *Pgdh* is common – its frequency was 0.22 in a population collected in 2001 (Konior et al., 2006), and 0.34 in a population we collected in 2003; however, a very low frequency of the F form was found among mites collected from the same site in 2004 (M. Zygadło, unpubl.). This suggests the presence of a strong selective force for maintaining the F allele in the wild, as well as an interaction with the environment with respect to total fitness. However, the nature of these processes remains to be elucidated.

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Population density and male polymorphism in the feather mite *Falculifer rostratus* (Acari: Falculiferidae)

H.C. Proctor, G. Williams & D.H. Clayton[1]
Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9.
E-mail: hproctor@ualberta.ca; and [1]Biology Department, University of Utah, Salt Lake City, UT 84112-0840, USA

Male polymorphism, in which two or more male morphologies occur within a species, is a widespread phenomenon in the Astigmata. Factors determining which morph a male will become have been studied for several free-living species. In *Sancassania berlesei* (Michael) (Acaridae), male nymphs develop into relatively unmodified homeomorphs at high population densities, and into highly modified, aggressive heteromorphs at low densities. We tested whether population density correlated with male morph ratio in the dimorphic feather mite *Falculifer rostratus* (Buchholz) (Falculiferidae). Twenty-one pigeons (*Columba livia* Gmelin) from Illinois, USA, were thoroughly washed and all *F. rostratus* extracted were identified to stage, sex, and male morph. Excluding four birds that had no *F. rostratus*, total densities per host ranged from 1-1155. Numbers of heteromorph males correlated positively with population density, but homeomorph numbers had no obvious relationship with density. Ratios of homeomorph:heteromorph were frequently higher at low population densities than at high densities – the opposite of the pattern observed for *S. berlesei*. An alternative hypothesis that quality of diet may determine morph ratio is tested and receives little support. It is possible that male morph is determined by density in *F. rostratus*, but that it is controlled at a finer physical scale (e.g., per feather) than could be measured in this study. Other possibilities are that morphs are determined genetically or by host variables we did not account for (e.g., moulting status).

Key words: Pigeon, heteromorph, homeomorph, Astigmata, population size

Male polymorphism is widespread in animals, both vertebrates (e.g., salmonid fishes) and invertebrates (e.g., scarab beetles), and is usually associated with different strategies for gaining access to females (Foote et al., 1997; Emlen 1997). Polymorphism in male mites occurs in a few Mesostigmata and Prostigmata, such as *Macrocheles* (GW Krantz, pers. comm.) and *Cheyletus* (Regev, 1974), but is most widespread and best studied in the Astigmata. The function and induction of different male morphs have been examined for several free-living species in the family Acaridae, especially in *Sancassania berlesei* (Michael). In *S. berlesei* there are two main morphs: a 'fighter' with hypertrophied legs III that are used as stabbing weapons (heteromorph), and a non-aggressive 'scrambler' with unmodified legs III (homeomorph) (Radwan, 1993; Łukasik et al., 2006). In other species of acarid there can be additional morphs (summarized in Timms et al., 1981). Heteromorphic male *S. berlesei* are aggressive and attack any other male encountered, whereas homeomorph males are non-aggressive and always lose in encounters with fighters. In this species, population density affects the development of a male into a fighter or scrambler: at low density, male nymphs become heteromorphic fighters, whereas at high density they become non-aggressive homeomorphs. The polymorphism is maintained because in small populations a fighter potentially kills all other males and monopolizes females, whereas in crowded conditions fighters spend all their time fighting rather than mating; thus scrambler males have greater reproductive fitness at high densities.

Although it has been best studied in free-living species, male polymorphism is also common in symbiotic astigmatans. In feather mites (Analgoidea, Freyanoidea, Pterolichoidea) it occurs in at least 30 genera in nine families (as calculated from illustrations in Gaud & Atyeo, 1996). Heteromorphic male feather mites frequently have enlarged legs III or IV, but in some species it is the forelegs, palps, chelicerae or setae that are hypertrophied relative to the state in the homeomorph male. The one species of feather mite for which functions of these modifications have been studied is *Falculifer rostratus* (Buchholz) (Pterolichoidea: Falculiferidae) (Witalinski, 2004). The host for this mite is the rock pigeon *Columba livia* Gmelin (Columbidae). In *F. rostratus,* legs I and II and the lower (movable) cheliceral digits are greatly elongated in the heteromorphic male. The heteromorph is also larger in overall body size and is more heavily sclerotized than the homeomorph. Witalinski (2004) observed that heteromorphs use their elongated chelicerae and legs to lever rivals off the feather and throw them from the barb channel.

Not all male morph expression is determined by population density. In some species, polymorphism is controlled through variation in nutrition during early development. In *Onthophagus* beetles (Coleoptera: Scarabaeidae), males reared on a low-quality diet have disproportionately larger horns at any body size than males reared on a high-quality diet (Emlen, 1997). Witalinski (2004) commented on the lack of biological data for *F. rostratus* that would help to explain the occurrence of male polymorphism in this species. In this paper we tested the hypothesis that ratios of homeomorphs and heteromorphs in *F. rostratus* are associated with population density, as occurs in *S. berlesei*. We also secondarily test whether there is evidence that nutritional quality of the host, as expressed by mean body size of mites, correlates with morph ratio.

MATERIAL AND METHODS

Ninety feral rock pigeons were collected over a 1-week period in the summer of 1999 by Dale Clayton and Brett Moyer (University of Utah). Birds were captured using walk-in traps

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Table 1 Characteristics of host pigeons (*Columba livia*) and numbers of different stages, sexes, and morphs of *Falculifer rostratus*, ranked from lowest to highest total number of mites.

Host characters			Counts of *Falculifer rostratus*					
Band no.	Sex	Body mass (g)	Hetero males	Homeo males	Adult females	Nymphs	Larvae	Total
801	female	305	0	0	0	0	0	0
802	male	325	0	0	0	0	0	0
813	male	340	0	0	0	0	0	0
845	male	340	0	0	0	0	0	0
810	female	295	0	0	0	0	1	1
818	male	330	0	0	0	1	0	1
807	male	390	0	0	0	1	2	3
882	unknown	160	0	0	2	2	1	5
879	female	345	7	0	13	30	8	58
839	male	315	3	0	22	33	3	61
822	female	325	4	0	22	31	10	67
851	female	320	5	4	32	35	11	87
890	male	340	4	2	39	40	9	94
842	male	275	13	3	48	54	4	122
803	male	345	5	7	38	78	10	138
876	female	305	5	14	48	69	19	155
819	female	285	33	0	115	206	64	418
884	male	380	22	14	84	283	32	435
848	male	365	27	0	130	248	55	460
859	female	345	29	27	156	357	104	673
837	male	325	90	0	315	625	125	1,155

baited with pigeon feed at locations within an 80-km radius of each other in Ford County, Illinois. Birds were humanely dispatched, and body mass and sex of each individual recorded. Body washing is a very accurate predictor of total louse abundance (Clayton & Drown, 2001), and was therefore used in this study to collect arthropods from the birds. Each pigeon was placed in a 3.8-l paint can containing a 1.0% solution of liquid dishwashing detergent. Next, the can was placed in a mechanical paint shaker (Fleming Gray, Ontario, Canada) that underwent a 10-min cycle. Upon completion, the can was opened and 95% ethanol was used to reduce the volume of foam. The bird was then deposited in a second paint can containing only water and inserted into the paint shaker for an additional 10 min. Upon completion of the second 10-min cycle, the bird was removed and rinsed over a 19-l bucket containing 95% ethanol. After sufficient rinsing, the contents of the two paint cans were poured into the 19-l bucket and rinsed several times using water to ensure that all arthropods had been removed. The contents of the bucket were then strained through a 180-mm-mesh stainless steel screen and transferred to a Büchner funnel lined with filter paper. The filter paper was scanned using a dissecting microscope and a rough estimate of ectosymbiont numbers was made, and then the paper was placed in a wide-mouth jar filled with 95% ethanol and sealed without any arthropods having been removed from the filter paper.

For this study we selected a subsample of 21 body-washings that had a broad range of estimated *F. rostratus* numbers to allow us to test the population-density hypothesis. For the exhaustive mite counts, the filter paper was removed from the jar in approximately 4 × 2 cm strips and placed in a 60 × 15 mm Petri dish filled with ethanol. The contents of the dish were carefully examined using a Leica MZ16 dissecting microscope at 20-110× magnification. *Falculifer rostratus* specimens were removed and mounted onto microscope slides using PVA Mounting Medium (Bioquip Products, Rancho Dominguez, CA, USA). Slides were cured on a slide-warmer (ca. 40 °C) for a minimum of 3 days before they were removed. Mites were categorized as heteromorph male, homeomorph male, female, nymph, or larva using a Leica DMLB compound microscope at 100-400× magnification. Linear regressions relating numbers of heteromorphs and homeomorphs to total number of mites on birds were performed using the statistics package SPSS version 11.5 (SPSS for Windows, 2002). We also tested for evidence of a correlation between body weight of host and mite number (Pearson r), and whether host sex influenced mite load (two-sample t-test).

To explore the hypothesis that the smaller-bodied homeomorph males may develop on hosts that provide poor nutrition, we selected four pigeons that had both homeomorph and heteromorph males (bird band numbers 803, 851, 859, 876) and four that had only heteromorph males (819, 837, 848, 879) (Table 1). We haphazardly selected 5-10 females and 4-11 heteromorph males per host for a total of 50 females and 50 heteromorphs (25 each from birds with homeomorphs and heteromorphs, and 25 each from those with only heteromorphs). The body length of each mite was measured ventrally from the anterior margins of epimerites I to the median indentation at the opisthosomal terminus. Mean body lengths were compared between mites from birds with both male morphs and those with only heteromorph males using a one-tailed t-test. A one-tailed test was used because the hypothesis was directional: mites from hosts with both male morphs should be smaller than those from hosts with only heteromorphic males.

RESULTS

Falculifer rostratus were found in 17 of the 21 body-washing samples (Table 1). Excluding those birds that had no *F. rostratus*, total densities per host ranged from 1-1155. Females and nymphs were always more abundant than males. Thirteen samples included heteromorphic males, and of these, seven also included homeomorphs. Body mass of host was not correlated with *F. rostratus* load (Pearson R = 0.17, P = 0.46), and there was no difference in load between male and female hosts (t = 0.18, d.f. = 18, 2-tailed P = 0.87). In addition to *F. rostratus*, we found mites from seven other families in the body washings: *Diplaegidia columbae*

(Buchholz) (Analgidae), Dermoglyphidae, Harpirhynchidae, Cheyletiellidae, Syringophilidae, *Tinaminyssus melloi* (Castro) (Rhinonyssidae), and *Dermanyssus gallinae* (De Geer) (Dermanyssidae). Three species of lice were also found: *Campanulotes compar* (Burmeister) and *Columbicola columbae* (Linnaeus) from the Philopteridae and *Hohorstiella lata* (Piaget) from the Menopodidae.

There was a significant positive relationship between total number of *F. rostratus* on a bird and the number of heteromorphic males (r^2_{adj} = 0.92, P<0.001), but there was no relationship between density and number of homeomorphs (r^2_{adj} = 0.042, P = 0.21) (Fig. 1). When the ratio of number of homeomorphs to heteromorphs is plotted against population density (Fig. 2), there is no evidence of the predicted increase in proportion of homeomorphs with total number of mites per bird. Instead, at low densities there is wide variation in morph ratio, with homeomorphs being either more common or rarer than heteromorphs, but at higher densities the proportion of homeomorphs is low.

Mean body length of heteromorphic males from birds with both types of male morphs did not differ significantly from that of heteromorphs from birds that had only heteromorphic males (t = 0.063, d.f. =48, 1-tailed P = 0.48) (Fig. 3A). Female body length was significantly, albeit only slightly, less on hosts with both morphs (t = 1.84, d.f. = 48, 1-tailed P = 0.036) (Fig. 3B).

DISCUSSION

We found no evidence that homeomorphic male *F. rostratus* were more likely to develop on hosts with high densities of mites. Instead, it appeared that homeomorphs declined in relative abundance at higher population densities, whereas heteromorph numbers mirrored total mite number (Fig. 1). The need to exclude cases in which there were no males at all resulted in a small sample size for the morph ratio plot (n = 11, Fig. 2), and therefore we are reluctant to place too much emphasis on the negative triangular shape of the distribution. Nevertheless, these data allow us to reject the hypothesis that homeomorphic males dominate at high total population densities. It is still possible that male morph is determined by density in *F. rostratus*, but that it is controlled at a finer physical scale (e.g., per feather) than could be measured in this study. This could be tested by a correlational study in which feathers are examined individually, either on living hosts or on hosts that had been frozen or plucked immediately after death (to prevent movement of mites among feathers).

It is also possible that the mechanism determining male morph is something different from that operating in *S. berlesei*. We found little evidence that the controlling factor was quality of nutrition provided by the host. Although females from birds with both male morphs were significantly smaller than those from heteromorph-only birds, as predicted by

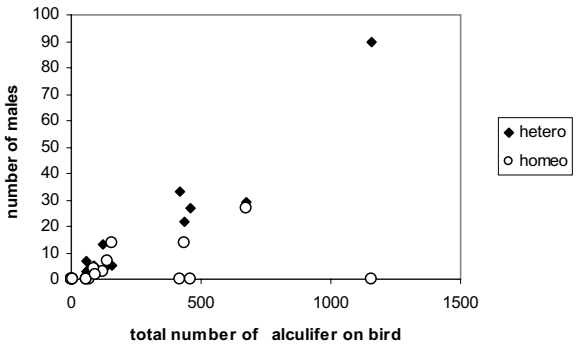

Figure 1 Relationship between total number of *Falculifer rostratus* per host pigeon and number of heteromorphic and homeomorphic males.

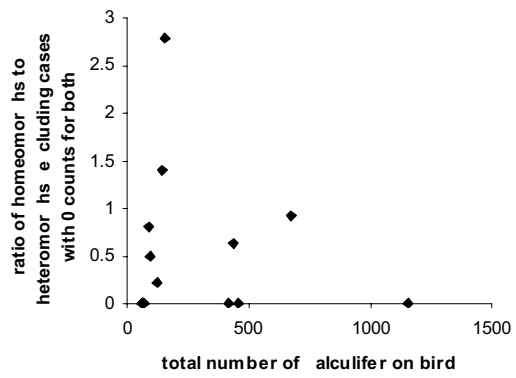

Figure 2 Ratio of homeomorphic to heteromorphic males plotted against total number of *Falculifer rostratus* per host pigeon.

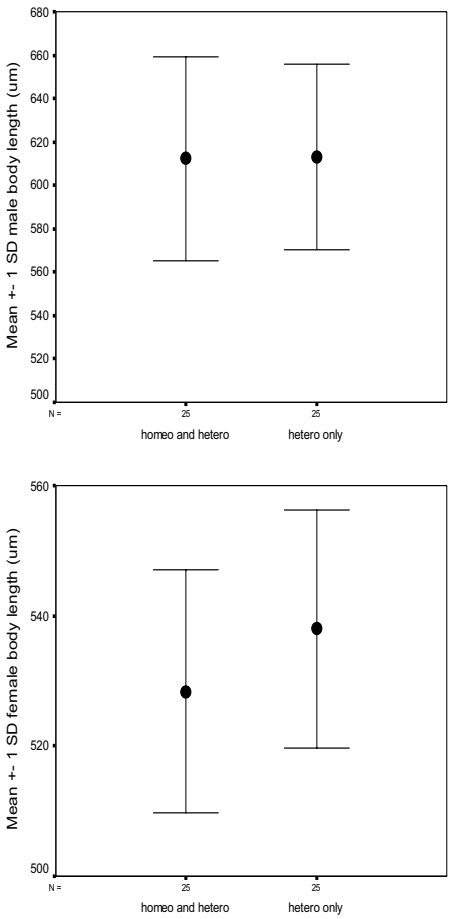

Figure 3 Mean (± SD) body lengths of *Falculifer rostratus* on hosts that had both male morphs, and those that had only heteromorphs: (a) body lengths of heteromorphic males; (b) body lengths of adult females.

our hypothesis, this difference was very small, and heteromorphic males did not show any difference in size (Fig. 3). It would be interesting to test the nutritional hypothesis in a controlled laboratory experiment in which the amount of preen oil provided to mites is manipulated. Other possibilities are that morphs are determined genetically or by host variables we did not account for (e.g., moulting status).

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Observations on reproduction, development, and sexual behaviour of stream-inhabiting water mites (Acari: Hydrachnidia)

Peter Martin
Christian-Albrechts-Universität zu Kiel, Zoological Institute, Olshausenstr. 40, D-24098 Kiel, Germany.
E-mail: pmartin@zoologie.uni-kiel.de

Details of reproduction, development, and sexual behaviour are known only for a few water mite species, mostly from standing water. This study reports laboratory observations on 20 species from two small streams in North Germany. Ovigerous females carried between 1.4 (*Aturus fontinalis*) to 69 eggs (*Sperchon setiger*). In most species, eggs were laid consecutively in distinct clutches, generally with <50 eggs per clutch. Separately deposited single eggs were exclusively found in containers housing the small species *Feltria rouxi*, *Ljania bipapillata*, and *A. fontinalis*. Lifetime reproduction was recorded for *Sperchonopsis verrucosa* (110 eggs in 3 years) and *Sperchon thienemanni* (122 eggs in 2 years). Development from egg until larval hatching took 2-10 weeks, eggs laid late in the year took up to 3 months. The deutonymphal stage generally takes several months, depending on food supply. The period between the immobile deutonymph and adult emergence takes 1-4 weeks. In the laboratory, life spans vary from several months (male *F. rouxi*, *A. fontinalis*) up to 3 years (female *S. verrucosa*, *Lebertia glabra*). In most cases, spermatophore deposition occurs even in the physical absence of females. For stream-living species in temperate climates, different patterns of overwintering are known: hibernation often occurs as adult. Overwintering exclusively as deutonymph has never been observed, but part of the population often hibernates at this stage. In species with parasitic larvae late in the year (*Wettina podagrica*, *L. glabra* and *Sperchon squamosus*), most specimens probably hibernate at the deutonymphal stage. Overwintering as a well-developed larva remaining within the clutch envelope has been observed only in *Atractides nodipalpis*. Hibernation at the egg stage is known from the literature for *Sperchon glandulosus*.

Key words: Rheobiontic water mites, behaviour, development, life cycle strategies, overwintering

Most water mites have a life cycle consisting of three free-living developmental stages: the larva, the (deuto-) nymph, and the adult (Böttger, 1977; Di Sabatino et al., 2002). The sexes are separated and reproduction occurs by various modes of sperm transfer, mainly indirectly by the use of spermatophores (Proctor, 1992). Although the principal life cycle is known, few monographic studies exist on the autecology of selected species. Most work has been undertaken on species from lakes and ponds. Comprehensive studies have been carried out on, for example, *Hydrachna globosa*, *Limnochares aquatica*, *Limnesia maculata* (Böttger, 1972a,b), some *Unionicola* species (Hevers, 1980), some *Arrenurus* species (Stechmann, 1978), *Hydrachna conjecta* (Davids, 1973), and *Hydrodroma despiciens* (Meyer, 1985). In contrast to this detailed work on species from standing water, little is known of the life history of water mites living in springs or of species from the hyporheic interstitial. Only one detailed study exists for stream-living water mites, dealing with species from a small stream in a German mountainous region (Ullrich, 1976, 1978). Other autecological results for rheobiontic species have dealt with parasitology (Efford, 1963; Gledhill et al., 1982), are based on phenological data only (Lundblad, 1968; Gerecke, 2002), or concern just one (*Feltria rouxi*; Efford, 1965) or a selection of species (Efford, 1966).

Here, results are presented that are based on various water mite species from two small streams in North Germany (Martin, 1998). Although some data are fortuitous and have resulted from the rearing of larvae of the respective species, several patterns have been revealed that are typical of rheobiontic water mites.

MATERIAL AND METHODS

The data for this article originated from an autecological study that dealt with water mite species from two small streams in the North German Lowland (cf. Martin, 1998). The adults and nymphs (including the ovigerous females) were derived from benthic samples of various stream sites, as documented in Böttger & Martin (1995), Martin (1996, 1997), and Martin & Speth (1996). Laboratory observations took place from 1994 to 1997. Eggs were counted by observation of intact females under the microscope (*Feltria rouxi*, *Ljania bipapillata*, *Aturus fontinalis*) or by opening the body and removal of the eggs (other species).

Since observation of small animals under running water conditions is difficult in general, and since the work of Ullrich (1976) has established that far-reaching results are achievable in standing water conditions, the live mites collected in the field were transferred into tissue-culture plates. For breeding of the mites, various numbers of specimens were used. The plates were placed in a climatic chamber (BBC-York) in the Zoological Institute of Kiel University. Temperature and light regimes were regulated and approximated to the conditions in the field. Temperature was adjusted monthly, based on the median monthly temperatures of a summer cold lowland stream (Albrecht, 1953): Jan, Feb 5 °C; Mar 6 °C; Apr 8 °C; May 10 °C; Jun 13 °C; Jul 14 °C; Aug 13 °C; Sep 12 °C; Oct 10 °C; Nov 8 °C; Dec 6 °C. The light-dark regime changed twice per month with minimum light in the second half of December (08:45-16:00 hours) and maximum light in the second half of June (03:45-21:00 hours). Observations were made using a stereo-microscope with a cold-light lamp in order to minimise any influences on the animals (for further details of the methods and the origin of the animals examined, see Martin, 1998).

Nomenclature of the developmental stages follows Böttger (1977): egg, prelarva, larva, protonymph, deutonymph, tritonymph, adult. As an adjustment to practical limitations in observing live mites, larval development encompasses the period between oviposition and hatching

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of the larva (ignoring the prelarval stage). Postlarval resting stage I is the period between cessation of activity of the larva and hatching of the deutonymph; postlarval resting stage II is the period between cessation of activity of the deutonymph and hatching of the adult. The only active nymphal stage, the deutonymph, is simply called the nymph.

RESULTS

Reproduction and development

In most species, eggs were laid in several distinct clutches and not in a single batch (e.g., in *Protzia eximia*, *Lebertia glabra*, *Atractides nodipalpis*). The mean number of eggs per ovigerous female differed between 1.4 (*Aturus fontinalis*) and 34.0 in *Hygrobates nigromaculatus* and the highest total egg numbers were recorded for *Sperchon setiger* and *H. nigromaculatus* (both 69) (Table 1). Egg numbers in clutches reached a maximum of 40 in *H. nigromaculatus* and 31 in *Protzia eximia*. A single egg was found in containers with the small females of *Feltria rouxi*, *Ljania bipapillata*, and *Aturus fontinalis*. A maximum of six eggs was observed for *L. bipapillata*. In these species, egg laying often occurred only if several females were kept together in one container. Furthermore, the maximum egg number per ovigerous female was distinctly higher than the observed single eggs in the containers (four eggs in *F. rouxi*, nine in *L. bipapillata*, and six in *A. fontinalis*). For *Sperchonopsis verrucosa* (n = 6) and *Sperchon thienemanni* (n = 1) lifetime reproduction was documented. One *S. verrucosa* female produced maximally 10 clutches in 3 years. Egg production per year was 36 (23-68), total reproduction amounted to 110 eggs (89-139). One *S. thienemanni* female produced six clutches with a total of 122 eggs in 2 years. The numbers of eggs produced in *S. verrucosa* and *S. thienemanni* were comparable to those observed by Ullrich (1976) for *Sperchon setiger* (110-128). For the other Sperchontidae and the Lebertiidae, Hygrobatidae, and Wettinidae in the present study, a similar scale of egg production is expected.

Egg development until emergence of the larvae took from 2 weeks in *Sperchonopsis verrucosa* to 10 weeks in *Sperchon thienemanni* and *Wettina podagrica*. For *Protzia eximia*, two patterns of egg development were observed. From eggs deposited early in the year, larvae hatched after about 4 weeks, whereas eggs laid late in the year went through a diapause stage and developed further with increasing water temperature under spring conditions; thus, the development from egg to larvae could take 3 months. The development of *Atractides nodipalpis* larvae took about 4 weeks but most larvae stayed in the clutch for up to 7 months. In the study of Ullrich (1976), the larvae of *A. nodipalpis* also did not hatch at a cultivation temperature of 13 °C, but only after at least 2 months at 6 °C and subsequent warming. A similar phenomenon was seen in the eggs of *Ljania bipapillata* laid late in the year: larvae developed within a few weeks but spent the winter in the clutch envelope. This resting period of completely developed larvae is considered as hibernation and probably enables them to find suitable hosts in the following early spring (cf. Martin, 1998).

The duration of the other developmental stages and of the active stages exhibited marked differences. For the prelarval stage, which was not examined in the present study, Ullrich (1976) reported a duration of 8-10 days at a water temperature of 13 °C for some stream-living water mite species. Thus, a similar time scale of a few days was assumed for the prelarval stages of the species studied here.

Normally, the larvae were active after leaving their clutch, crawling on the surface of the substrate or briefly swimming (e.g., *Sperchon thienemanni*, *Atractides nodipalpis*, *Aturus fontinalis*). Larvae of the two main inhabitants of lentic substrates of streams, viz. *Hygrobates nigromaculatus* and *Wettina podagrica*, proved excellent swimmers. Without reaching a host species, the emerged larvae died after 2-5 weeks (own observations; Ullrich, 1978). Single larvae of *A. nodipalpis* survived a period of 8 weeks after leaving the clutch. A particular characteristic of *S. thienemanni* was the aggregation of emerged larvae that often was found and without movement. Ullrich (1978) interpreted this behaviour as a survival-prolonging adaptation to the lack of hosts.

No observations regarding the period of parasitic association or the duration of postlarval resting stage I were made in the present study.

Since all hosts determined for the mite species under study are relatively short-lived insects (mainly chironomid dipterans; Martin, 2000), parasitic life probably only occurs for a few days to about 1 week (Ullrich, 1978). After leaving the host, the larva returns to the water, looks for a sheltered place where it often wedges itself into the substrate, and enters the resting stage. Ullrich (1976) measured the duration of the postlarval resting stage I as 13-24 days (water temperature 13 °C).

The period of the nymphal stage in the present study varied considerably between and within species. The nymphal period took a few months, e.g., in *Lebertia glabra*, and up to 2 years in some individuals of *Sperchonopsis verrucosa*. Similar differences were found by Ullrich (1976) who recorded nymphal stages taking 6 weeks to 8 months. These observations lead to the conclusion that the duration of the nymphal period is strictly dependent on the food supply. For many water mites, the nymph is the most important growth stage (Smith et al., 2001). Indeed, nymphs that lived longer in the laboratory had a poor food supply, e.g., the nymphs of small species requiring small chironomid larvae that were difficult to obtain. Obviously, entry into postlarval resting stage II requires a minimum quantity of food incorporation. My own phenological data (Martin, 1998) confirmed that the usual duration of the nymphal stage amounts to several months, provided a sufficient supply of food in the field. Several times, hibernating nymphs have been recorded (e.g., *Lebertia inaequalis*, *L. stigmatifera*, *Aturus fontinalis*) but the main part of the population seldom spends the winter in this stage. Only species whose larvae parasitise late in the year seem to hibernate as nymphs (*Wettina podagrica*, *L. glabra*). Before losing their mobility, the nymphs in the laboratory squeezed themselves into interstices of the substrate and anchored themselves therein with their palps.

The last metamorphosis during the development of the water mites observed in the present study, viz., postlarval resting stage II, only took a short time. This stage lasted between 1 week (e.g., *Aturus fontinalis*) and 4 weeks (*Sperchonopsis verrucosa*, *Sperchon thienemanni*).

As for nymphs, the duration of the adult stage is strictly dependent on the food supply and can be shortened by feeding at libitum (Martin, 2005). Although food was offered at possibly the lowest level in the laboratory, sexual activity of the adults (deposition of spermatophores by males and oviposition by females) was seen as a clue that

Table 1 Observations of egg numbers, development, and life duration of various stages and of sexual behaviour. Data in parentheses, uncertain. Abbreviations used: d, days; DN, deutonymph; ind., indirect; n, no. specimens; PR = postlarval resting stage.

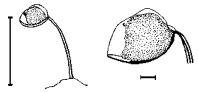
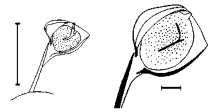
Species	Mean no. eggs per ovigerous female (min-max)	Clutch size, total no. eggs laid per female	Egg development until hatching (weeks, if not otherwise indicated)	Duration of developmental stages/life span	Sexual behaviour
Panisellus thienemanni (K.Viets)				(male 15 months) Boehle (1996): parasitism about 3 weeks	unknown
Thyopsis cancellata (Protz)	n = 1; 4 eggs			(12 months)	unknown
Protzia eximia (Protz)	n = 18; 10.3 (1-23)	6-31 Ullrich (1976): 12 eggs/clutch	4-12 Ullrich (1976): 40 d	adults: 13 months	deposition of spermatophores in small fields (4-20) also in the absence of female  [scale bar: 100 µm, detail 10 µm]
Sperchonopsis verrucosa (Protz)	n = 250; 7.6 (1-21)	2-29 eggs/year (n = 30): 36 (range: 23-68); eggs/female (n = 6): 110 (range: 89-139) in 10 clutches max	2-4	male 24 months female 36 months	deposition in large fields (about 100) also in the absence of female, duration of spermatophore deposition 30-60 s  [scale bar: 50 µm, detail: 10 µm] pairing behaviour?
Sperchon clupeifer Piersig	n = 19; 10.3 (1-29)	n = 7: 13 eggs (4-25)	3-7	Bader (1980): adults ca. 12 months	pairing behaviour?
Sperchon setiger Thor	n = 208; 14.5 (1-69)	Ullrich (1976): 38	4-5 Ullrich (1976): 36 d	(female 38 months, male shorter?) PRII 6 weeks Ullrich (1976): parasitism 2-12d, PRI 16d, DN 42-196d, PRII 22d	Ullrich (1976): deposition also in the absence of female, but increased in the presence of female, after pairing behaviour immediate deposition of spermatophores by the male; documented spermatophore: Ullrich (1976)
Sperchon squamosus Kramer	n = 32; 9.4 (1-29)		4-5 Ullrich (1976): 26 d	(male 32.5 months)	deposition also in the absence of female
Sperchon thienemanni Koenike	n = 168; 4.8 (1-18)	n = 25: 16.2 (3-25) eggs/year (n = 5): 50-70 eggs/female (n = 1): 122 in 6 clutches during 2 years	5-10	DN 1-4 months, PRII 2.5-3.5 weeks, adults 24 months	deposition of spermatophores in small fields (ca. 20) also in the absence of female, duration of deposition 120 s  [scale bar: 100 µm]
Lebertia fimbriata Thor	n = 6; 5.3 (1-16)	up to 15	5-7	(female 34 months)	ind. sperm transfer by spermatophores

Table 1 Continued

Species	Mean no. eggs per ovigerous female (min-max)	Clutch size, total no. eggs laid per female	Egg development until hatching (weeks, if not otherwise indicated)	Duration of developmental stages/life span	Sexual behaviour
Lebertia glabra Thor	n = 37; 4.1 (1-22)	n = 5: 8.8 (6-12) Efford (1966): 6.5	5-6 Efford (1966): 20.4 d (20 °C)	PRII 2-3 weeks, >12 months (female 34 months)	deposition also in the absence of female documented spermatophore: Efford (1966)
Lebertia inaequalis (Koch)	n = 82; 8.0 (1-49)		5-6	adults ca. 18 months	ind. sperm transfer by spermatophores
Lebertia rivulorum K.Viets	n = 39; 8.8 (1-24)	ca. 15	ca. 5		unknown
Lebertia stigmatifera Thor	n = 13; 2.7 (1-5) Scheffler (1967): up to 24	n = 1: 7	3.5		unknown
Hygrobates fluviatilis (Ström)	n = 299; 10.6 (1-62)	up to 16	5-6 Ullrich (1976): 32 d	female max. 12 months	unknown
Hygrobates nigromaculatus Lebert	n = 36; 34.0 (2-69)	n = 1: 5-40	3	(male few months, female 16 months)	ind. sperm transfer by spermatophores, duration of spermatophore deposition: about 20 s Ullrich (1976): deposition also in the absence of female; documented spermatophore: Ullrich (1976)
Atractides nodipalpis (Thor)	n = 182; 5.0 (1-27)	n = 5: 10.8 (6-16) Ullrich (1976): 20	development ca. 4 weeks, hatching ca. 7 months; Ullrich (1976): 35 days, survival for 6 months in clutch envelope; hatching after 2 months in 6 °C and then warming	PRII 2 weeks, adults max. 6 months Ullrich (1976): parasitism 3-4 days, PRI 11-13 d, nymphs 196-224 d, PRII 13-16 d, supposed other life cycle: Gerecke (2002)	ind. sperm transfer by spermatophores; documented spermatophore: Ullrich (1976)
Feltria rouxi Walter	n = 8; 2.5 (1-4)		5	female 10 months, male 3-4 months Efford (1965): 3 weeks	unknown Motas (1928) for *F. setigera*: direct sperm transfer by copulation; Efford (1965): no such observation
Wettina podagrica (Koch)	n = 48; 7.9 (2-16)	n = 1: 5	4-10	female 3-4 months	unknown; Smith & Oliver (1986) for *Wettina*: expectation of direct sperm transfer
Ljania bipapillata Thor	n = 175; 3.0 (1-9)	up to 6 idem: Efford (1966)	4 weeks to 5 months Efford (1966): 23 d (18-20 °C)	PRII 4 weeks, male 5 months, female 9 months	ind. sperm transfer by spermatophores Smith & Oliver (1986): expectation of ind. sperm transfer; Schwoerbel (1967): for *Ljania bipapillata* subsp. and other *Ljania* species: direct sperm transfer by copula
Aturus fontinalis Lundblad	n = 1,149; 1.37 (1-6)		4-9	DN 7 months, PRII 4 d to 3 weeks, male 5 months, female 7 months	ind. sperm transfer during a pairing position, small fields of 10-15 spermatophores, behaviour see text; Proctor (1992): expectation of ind. sperm transfer during a pairing position; Lundblad (1929a): for *A. scaber* speculation of direct sperm transfer during copulation; Smith & Oliver (1986): for *Aturus* observation of a mating position

Table 2 Mean dorsal length (µm) of idiosoma of larvae, nymphs and adults of 13 water mite species. n, sample size.

	Larvae		Nymphs		Males		Females	
Water mite species	Mean (range)	n	Mean (range)	n	Mean (range)	n	Mean (range)	n
Protzia eximia	201 (135-288)	98	493 (312-608)	7	746 (686-827)	17	1,003 (780-1,186)	21
Sperchonopsis verrucosa	195 (173-243)	25	421 (203-608)	219	593 (359-780)	568	749 (374-998)	576
Sperchon clupeifer	237 (160-396)	23	457 (296-640)	30	605 (530-655)	9	775 (437-1,123)	49
Sperchon setiger	201 (180-336)	13	623 (452-749)	21	710 (468-1,123)	127	1,070 (515-1,513)	208
Sperchon thienemanni	258 (208-328)	29	425 (218-702)	363	778 (421-1,123)	432	892 (390-1,295)	407
Lebertia fimbriata	315 (272-392)	67	390 (312-468)	4	768 (655-967)	4	964 (936-998)	8
Lebertia glabra	325 (256-404)	265	493 (312-702)	195	720 (562-1,295)	91	859 (577-1,170)	112
Hygrobates nigromaculatus	305 (276-344)	213	493 (281-733)	33	1,163 (811-1,420)	64	1,145 (764-1,638)	90
Atractides nodipalpis	271 (224-316)	472	406 (234-671)	121	562 (374-686)	254	733 (452-1,076)	286
Feltria rouxi	181 (153-205)	214	230 (203-281)	4	293 (281-312)	5	406 (374-437)	17
Wettina podagrica	256 (233-348)	53	437	1	602 (515-780)	45	724 (484-877)	67
Ljania bipapillata	238 (210-296)	43	365 (250-515)	59	504 (452-546)	157	610 (499-686)	266
Aturus fontinalis	198 (163-228)	853	312 (218-437)	239	374 (265-452)	1136	421 (343-468)	2227

food supply was adequate. Still, whether a laboratory lifetime estimate reflects the situation in the field remains unclear. At temperatures lower than those in the field, the life span of some species was distinctly prolonged (unpubl.). Thus, temperatures adapted to field conditions and the provision of sufficient food may best reflect life duration in the field. In the present study, the age of adults ranged between several months (e.g., males of *Feltria rouxi* and *Aturus fontinalis*) to about 2-3 years in some specimens of various species (females of *Sperchonopsis verrucosa*, *Lebertia fimbriata*, *L. glabra*: 3 years; both sexes of *Sperchon thienemanni* and males of *S. verrucosa*: 2 years). Differential life spans between sexes were observed in many species (*S. verrucosa*, *F. rouxi*, *Ljania bipapillata*, *A. fontinalis*, and possibly *Sperchon setiger* and *Hygrobates nigromaculatus*). The phenomenon of short-lived males was previously observed for many species in laboratory studies (Ullrich, 1976) and was used as an explanation of a female-biased sex ratio in the field (e.g., Bader, 1965, 1980). For most of the species in the present study, the adults represented the overwintering stage (cf. Martin, 1998). For *Sperchon clupeifer* and probably for *F. rouxi*, the adults were the single stage found during the winter, whereas in the populations of *Protzia eximia*, *S. verrucosa*, *S. setiger*, *S. thienemanni*, *Lebertia inaequalis*, *Hygrobates fluviatilis*, and *A. fontinalis*, also a small proportion of nymphs overwintered.

In Table 2, the sizes of larvae, nymphs, and adults of both sexes are presented from individuals obtained in field samples. For most species, most growth occurs during the nymph stage. In all species, the females show a distinctly higher maximum length than the males. A comparison of the length of the females with the maximum number of eggs within the ovigerous females (Table 1) shows a clear positive relation; this is probably also reflected in the reproduction rate of most of the species.

Sexual behaviour

Sexual maturity is obviously reached soon after the emergence of the adults. Male *Sperchonopsis verrucosa* can be observed depositing spermatophores 6 weeks after the end of postlarval resting stage II. In females, the period until oviposition is presumably prolonged to allow the maturation of the eggs, but actual observations of stream-living water mites are lacking. Sperm uptake by the females and oviposition can be several months apart, as Meyer (1985) has shown for a lake-inhabiting mite species.

In water mites, an indirect and a direct mode of sperm transfer is possible (see Proctor, 1992). Direct sperm transfer is realised by a true copulation posture: both partners have their genital openings attached to each other. True copulation with sperm transfer from the male into female genital opening has been observed for several stream-living species (e.g., *Kongsbergia*, see Proctor, 1992). One case of copulation has been reported by Schwoerbel (1967) for one of the species studied here (*Ljania bipapillata*). In the present study, such pairing behaviour has never been observed, neither for the latter species, nor for any of the others. Often, pairing postures in which the female has been observed in a fixed position on the male's dorsal idiosoma has inaccurately been called 'copulation', but with no documented sperm transfer. Many of these observations reflect an indirect mode of sperm transfer (see below). Distinct sexual dimorphism often serves as a hint with regard to copulation behaviour.

The mode of indirect sperm transfer by spermatophores can be assessed from the necessity of the presence of the female for the deposition of the male spermatophore. In the laboratory, the males of *Protzia eximia*, *Sperchonopsis verrucosa*, *Sperchon setiger*, *S. squamosus*, *S. thienemanni*, and *Lebertia glabra* deposited spermatophores on the substrate without the physical presence of conspecific females (Table 1). Since water from the field was used in the laboratory, pheromones might have been responsible for the behaviour of these males. For *P. eximia*, *S. verrucosa*, and *S. thienemanni*, the small spermatophores were drawn (see Table 1) and measured. No morphological irregularities were shown, all fitting into the types documented by Proctor (1992). Total height of the spermatophores (*P. eximia*: 100 µm, *S. verrucosa*: 50 µm, *S. thienemanni* 150 µm) seemed to be smaller than most of those observed by Ullrich (1976) in *S. glandulosus*, *S. setiger*, *L. salebrosa*, *H. calliger*, *H. nigromaculatus*, and *A. nodipalpis* (total length up to 715 µm). Ullrich (1976) also registered spermatophore deposition by the males in the absence of females for most of these species. Usually, the spermatophores examined here were deposited in distinct fields of variable numbers. The field could be small (as in *P. eximia*, 4-20 spermatophores) or larger (*S. verrucosa*, up to about 100 spermatophores), and field size probably also depended on the size (and costs) of a single spermatophore. This is in agreement with the data of Ullrich (1976) who also found fields of spermatophores of variable numbers (up to 50 spermatophores). Ullrich (1976) also described details of spermatophore deposition behaviour, especially for *S. setiger*.

If the presence of conspecific females (mediated by physical contact or by chemical substances) is necessary for the activity of males, then a graduated contact of the sexes occurs together with a more and more complex sexual behaviour (cf. Proctor, 1992; Proctor & Wilkinson, 2001). A mode in which the presence of the female causes spermatophore deposition by the male but no further changes in behaviour, has been observed in a specimen of a North American population of *Atractides nodipalpis* (Proctor, 1992). However, this was not seen in that species examined here. Ullrich (1976) reported increased deposition of spermatophores in presence, compared to absence, of the female in *A. nodipalpis*.

Pairing behaviour can occur with indirect sperm transfer, wherein a male deposits spermatophores on a substrate, remains in the vicinity, and guides a female towards them. Such a behavioural pattern was not observed for the species under study. Sperm transfer during a fixed pairing position (often misleadingly and vaguely called 'copulation' in the literature too) is characterised by a transfer of sperm by the male using his modified extremities to adhere to the female or by steering the female onto spermatophores deposited by the male beforehand. Sperm transfer by using modified legs could not be observed in this study, although a pairing posture has been reported in various *Feltria* species (Motas, 1928; Uchida, 1932). Proctor (1992) assumed that, at least for the subgenus *Feltria*, the modified third legs of the males are used for sperm transfer. A similar pairing position has also been reported by Smith & Oliver (1986) for the genus *Aturus*, Lundblad (1929a) described it for *Aturus scaber* as 'copulation'. Lundblad (1929a) assumed that sperm transfer occurs during the pairing posture by the modified third legs of *A. scaber*. In the present study, the pairing posture did not seem to be connected with direct sperm transfer for the related species *Aturus fontinalis*: Usually, the partners ignored each other, although sometimes a fixed pairing posture between a female on the dorsal idiosoma of a male could be seen, stabilised by the morphologically modified fourth legs of the male. After the apparently accidental meeting and brief physical contact between the partners, the male started to deposit spermatophores, the female remaining nearby. After about 2 min, a small field of 10-15 short-stalked spermatophores was seen. The female then approached the male who vibrated his caudal dorsal idiosoma with his first legs. The female tried to climb actively upon the male's back but was assisted by the fourth legs of the male. A fixed pairing posture was achieved after a few seconds so that the female overlapped two thirds of the back of the male, as observed from the top (much like the drawing for *A. scaber* in Lundblad 1929a). Suddenly, the male made shaking movements such that the genital opening of the female was repeatedly pressed onto different areas of the spermatophore field. The male did not leave his initial position during this behaviour. After sperm transfer, which took about 5 min, the male moved a few steps forward and the female climbed down from his back and left his vicinity. The male stayed near the spermatophore field for several minutes before also leaving the scene of the action. Thus, the sperm transfer of *A. fontinalis* was reminiscent of that in the genus *Arrenurus* (Lundblad, 1929b; Münchberg, 1952; Böttger, 1962, 1965; Proctor, 1992). Although different modes of sperm transfer may take place within *Aturus*, the observed behaviour in *A. fontinalis* is probably more representative for *Aturus* species. Based on the observed pairing posture, Proctor (1992) hypothesised the following behaviour for *A. fontinalis*: the deep genital slit of the male serves as a guide rail for the introduction of the spermatophore head into the female genital opening.

For two Sperchontidae in the present study, a peculiar behavioural pattern was observed that presumably plays a role in the sexual biology. Males, which are distinctly smaller than the females in these species (Table 2), were sometimes found on the dorsal idiosoma of the females. In *Sperchonopsis verrucosa*, the male holds on to the caudal dorsal idiosoma of a female with the first three pairs of legs. The females transport the males for several hours, occasionally even for several days to a few weeks. A similar pairing of one male on a female has been observed in *Sperchon clupeifer*. The only other reported case of such behaviour was for *Sperchon setiger* (Ullrich, 1976): males climb up onto the dorsal idiosoma of the female and remain attached there for several minutes to several hours. Ullrich (1976) also observed that the males often deposit spermatophores just after climbing down from the female. Interestingly, the males that also deposit spermatophores in the absence of females are stimulated to increased deposition activity by their presence. Thus, this kind of pairing behaviour may increase the reproduction success of the male. From the female's point of view, tolerance of the transported male may increase the fertilization of eggs in environments with a low abundance of the species. Consequently, the pairing of the sexes may increase their reproduction effort.

The mode of sperm transfer remains open for *Panisellus thienemanni*, *Thyopsis cancellata*, *S. clupeifer*, *Lebertia rivulorum*, *L. stigmatifera*, *Hygrobates fluviatilis*, *Feltria rouxi*, and *Wettina podagrica*. Since no other way of transfer is reported in the literature, indirect sperm transfer by deposited spermatophores probably occurs in these species, with the exception of *F. rouxi*. Smith & Oliver (1986) also assumed indirect sperm transfer for the genus *Wettina*.

DISCUSSION

The observations presented here and the knowledge on the development and sexual behaviour of water mites previously reported in literature prompt the question as to whether species from running waters have evolved characteristic adaptations for their lotic habitat. The diversification in water mites in general, the large number of lotic water mites of the various taxa (Di Sabatino et al., 2000), and the limited knowledge of the autecology of stream-living water mites provide partial answers to this question. For the evolution in life histories in Parasitengona in general, see Wohltmann (2001).

Modes of sperm transfer have been reviewed by Proctor (1992). The rheobiontic species obviously do not prefer selected strategies of sperm transfer. The multitude of modes may provide clues to the systematic origins of the stream-living species. In the lotic environment water-soluble and volatile chemical cues may play a less important role as indicated by the fact that spermatophore deposition commonly occurs in the absence of females. However, there are many pheromones in Acari as a whole (Sonenshine, 1984) and hence sex pheromones may also exist for stream-living water mites. Recently, Smith & Florentino (2004) published direct and indirect evidence for sex pheromones in various species from standing water. The interesting sexual behaviour of *Aturus fontinalis* prompts the question as to whether

the mating behaviour of *A. fontinalis* represents female choice, and thus *Aturus* species seem to be suitable objects for studies of sexual selection as carried out by Proctor & Wilkinson (2001) for species from standing water.

The egg stage is a risky phase as it is a defenceless part of the mite's life cycle. No reports of predation on water mite eggs have appeared, but the eggs seem to be important in the diet of some water mite species (Martin, 2005). Moreover, small and particularly single eggs without a clutch envelope (*Feltria*, *Aturus*, *Ljania*) might become victims of grazing macro-invertebrates. This risk together with the mechanical stresses produced by the water current may serve as an explanation for the observation that, in most species of the present study, egg laying occurs on the underside of small pebbles offered in the mini-aquaria. This behaviour is seldom seen in species from standing water or from springs (pers. obs.) and thus oviposition may be photophobic, especially in rheobiontic species. Together with the solid clutch envelope, the position of the eggs may reduce their chances of becoming victims, e.g., of larger grazers mainly present on the light-influenced benthos surface of streams. In terrestrial parasitengonid mites clutch envelopes have been interpreted as providing protection against drying out (Wohltmann, 1998), but their prime function may be to serve as mechanical shelter for water mites.

Diapausing eggs may be less important in stream-living than in spring-living water mites (unpubl.). However, for some lotic species, such as *Sperchon glandulosus* (Ullrich, 1976) and some portions of egg populations (*Ljania bipapillata*), dormancy phenomena seem to regulate the synchronisation between the life histories of mites and their hosts. Egg diapause has often been reported for mites in general (Walter & Proctor, 1999) but, in water mites, no clear report has previously been published. Gerecke (2002) hypothesised partial overwintering of the population at the egg stage for *Torrenticola amplexa* in a stream in southwest Germany. The well-developed larvae of *Atractides nodipalpis* as the remaining resting stage in the clutch envelope are the only reported hibernating larvae in water mites. Whether similar observations in spring-living species (e.g., *Hygrobates norvegicus*, *Atractides panniculatus*; unpubl.) are laboratory artefacts or express a life-history strategy of the Hygrobatidae is unclear. The 'chilling' of eggs (or other developmental stages) as a necessary factor for further development has been shown for terrestrial Parasitengona (Eggers, 1995). This phenomenon has been shown as a stimulus only for the diapausing larvae of *A. nodipalpis*. Further studies on this would be interesting as it might also be important for other species from lotic environments. Thus, chilling could also play an important role in synchronization the mite's with the host's life cycle.

The number of eggs produced seems to be extremely low in the Feltriidae and Aturidae. Because Mitchell (1954) found only one egg in most ovigerous *Aturus* females of various species, he assumed a loss of larval parasitism in that genus. Since then, not only *Aturus*, but also *Feltria* and *Ljania* have been shown to be parasites of chironomids (Smith & Oliver, 1986; Martin, 2000; Martin & Stur, 2006). In any case, the small egg numbers in ovigerous females perhaps reflect the small body size of these species, in that only a few eggs can be produced at the same time. The maintenance of high abundance in their habitats is only possible by the production of a sufficient number of consecutive eggs and/or by the high survival of the parasitic larval stage. Species with such low reproduction rates have not been found to accumulate in diverse habitats; such representatives are also found in standing water (e.g., *Axonopsis*) or in interstitial environments (e.g., *Stygohydracarus*). In a survivorship analysis for *Feltria rouxi*, Efford (1965) has assumed a total production of about 10 eggs per female. With a reproduction rate of this dimension, losses in the juvenile stages have to be extremely low, i.e., high survival of larvae occurs during and after parasitism. The numbers of eggs in the ovigerous females (maximum: 69 eggs) together with the maximum lifetime numbers of eggs observed in the present study (about 120 eggs) and similar data in the literature (Ullrich, 1976) allow the conclusion that the reproduction rate is low in stream-living water mites, at least in the families Sperchontidae and Lebertiidae. Similar reproduction rates have been reported for species from standing water (about 200 eggs per female in *Hydrodroma despiciens*; Meyer, 1985). However, high egg production has also been reported. *Eylais discreta* females produce up to 13,800 eggs (Davids, 1973). This supposed excess is presumably connected with the high loss of larvae during parasitization and during postlarval life (Davids, 1997). To date, no instances of such enormous egg numbers have been documented in stream-living mites, but it might occur in some species of high order streams, e.g., in *Hydrodroma torrenticola*. In conclusion, also in the larger mite species of the present study, the losses in juvenile and especially in larval stage are probably not severe.

In the past, the water mites of running water were considered as species that have abandoned their parasitic larvae because of their turbulent environment (Martin, 2000). For most lotic water mites, this hypothesis has been rejected (Smith & Oliver, 1986; Smith, 1998; Smith et al., 2001) and hosts have been reported for nearly all species in the present study (Martin, 2000). An interesting aspect concerns assumed differences in the life cycle of species from different populations. The parasitic larvae of *Hygrobates nigromaculatus* in the present study have non-parasitic larval counterparts in populations from lake shores (van Hezewijk & Davids, 1985). This difference in life cycle seems to be a stable character for this taxon and serves as an argument to separate the species (Martin & Davids, 2002; Martin et al., in prep.). Other species with an ecological distribution in pools of running water and in standing water also exhibit differences in their life cycle (Gerecke, 2002). Perhaps, this kind of distribution in similar microhabitats in both standing and running water might serve as a centre for the creation of new species.

No evidence has been found for prolonged resting stages I or II. None of the rheobiontic species studied here seems to use these stages as a resistant stage or for hibernation. The resting stages are seen as an endangered key stage in the life cycle (R. Gerecke, pers. comm.) but, to date no specific studies investigated their requirements.

The duration of nymph and adult stages obviously depends on their food supply (Martin, 2005). Thus, comparing estimations of the lifespan of a water mite in the field with laboratory observations is difficult. The laboratory finding that some species can live for several years, as was obtained in the present study, has also been noted in phenological studies of water mite species living in springs and spring brooks (Bader, 1977, 1980). However, whereas Bader assumed that females die after oviposition (semelparity) or can lay eggs only during one period of their lives, the present study has shown that females of some species are able to

oviposit in several consecutive years (e.g., *Sperchonopsis verrucosa*, *Sperchon thienemanni*) and thus reflect iteroparity. Some species from standing water have also been found to live for several years, e.g., some *Arrenurus* spp. (Stechmann, 1978).

Thus, the development and life cycle of water mites from running water seem to be varied and flexible. The distinct seasonality in the life cycle of the water mite is based on synchronisation between the occurrence of larvae and the presence of suitable hosts. Often, some of the developmental stages of a population differ in their strategies from the majority of the population (Martin, 1998); this is also reflected in the host spectra of the mites [see Martin (2000) for the species presented, and Martin & Stur (2006) for spring-living species]. The use of this heterogeneity may lay in the spreading of risk – chances of survival in cases of strong disturbances, for example, may be higher if different strategies are expressed.

A few examples exist of different life cycle strategies for different populations of one species (e.g., Efford, 1966; Ullrich, 1976; Meyer, 1994; Martin, 1998; Gerecke, 2002). Differences in life cycle strategies of a species at different sites lead to the hypothesis that water mite species are flexible in their life cycle adaptations and that the developmental stages can be adjusted to the specific requirements of the respective stream. Strong differences in host spectra will also be relevant to this flexibility (Martin & Stur, 2006). Furthermore, taxonomic imprecision may lead to differences in supposed life cycle strategies as Gerecke (2002) mentioned for *Atractides nodipalpis*. Similarities exist between the supposed life cycles by Ullrich (1976) and the population presented here, whereas different life-history strategies have been described by Efford (1966) and Gerecke (2002). Gerecke (2002) suggests that another *Atractides* species is involved in the population of Efford (1966), and similar taxonomic confusion might be surmised in the description of Imamura (1951). Furthermore, the data pool behind the results published up to now is markedly different and rarely have both field and laboratory data been employed as in the present study.

From the results presented above, various patterns of life-cycle strategies and overwintering can be summarised for stream-living water mites at temperate latitudes; these are valid for most specimens of the populations (see Fig. 1):

(1) For most species, hibernation takes place at the adult stage (Fig. 1A). Oviposition takes place at various times from spring onwards. Larvae hatch after a few weeks, parasitise their host, and return normally to their water of origin. The nymphs only live a few months and transform in a given year into adults. The females of this generation hibernate and lay eggs in the next year. This pattern has been found without exception for *Sperchon clupeifer* and is assumed for *Feltria rouxi*. For the main proportion of the population of *Protzia eximia*, *Sperchonopsis verrucosa*, *Sperchon setiger*, *S. thienemanni*, *Lebertia inaequalis*, *Hygrobates fluviatilis*, *H. nigromaculatus*, and *Aturus fontinalis*, this kind of development is valid. *Lebertia stigmatifera*, *L. rivulorum*, and *Ljania bipapillata* overwinter probably predominately as adults too.

(2) Overwintering at the nymphal stage alone has never been observed for any of the species discussed (see Fig. 1B). For species with parasitic larvae occurring late in the year (*Wettina podagrica*, *Lebertia glabra*, and *Sperchon squamosus*), I assume that most specimens hibernate at the nymphal stage. In *Lebertia fimbriata*, about half of the population hibernates as adult (originating from larvae parasitizing early in the year) and the other half hibernates at the nymphal stage (originating from larvae parasitizing late in the year).

(3) Overwintering as well-developed larva in the clutch envelope has been observed only for *Atractides nodipalpis* (Fig. 1C). Single larvae of *Ljania bipapillata* have also been found resting in the clutch until spring conditions of temperature and/or photoperiod.

(4) Another mode of hibernation is absent in Fig. 1: overwintering at the egg stage, as shown for *Sperchon glandulosus* (Ullrich, 1976). Although this mode of hibernation is obviously the rule for this species in the present study, a few eggs of *Protzia eximia* have also been found as a resting stage throughout the winter (see above).

Gerecke (2002) reported data that were based on phenological benthos samples only and on the life cycle of the most abundant species of water mite in a small stream. He found partly differing strategies. For some species, he has also assumed overwintering in the resting stages, as for species in streams in Sicily (Gerecke, 1991) or for temporary pools (Wiggins et al., 1991).

The hibernation strategies in streams are obviously similar to those in other habitats at temperate latitudes. Pieczynski (1976) and Meyer & Schwoerbel (1981) have also found overwintering adult stages in lakes for most species. How tropical species in constantly tempered waters synchronise their life cycle with that of their hosts remains unclear. The hibernation of species on their hosts has often been observed for species in temporary pools (Wiggins et al., 1991; Davids, 1973, for *Hydrachna*), but has not been reported for stream-living mites. Such a strategy could be undertaken by species in temporary streams but this has not yet been studied.

The sensitivity of the mites to various disturbances (such as pollution by nutrients, heavy metals, degradation of substrata) is probably attributable to the complexity of their life cycle and the different demands of the various developmental stages. This diversity in water mite biology may be the main reason for the proposal of water mites as bioindicators in stream limnology (Di Sabatino et al., 2000). As in limnology in general, the species composition of water mites in larger streams and/or rivers is not well known. New biological data are required regarding the species from such habitats. Other lotic habitats (springs and hyporheic interstitial) are

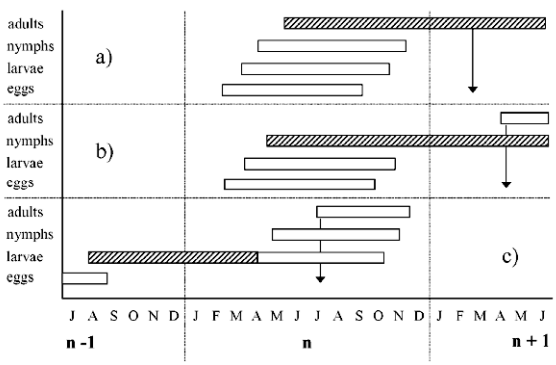


Figure 1 Schematic life cycles of water mites from two small streams in Northern Germany. Valid only for the main part of the population. A) hibernating adults, B) hibernating nymphs, C) hibernating as developed larvae in the clutch envelope. For explanation and species, see text.

also known to have a peculiar species composition. Although various studies of the faunistics and biology of spring-living mites are in progress (e.g., Gerecke & Martin, 2006; Martin & Stur, 2006), the biology of species adapted to live in the interstices below the stream benthos is virtually unknown.

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Nest microflora in the social spider mite, *Stigmaeopsis longus* (Acari: Tetranychidae)

Ayumi Kikuchi & Michiko Tanaka[1]
Lab. Animal Ecology, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan.
E-mail: kikuayumm21@yahoo.co.jp; and [1]Lab. Applied Microbiology, Microbial Resources and Ecology, Graduate school of Agriculture, Hokkaido University

Group-living has many benefits, but it also has some costs. The social spider mite *Stigmaeopsis longus* weaves nests on the undersurface of dwarf bamboo leaves and deposits fecal piles at specific sites for nest sanitation. These nests are hypothesized to have a protective function against predators. However, predators are not the only natural enemies, pathogens are also important. If the nest has a protective effect against microbes, and if waste management plays a role in nest sanitation, it is expected that the number and diversity of phylloplane fungi inside the nest should differ from the outside. As such, we attempted to compare the fungi inside and outside nests. Our results show that fungal populations inside differed significantly from those outside the nests, and *Cladosporium cladosporioides* was the dominant species both inside and outside, but it was more abundant inside. Furthermore, there was a significant difference in the correlation of *C. cladosporioides* with other fungi inside vs. outside nests. We conclude that *C. cladosporioides* may have a suppressing effect on other, more harmful, fungi in *S. longus* nests.

Key words: *Stigmaeopsis longus*, phylloplane fungi, *Cladosporium cladosporioides*, nest sanitation

Group-living offers animals several advantages, such as effective foraging, increased group defense, enhanced reproduction, and better access to suitable environments for survival and reproduction (Krause & Ruxton, 2002). Such life styles inevitably also incur costs, however, such as competition for habitat and food resources, and increased parasite burden (Rosengaus et al., 1998; Krause & Ruxton, 2002), because the increased proximity of individuals is likely to increase the likelihood of transfer of pathogens.

To decrease the risk of infection, eusocial insects (bees, ants, termites) have various defense mechanisms, such as grooming and antibiotic secretions (Rosengaus et al., 1998; Hart & Ratnieks, 2002; Little et al., 2003). In nest-building animals, negligent defecation habits may increase the probability of disease infection. In this respect, nest sanitation behavior may well be an important adaptation (Sato et al., 2003; Sato & Saito, 2006). For instance, in ants and bees, workers carry the defecation wastes of their queens and larvae out of the nest (Hölldobler & Wilson, 1990).

Stigmaeopsis longus weaves nests by silken threads on the undersurface of dwarf bamboo (*Sasa senanensis*) leaves, and lives within them in large groups. Nest members pierce and feed upon leaf cells within the confines of their nest, and deposit piles of fecal pellets at specific sites to achieve some form of nest sanitation (Sato & Saito, 2006). Recently, it was hypothesized that defecation behavior in this mite follows a simple rule: if the first faeces are deposited near the nest entrance, then volatile cues emanating from these faeces will trigger others to deposit their faeces nearby as well (Sato et al., 2003). The nest woven by *S. longus* does not only help to prevent predators from intruding (Mori et al., 1999), but it also defines the arena within which *S. longus* adults defend their own offspring by counterattacking any predatory mites intruding the nest (Saito, 1986a,b). However, predators are not the only natural enemies. Pathogens such as fungi and bacteria may also be important enemies of *S. longus*. Hence, we asked whether the web confers any advantages in combating pathogens. We hypothesized that the nest has a direct antimicrobial function. Alternatively, the nest indirectly influences which microbes grow within.

For either of these hypotheses, waste management is expected to play a role in nest sanitation, and the number and diversity of phylloplane fungi inside the nest is expected to differ from the outside. Here we compare the number and diversity of fungi inside and outside the nests.

MATERIALS AND METHODS

Sasa leaves with *S. longus* nests were collected from the campus of Hokkaido University. The upper side of a leaf was sterilized with a 5% sodium hypochlorite solution (note that we subjected to test the leaf undersurface), and a single 2 × 10-mm leaf section was removed from inside each nest and another leaf section from the non-nest area just adjacent to it.

The leaf-washing and dilution-plating method (Jager et al., 2001) was used to isolate and quantify phylloplane fungi from washed leaves. Ten leaf pieces were shaken in sterile physiological sodium chloride water, and then the suspensions were plated onto potato-dextrose agar (PDA) and incubated at 25 °C. Seven days after inoculation, any fungal colonies that had appeared on the plates were counted. We replicated this treatment 15 times.

In addition, frequently observed species were identified based on morphological features and the information from rDNA sequencing (26S rRNA1-4, ITS region including 5.8 rDNA ITS1-4).

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Table 1 The result of identification by rDNA sequencing.

Species no.	Identification	Species no.	Identification
1	*Cladosporium cladosporioides*	14	mitosporic Hypocreales
2	*Aureobasidium pullulans*	15	mitosporic Tremellales
3	mitosporic Nectriaceae	16	-
4	-	17	Sordariomycetes
5	*Acremonium* sp.	18	-
6	*Sydowia polyspora*	19	-
7	-	20	-
8	*Arthrinium* sp.	21	*Meria geulakonigii*
9	-	22	*Gleomella lagenaria*
10	*Acrodotium* sp.	23	-
11	-	24	*Phaeosharia* sp.
12	*Phoma* sp.	25	-
13	*Diosezegia takashimae*	26	*Altanaria* sp.

-, non-identified species.

Table 2 Analysis of covariance for number of fungal colonies.

	df	SS	MS	F	P
Site (inside, outside)	1	1.817	1.817	5.672	0.025
Cladosporium cladosporioides (present, absent)	1	0.068	0.068	0.212	0.649
Site**C. cladosporioides*	1	1.383	1.383	4.319	0.048
Residual		26	8.327	0.320	

RESULTS

There were significantly more colonies inside the nest than outside (Fig. 1) according to a paired t-test. Figure 2 shows the diversity of fungal species, and Table 1 is the result of identification by rDNA sequencing. Sixteen out of 26 species were observed frequently. They were identified based on morphological features and rDNA sequences. The dominant species (no. 1 in Table 1 and Fig. 2), both inside and outside nests, was identified as *Cladosporium cladosporioides*. On the inside 902 cfu/ml were counted and 135 cfu/ml on the outside.

As the frequency of *C. cladosporioides* increased outside the nest, other fungi also increased. On the other hand, inside the nests, other fungi rather decreased with the increase of *C. cladosporioides* (Fig. 3). An analysis of covariance (ANCOVA) indicated that there was a significant difference in the correlation of *C. cladosporioides* and the other fungi between leaf sections inside and outside the nests (Table 2).

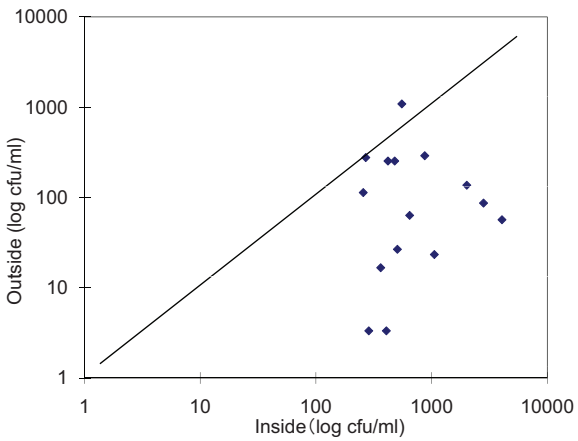

Figure 1 Number of fungal colonies inside and outside nests. The dots indicate fungal population inside and outside for each replicate. There were more colonies/ml inside than outside the nests (P<0.05, paired t-test).

DISCUSSION

Our result demonstrated that there were about eight times more colonies of fungi inside than outside the nests. The coprophilous fungus *C. cladosporioides* was the dominant species both inside and outside, and it was more abundant inside. These findings suggest that *C. cladosporioides* was associated with the fecal piles deposited by *S. longus* inside the nest.

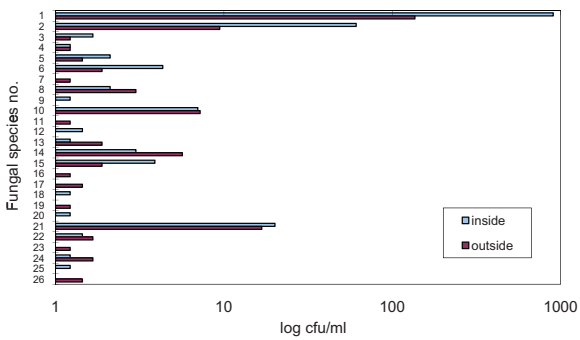

Figure 2 Diversity of fungal species. The horizontal axis represents the total number of fungal colonies for 15 replicates.

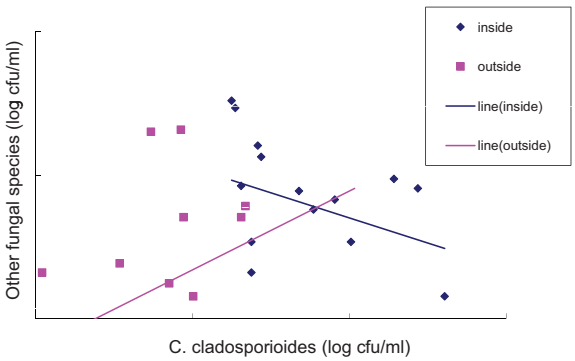

Figure 3 The influence of *Cladosporium cladosporioides* on other species. There was a significant difference in the correlation of *C. cladosporioides* and the other fungi inside and outside nests (P<0.05, ANCOVA).

Cladosporium cladosporioides is known as a typical phylloplane fungus, and it is widely distributed on plant surfaces, withered plants, fallen leaves, soil, and excrement. *Aureobasidium pullulans*, *Acremonium* sp., *Arthrinium* sp., *Phoma* sp., *Gleomerella lagenaria*, *Phaeosharia* sp. and *Altanaria* sp. are plant-pathogenic fungi. Some plant- or entomopathogenic species are known in the genus *Nectria,* including mitosporic Nectriaceae. We do not yet know whether they are pathogenic towards dwarf bamboo and spider mites.

There was a significant difference in the correlation of *C. cladosporioides* with other fungi inside vs. outside nests. The stable and fertile microclimatic conditions within the nest may facilitate the development of phyllosphere fungi, including pathogens, which could bring a considerable cost to group-living animals (Wilson, 1971). However there are not only pathogenic fungi inside nests on leaves. Because *C. cladosporioides* was isolated frequently, we suppose it may have some suppressing influence on other, more harmful, fungi in *S. longus* nests.

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Evolutionary and Ecological Acarology: Demography, Diapause and Dispersal

Seasonal adaptations in the life cycles of mites and ticks: comparative and evolutionary aspects

Valentin N. Belozerov
Department of Entomology, Biological Research Institute, St. Petersburg State University, Stary Peterhof, St. Petersburg, 198504, Russia. E-mail: val.belozerov@mail.ru

Data on seasonal adaptations (in particular on distribution of dormant stages) within the life cycles of the Acari (Chelicerata, Arachnida) are reviewed and compared with similar traits in mandibulate arthropods (insects and crustaceans). They all share responses to photoperiod and temperature, but there are some similarities and essential peculiarities with regard to the ontogenetic distribution of diapausing stages for each of these groups. The main similarity concerns the species-specifity of the dormant stage position, whereas differences concern the number and ontogenetic distribution of these stages within life cycles. Plesiotypic traits of seasonality in the Acari are expressed in the presence of complex 'systems of seasonal control' (SSC) with various dormant stages enabling the control of perennial, semivoltine, and monovoltine development, whereas apotypic traits are characterized by simple SSC with a limited number of dormant stages for control of mono- and polyvoltine development. The Oribatida, a major part of the Prostigmata, and all Ixodida show evolutionary transformations from plesiotypic to apotypic traits of seasonality, but Astigmata, some Prostigmata, and Mesostigmata show fixed apotypic traits only, which pleads for their derived state. Primary adaptations enabling seasonal control of development in arthropods emerged most likely at the earliest steps of the origin of life on earth, and evolved thereafter from the ancestral devices within various taxa of animals according to their organization, manner of life, geographic distribution, and environmental heterogeneity. The evolution of seasonal adaptations in the Acari occurred through transformation of ancestral systems of seasonal control with numerous dormant stages into systems with a reduced number of dormant stages, or even a single dormant stage.

Key words: Dormant stages, ontogenetic distribution, diapause, seasonal adaptations, Acari, insects, crustaceans

Animals and plants generally exhibit adaptations to seasonal climate changes. They require season-dependent switching from active to dormant stages in their life cycles or vice versa. The dormant stages serve to survive adverse seasons, and to synchronize the switch to active stages with seasons favourable for development and reproduction.

In arthropods the most important type of dormancy anticipating the approach of an adverse season is diapause. This involves reduced physiological activity and an arrest of development and reproduction in response to environmental factors (e.g., temperature decline) or signals thereof. Studies of the mechanisms underlying induction, maintenance, and termination of diapause have shown the primary role of photoperiod as an environmental signal. Whereas even to date much of the diapause research concerns the response of arthropods to this signal (termed photoperiodism), less attention is paid to the type of dormancy (termed quiescence) that is induced under the direct impact of adverse climate conditions.

This article is on seasonal adaptations in the life cycles of mites and ticks (Acari). It does not concern the physiological mechanisms of life-cycle control and photoperiodism. Instead it will focus on the ontogenetic distribution of dormant stages within their life cycles. The comparative study of these traits of diapause and their correlation with taxonomic data may help to clarify the evolution of seasonal adaptations in the Acari, as representatives of chelicerate arthropods, and enable comparison with other (mandibulate) arthropods (Belozerov, 2007).

A history of diapause research in insects and acarines

Seasonality has received much more attention in insects than in other taxa. The first experimental demonstration of the phenomenon of 'diapause' was made in eggs of the silkworm (*Bombyx mori*) by Duclaux (1869), but the term 'diapause' was first introduced by Wheeler (1893) to describe a particular stage in the embryonic development of a grasshopper (*Xiphidium* sp.). It was Henneguy (1904) who used the term to refer to the physiological state of dormancy or arrested development in insects. In this latter sense (see Andrewartha & Birch, 1954), the term 'diapause' became widely used in entomology, even more so after discovery that photoperiod played a primary role of in diapause control (Kogure, 1933; Danilevsky, 1948). By now, there is a vast literature on seasonality and photoperiodism in insects, including a number of excellent monographs (Lees, 1955; Danilevsky, 1961; Tyshchenko, 1977; Beck, 1980; Saunders, 1982; Zaslavsky, 1984; Tauber et al., 1986; Danks, 1987). The first case of 'diapause' among the Acari was described not until 1907, 40 years later than in insects, when Beynarovitch discovered a long delay in development of the autumn-engorged larvae of the tick *Ixodes ricinus*. In Russia, Alfeev (1948) and Serdjukova (1951) highlighted diapause and life cycles in ixodid ticks. They described the diversity of their diapause stages (immature, adult, and egg stages). Belozerov (1968, 1982) later showed that two main types of diapause, one developmental and the other behavioural, occur in these parasitic Acari, i.e., in engorged and unfed specimens, respectively, and that both types emerge in response to photoperiod. That seasonal activity in ticks depends on day length, was discovered as early as 1941 by Smith & Cole for the case of the American dog tick (*Dermacentor variabilis*) and later for development of engorged immature *Ixodes ricinus* by Loew (1962), Belozerov (1963, 1968), and others. Photoperiod-dependent diapause was also shown in plant-feeding tetranychid mites (Lees, 1950; Bondarenko, 1950), in plant-feeding eriophyoid mites (Sapozhnikova, 1982), as well as in predatory phytoseiid mites (Putman, 1962). The available data on diapause and its control have been reviewed for ixodid ticks by Belozerov (1982) and Sonenshine (1988), for

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Table 1 Groups of Acari considered with regard to their seasonal adaptations [classification follows Evans (1992), with some changes].

Superorder	Order	Suborder	Lower taxa
Acariformes (= Actinotrichidia)	Oribatida (= Cryptostigmata)		representatives of different oribatid mites
	Astigmata (= Sarcoptiformes)	Acaridia	representatives of some tyroglyphoid mites
		Psoroptidia	representatives of feather mites (Analgesoidea)
	Prostigmata (= Actinedida; Trombidiiformes)	Eupodina	*Halotydeus*, *Penthaleus* (Penthaleidae) + Halacaridae
		Eleutherengona (= Rhaphignathina)	Tetranychoidea + Eriophyoidea
		Parasitengona	Trombidia (Trombidioidea, Trombiculoidea) + Hydrachnidia
Parasitiformes (= Anactinotrichidia)	Mesostigmata	Gamasina	Phytoseiidae, Macrochelidae, Parasitidae
		Uropodina	representatives of uropodid mites
	Ixodida (= Metastigmata)		representatives of main families of Ixodida

tetranychid and phytoseiid mites by Veerman (1985, 1992, 1994) and Overmeer (1985), for eriophyid mites by Manson & Oldfield (1996), and for Parasitengona by Wohltmann (2001). It should be noted that among the Acari ixodid ticks, tetranychid mites, and phytoseiid mites have been more thoroughly studied with regard to photoperiodic control of diapause and that these taxa share much of the mechanisms observed in insects (Belozerov, 2007).

Comparative aspects in distribution of dormant stages in life cycles of the Acari

As a polyphyletic group, the Acari are very diverse in their dormant stages with respect to number, distribution, and properties that affect life cycle duration and phenology. Reviewing the data available for all groups of Acari studied (Table 1), shows that even in the same ordinal (or subordinal) taxon, some representatives possess complex 'systems of seasonal control' (SSC), with many dormant stages and long (semivoltine and perennial) life cycles, whereas others have simple SSC, with a limited number of dormant stages (only one or two), and rather short life cycles, enabling mono- and polyvoltine development. This is typically the case in oribatid mites, but also exists in some prostigmatic mites from the superorder Acariformes (Table 2A,B), and in the specialized group of blood-sucking ixodid ticks from the superorder Parasitiformes (Table 3). However, other orders (and lower taxa) are more uniform in having a simple SSC**,** such as the Astigmata in the Acariformes (Table 2A), and the Mesostigmata in the Parasitiformes (Table 3).

Most representatives of the order Oribatida have the ancestral – plesiotypic, according to Norton (1994) – characteristics in their life-history, ontogenetic, and eco-physiological traits. The same plesiotypic traits are characteristic also for the SSC of their life cycles. This hypothesis is well supported by data on distribution of dormant overwintering stages within the life cycles of Oribatida (Table 2A). Most of the soil mites from plesiotypic habitats (soil and litter in forests of temperate climate) are capable of monovoltine, semivoltine, and perennial development, and can overwinter in a dormant state practically in any stage during ontogeny (mainly as adults, proto-, and deutonymphs, but also as eggs, larvae, and tritonymphs) and can still ensure the synchronization in development of their successive generations. Such a complex SSC is also an adaptation of oribatid mites to extreme conditions involving long winters and short summers in the Antarctic (*Alaskozetes antarcticus*) and the Subarctic (*Ameronothrus lineatus*). These oribatids have life cycles of 5-6 years and can overwinter in a state of diapause (or quiescence?) in every stage. In contrast, some small-sized oribatids (*Oppia*, *Oppiella*) from temperate habitats are characterized by polyvoltine development with a transition from rapid spring-summer generations to an autumn generation that overwinters in the egg or adult phase (i.e., simple apotypic SSC with only one or two dormant stages). Unfortunately, the physiological properties of dormant stages in oribatids are investigated rather poorly (the only exception is probably presented by *A. antarcticus* with strong dormancy at every stage), but it is impossible as yet to be sure, what type of dormancy (diapause or quiescence) prevails in these mites with plesiotypic life cycles..

A quite different situation is observed in the related, but derived order of Astigmata, presented by free-living Acaridia and parasitic Psoroptidia mites, which are small in size, develop rapidly, and have a simple SSC with only one (Acaridia) or one-to-two dormant stages (Psoroptidia). In Acaridia dormancy is often associated with phoresy and it occurs in the heteromorphic facultative deutonymph (hypopus), the presence or absence of which is controlled in preceding stages depending on environmental and genetic factors (Knülle, 1999). This ontogenetic development of diapause in response to predictable factors is strong evidence for diapause in the hypopus stage (Danks, 1999), but it should be noted that the hypopus is usually an adaptation to irregular, non-predictable environmental changes. Parasitic Astigmata differ from their free-living relatives by the permanent loss of the deutonymphal stage (they retain only the proto- and teleonymph stage), by more expressed seasonality (in feather mites it is connected with regular seasonal events of their host birds) and by the possibility to hibernate in one of two stages (teleonymph and egg) (Dubinin, 1951). Undoubtedly, Acaridia and Psoroptidia represent sister groups with apomorphies concerning two ontogenetic traits: (1) the facultative or obligate absence of the deutonymph stage, and (2) the possibility for dormancy in either the deutonymph or the teleonymph stage. More research is needed to assess which types of dormancy occur in the Astigmata, as well as in the Oribatida.

The Prostigmata are especially diverse with respect to their life cycles and SSC. Below various taxa in this order are reviewed using abbreviations for terms describing the various stages in their life cycle (E for egg, PL for prelarva, L for larva, PN for protonymph, DN for deutonymph, TN for

Table 2 The presence of different types of diapause in life cycles of acariform mites from various orders.

Taxa	Subtaxa and voltinism	Egg	L	PN	DN	TN	Ad	Main references
A. Oribatida, Astigmata and partly Prostigmata								
Oribatida	Perennial development	+	+	+	+	+	+	Block & Convey, 1995
	Mono- and semivoltine	+	+	+	+	+-	+	Weigmann, 1975
	Polyvoltine development	+	-	-	-	-	+	Woodring & Cook, 1962
Astigmata	Suborder Acaridia	-	-	-	Hyp	-	-	Knülle, 1999
	Suborder Psoroptidia	+	-	-	-	+-	-	Dubinin, 1951
Prostigmata	Suborder Eupodina							
	– Penthaleidae	+	-	-	-	-	-	Umina & Hoffmann, 2003
	– Halacaridae	-	-	+-	+-	+-	+	Straarup, 1968
	Suborder Parasitengona							
	– Erythraeina	+-	-	+?	+-	+?	+-	Wohltmann, 2001
	– Trombidia – Trombidiidae	+-	-	-	+	-	+	Zhang, 1999
	– Microtrombidiidae	+	-	-	+	-	+	Wohltmann, 2001
	– Trombiculidae	+	+	-	+	-	+	Daniel,1961; Sasa,1961
	– Johnstonianidae	+	-	-	+	-	+	Eggers, 1995; Wohltmann et al., 1994
	– Hydrachnidia	+-	+-?	?	+	?	+	Wohltmann, 2001
B. Suborder Eleutherengona (Prostigmata)								
Tetranychidae	Bryobia et al.	+	-	-	-		-	Veerman, 1985
	Tetranychus et al.	-	-	-	-		+	Veerman, 1985
Taxa	Habits	Egg	N1	N2	Ad, Protogyne		Ad, Deutogyne	
Eriophyidae	On coniferous (hidden)	+	+	+	+-		+	Bagnjuk, 1975
	On coniferous (open)	+	-	-	-		-	Löyttyniemi, 1971
	On foliary trees	-	-	-	-		+	Shevchenko, 1957
	Grass (Deschampsia)	+	+	+	+		no	Sukhareva & Sapozhnikova, 1975
	Grass (Calamagrostis)	-	-	-	+		no	Sukhareva, 1992

The presence (+) and absence (-) of ability for diapause at the stages of egg, protonymph (PN or N1), deutonymph (DN or N2), tritonymph (TN), and adult (Ad). There are two types of adult mites in Eriophyidae – protogyne (Ad protog.) and deutogyne (Ad deutog.). Hyp, hypopus in the deutonymphal stage of Acaridia.

Table 3 The presence of different types of diapause in the life cycles of mesostigmatic mites and ixodid ticks (superorder Parasitiformes).

Taxa		Egg	L	PN	DN	Ad	Main references
Suborder Gamasina	Phytoseiidae	-	-	-	-	+	Veerman, 1992
	Macrochelidae	-	-	-	-	Ph	Evans, 1992
	Parasitidae	-	-	-	Ph	-	Evans, 1992
Suborder Uropodina	Majority of uropods	-	-	-	Ph	-	Evans, 1992
	Uropoda repleta	+	-	-	-	-	Evans, 1992
Taxa		Egg	L		N	Ad	
Order Ixodida	Many *Ixodes*, *Haemaphysalis*	+	+		+	+	Belozerov, 1976, 1988
	Nearctic *Dermacentor*	-	+-		+-	+-	Belozerov, 1976, 1988
	Palearctic *Dermacentor*	-	-		-	+	Belozerov, 1976, 1988
	Some *Rhipicephalus*	-	+-		-	+-	Belozerov, 1976, 1988
	Boophilus	-	?		-	-	Belozerov, 1976, 1988

The presence (+) and absence (-) of ability for diapause at the stages of egg, larva (L), protonymph (PN), deutonymph (DN), nymph (N), and adult (Ad). Ph indicates the presence of phoresy combined with dormant state.

tritonymph, Ad for adult). Among the groups studied sufficiently thoroughly, are mites of the suborder Parasitengona with terrestrial Trombidia and fresh-water Hydrachnidia (Wohltmann, 2001). Both these groups originated from terrestrial predatory mites with a modified ontogenesis, involving alternation of active feeding stages (L, DN, Ad) and non-feeding calyptostases (PL, PN, TN). This modified ontogenesis is retained in recent terrestrial and aquatic Parasitengona, however with larvae adapted to parasitism on invertebrates and vertebrates (Belozerov, 2008a). The chigger mites (Trombiculidae) are best known among the Trombidia, because their larvae are parasites of rodents and vectors of some human rickettsioses. The chigger mites are usually monovoltine, but with overlapping generations, each well synchronized with the seasons because they can overwinter in most of their stages (E, L, DN, Ad), and because the adult mites have a long life span. Such a complex SSC is characteristic for the European species *Neotrombicula autumnalis* with mixed mono- and semivoltine development (Daniel, 1961), though *Leptotrombidium* spp. in Japan have mixed monovoltine generations, the synchronizing control of which is ensured by the simple SSC with two dormant stages (N and Ad females in *L. akamushi*; Takahashi et al., 1995; L and Ad in *L. pallidum*;Takahashi et al., 1993). But the SSC in representatives of the related trombiculoid family Johnstonianidae includes three dormant stages (E, DN, Ad) synchronizing monovoltine development of their overlapping generations (Eggers, 1995). In these mites, the dormant state of eggs laid in autumn is a facultative diapause controlled by temperature (Wohltmann, 2001). Similar traits of seasonality (mono- or semivoltine development, dormancy in long-lived DN and Ad, as well as E) are characteristic of representatives of two related families of terrestrial trombidioid mites – Trombidiidae and Microtrombidiidae, larvae of which do not parasitize mammals (as in the Trombiculidae), but insects (Zhang, 1999; Wohltmann, 2001).

Some important differences in regard to seasonality and its control appear in the Hydrachnidia, a specialized group of Parasitengona adapted to live in fresh water. They retain the type of ontogenesis known in terrestrial Trombidia – with alternation of active (feeding) and passive stages (calyptostases), as well as the switching of larvae from a predatory life style to parasitism on water insects. Here, the diversity of dormant stages is richer than in their terrestrial relatives, in that dormancy occurs not only in Ad, DN, L, and E, but also in calyptostases (PN, TN). This allows for a better synchronization of their larvae with the appearance of their insect hosts.

It is no surprise that the monovoltine life cycle in the group Erythraeina, phylogenetically derived from other Parasitengona (Wohltmann, 2001), is regulated usually by means of the simple SSC with a single dormant stage (though this stage can have a different ontogenetic position even in species that are related): Ad in *Erythraeus cinereus*, DN in *E. regalis*, or E in *E. phalangoides.* There are also some species with two dormant stages: PN and E in *Leptus funandezi*, PN and Ad in *L. ignotus* and *L. trimaculatus.* However, the majority of erythraeine mites (including monovoltine *Balaustium murorum* and polyvoltine *B. putmani*) have a diapause in the egg stage. It should be noted that Erythraeina represent another group of prostigmatic mites with dormancy in the calyptostasic stages, a phenomenon not known for other terrestrial Parasitengona (Belozerov, 2008a).

Similar to the Parasitengona (with more complex SSC in fresh-water Hydrachnidia, in contrast to terrestrial Trombidia) is the situation in the Eupodina**,** another suborder of prostigmatic mites (Table 2A), where predatory mites of the marine family Halacaridae have mono- and semivoltine development with overwintering Ad, PN, DN and TN, whereas herbivorous terrestrial Penthaleidae (*Halotydeus destructor*, *Penthaleus major* and others) in Australia have polyvoltine winter development with seasonal control ensured at the one stage only (facultative summer egg diapause induced by increasing photoperiod and terminated by high temperature).

Similar diversity occurs also in the suborder Eleutherengona (Table 2B). The group of four-legged mites (family Eriophyidae) have tight co-evolutionary connections with their host-plants, and exhibit deep morphological and ontogenetical specialization. It reveals a large diversity with respect to their life cycles (semi-, mono- and polyvoltine), as well as with respect to their SSC**:** many overwintering stages in the refuge-inhabiting mites *Trisetacus piceae* and *T. kirghizorum* living on coniferous plants, but only eggs overwintering in the vagrant mite *Nalepella haarlovi* (Loyttyniemi, 1971) from conifers, and only deutogyne females overwintering in *Aculus schlechtendali* and *Epitrimerus vitis* (Manson & Oldfield, 1996) from apples and grapes, and in the gall mite *Eriophyes laevis* from alder (Shevchenko, 1957). Many eriophyids exhibit this so called deuterogyny which involves the presence of morphologically and functionally different adult females: protogyne females capable of reproduction in summer and deutogyne females with a diapause arrest of reproduction in winter. It is now known that in some eriophyids an arrest of summer development through the appearance of deutogyne females is controlled by photoperiod (Sapozhnikova, 1982). Strikingly, there are no true deutogyne females in grass-inhabiting eriophyids. They overwinter either as protogyne females, or at any other stage in their life cycle (Sukhareva & Sapozhnikova, 1975). It is quite possible that in these species hibernation is realized in a state of quiescence.

Much more uniform with regard to the SSC of life cycles is another group of plant-feeding eleutherengone mites, the so called spider mites (family Tetranychidae). They are also important agricultural pests with rapid polyvoltine development leading to 5-7 and even more generations per season (one generation lasts ca. 2 weeks; Sabelis, 1991), that is stopped either by embryonal diapause of eggs (i.e., in the genera *Bryobia*, *Petrobia*, *Schizonobia*, *Aplonobia*, *Eurytetranychus*, *Schizotetranychus*, *Panonychus*, and *Oligonychus*), or by reproductive diapause of adult females (i.e., in the genera *Tetranychus*, *Eotetranychus*, *Platytetranychus*, and *Neotetranychus*, plus one species from the genus *Oligonychus*). Both types of diapause in spider mites are facultative, and have a lot in common with insects in their photoperiodic control (Veerman, 1985, 1994). The role of photoperiod in diapause induction was discovered simultaneously in *Panonychus ulmi* with egg diapause (Lees, 1950), and in *Tetranychus urticae* with imaginal diapause (Bondarenko, 1950). The latter species, owing to easy culturing, rapid development, and sharp photoperiodic responses, is one of the main models (together with some insects) in the study of mechanisms of seasonal photoperiodism in arthropods (Veerman, 1985). An important contribution to the study of diapause in spider mites in Russia was realized by KF Geyspitz and her students.

Only two orders of parasitiform acarines, Ixodida (= Metastigmata) and Gamasida (= Mesostigmata), are studied more or less sufficiently to be considered in this review. The order Mesostigmata includes two suborders, the Gamasina (with a broad range of life styles, ranging from predatory to phytophagous and parasitic) and the Uropodina (sharing some ecological similarity with oribatid mites). Both taxa are identical in their ontogeny (E, L, PN, DN, Ad). Development is more rapid in gamasid mites (1-3 weeks) than in uropodines (3-6 months), though their voltinism is similar (mono- and polyvoltine). In both suborders the state of dormancy is usually associated with phoresy on insects and other arthropods for dispersal. This occurs in stages of homeomorphic DN or Ad in Gamasina, and heteromorphic DN in Uropodina. Such a phoretic dormancy may be either obligate (appearing every generation), or facultative (depending upon an interaction of genetic and environmental factors, similar to the hypopus formation in astigmatic mites). In both groups of mesostigmatic mites, hibernation occurs in the same stages of DN or Ad as in which they are phoretic. However, in some uropodines dormancy may also occur in E.

In contrast to most mesostigmatic mites, there is much known on seasonal adaptations of the predatory mites of the family Phytoseiidae, well known for their ability to control plant-inhabiting spider mites (Veerman, 1992). Similar to their prey, they have polyvoltine development arrested in autumn by diapause in the adult females. The main role of photoperiod in the control of phytoseiid reproductive diapause was discovered by Putman (1962) in *Typhlodromus caudiglans*, and by Sapozhnikova (1964) in *Amblyseius similis.* Similarity of responses to photothermic factors in phytoseiids and tetranychids ensure the synchronization of their seasonal events and, as a result, the efficiency in control of spider mites by phytoseiids. With regard to photoperiodic control of diapause these two groups of mites reveal great similarity with insects, and owing to their rapid development they represent extremely convenient objects for experimen-

tal study of photoperiodism and the underlying physiological mechanisms. Except for some differences in seasonal traits, both suborders of mesostigmatic mites exhibit great similarity in the SSC of their life cycles, as revealed in the reduced number of dormant stages (usually DN or Ad). These dormant stages are associated with phoresy (without photoperiodic control) in Uropodina and most Gamasida, but with strong photoperiodic regulation of reproduction in adult females of phytoseiid mites.

The special position among the Acari is occupied by ixodoid ticks (order Ixodida). Owing to their great medical and veterinary importance, ixodoid ticks (especially the family Ixodidae) represent one of the most thoroughly studied groups of arthropods (Balashov, 1967, 1998; Sonenshine, 1991, 1993). The seasonal events and the regulatory mechanisms in ixodid ticks were subjects of my experimental investigations during more than 40 years (Belozerov, 1963, 1976, 1982, 1988, 1991, 1999, 2002). In spite of the great biological and developmental specialization of ixodids, revealed in oligomerization of ontogenesis with a single nymphal stage and a single engorgement at every feeding stage (L, N, Ad), ixodid ticks possess a wonderful diversity of life cycles, phenology and seasonal adaptations (Belozerov, 1976; Balashov, 1998). The SSC in primitive representatives of this family (genera *Ixodes* and *Haemaphysalis*) are characterized by a multitude of regulatory adaptations (Table 3) enabling the induction of dormant state and its termination in unfed larvae, nymphs, and adult ticks (behavioural diapause), the arrest of development in eggs and engorged larvae, nymphs, and adult ticks (developmental, or morphogenetic diapause), as well as in ticks attached to their hosts (delay of feeding and engorgement). Populations of these ticks in forest landscapes of temperate climate have, as a rule, overlapping generations and perennial development. More derived species of the same genera reveal acceleration of development (to semi- and monovoltine) due to reduction of their SSC by means of transformation of some regulatory stages into the so called transitory stages, which have lost the regulatory functions, but gained the ability for rapid non-diapause development. The same changes in voltinism and the type of SSC occur in other genera of more derived ixodid ticks adapted to open landscapes and warmer climate (*Dermacentor*, *Hyalomma*, *Rhipicephalus*, and even tropical *Amblyomma*). Extreme cases are the single-host ticks *Boophilus* and *Anocentor* adapted to tropical and subtropical conditions by continuous polyvoltine development and absence of real dormant stages. However, in the palearctic *Hyalomma scupense* and nearctic *Dermacentor albipictus* with single-host parasitism and the retention of diapause ability (unfed larvae and slow-feeding nymphs) we observe the opposite phenomenon, in that these ticks modify the season of their feeding and developmental activity: they are parasitic not in summer, but in winter.

Two types of seasonal control systems in acarine life cycles

The review of available data on dormant stages within life cycles of the Acari shows that these chelicerate arthropods can be divided into two groups according to their systems of seasonal control (SSC):

The first group is characterized by variable SSC with various numbers of dormant stages (ranging from many to just a single one) and by great diversity of voltinism (from perennial up to polyvoltine development). The diversity of this complex group is distributed over the full range from 'K-selected' to 'r-selected' acarines. It comprises the whole order Oribatida, some taxa of the order Prostigmata, namely the suborder Parasitengona, and partly the Eupodina (family Halacaridae) and the Eleutherengona (superfamily Eriophyoidea), and especially the whole order Ixodida (=Metastigmata).

The second group is characterized by a simple SSC with a limited number of dormant stages (usually single, but sometimes two) determining the duration of polyvoltine development or the seasonality of monovoltine development. This group comprises the whole order Astigmata (both Acaridia and Psoroptidia), partly the order Prostigmata (family Penthaleidae from the suborder Eupodina, and superfamily Tetranychoidea from the suborder Eleutherengona), as well as the order Mesostigmata (Gamasina and Uropodina). They are represented by 'r-selected' species mainly.

Evolutionary aspects of dormancy and life-cycle control in the Acari

Russian entomologists, such as Ushatinskaya (1976) and Tyshchenko (1983), usually considered the evolution of seasonal adaptations in insects and the phylogeny of insects as separate processes, not related to each other, despite the high degree of correlation between the diapausing stage and the phylogenetic classification of insects (Tauber et al., 1986; Danks, 1987). This independence was motivated by assuming an easy, independent, and de novo evolution of diapause, the properties and position of which are determined by ecological demands (Danilevsky, 1961; Alexander, 1968; Kozhanchikov, 1976; Danks, 1987). But in Crustacea the evolution of diapause is clearly connected with their phylogenesis, as well as with environmental changes during their transition from the sea into fresh waters (Alekseev, 1990; Alekseev & Starobogatov, 1996). In my view, Acari are much like Crustacea in this respect.

As a rule, the seasonal control in mandibulate arthropods is realized apotypically, i.e., at a single diapausing stage in a species-specific position during ontogeny. However, in insects this may be any ontogenetic stage, depending on the particular taxon. The majority of Crustacea are capable of dormancy at only one particular stage: the egg in the lower palaeolimnic, the adult in the higher neolimnic Crustacea. The mesolimnic Maxillopoda are an exception among the Crustacea, in that they can be dormant in all three stages including the larva.

In the Acari, as representatives of chelicerate arthropods, the number of dormant stages can vary, in that the majority of mites and ticks ('K-selected' organisms with slow development) possess a complex SSC with numerous regulatory stages, capable of diapause in different (but species-specific) ontogenetic stages. However, some mites have 'r-selected' properties (with rapid development) and a simple SSC with a limited number of regulatory diapausing stages (one or two) – these are either species from otherwise 'K-selected' taxa, or species from ordinal (Astigmata, Mesostigmata) and lower taxa (Tetranychoidea, Penthaleidae) consisting of 'r-selected' species only, with uniformly a simple SSC.

Just like the specialized monophyletic group of ixodid ticks (Parasitiformes: Ixodidae), thoroughly analyzed with respect to their adaptations to climate seasonality (Belozerov, 1976, 1977, 1982, 1988, 1991, 1999, 2002), the evolution of life cycles and their SSC in mites may be realized through similar transformation from ancestral polymeric SSC with

numerous dormant stages, into oligomeric and monomeric SSC with fewer diapausing stages (Fig.1). This transformation probably first involved modification of some dormant stages into transitory non-diapause stages that enable rapid development. Subsequently, changes occurred in the direction of oligomerization (Dogiel, 1954; Beklemishev, 1970) or simplification (Danks, 1987) of SSC in life cycles. The end product of the transformation is rationalization (Schmalhausen, 1968). Together with increasing reduction of diapausing stages, primitive perennial cycles transform into semi-, mono-, and polyvoltine cycles, and in extreme cases even into continuous development. This view is in good agreement with that of the German acarologist Andreas Wohltmann (Wohltmann et al., 1999; Wohltmann, 2001), who considered monovoltinism and polyvoltinism in Hydrachnidia as plesiomorphic (plesiotypic) and apomorphic (apotypic) traits, respectively. These successive transformations of seasonal events and adaptations can be observed in Ixodida, as well as in Oribatida and Prostigmata.

The patterns of evolutionary transformations in life cycles of the Acari due to climate seasonality are in conflict with the views of many ixodologists (Alfeev, 1948, 1951; Serdyukova, 1960) and entomologists (Kozhanchikov, 1959; Alexander, 1968; Masaki, 1978; Tyshchenko, 1983; Zaslavsky, 1988), who consider the evolution of insects and ticks as a pathway from continuous non-diapause development in ancestral, uniformly warm and humid climate, without seasonality, to monovoltine and perennial development with seasonal adaptations to more harsh climate. However, as was noted by Tauber et al. (1986), there are no cases illustrating the de novo evolution of diapause from completely non-diapause population in insects. All experimental and comparative entomological studies indicate that seasonal adaptations involve modification of diapause that already existed in the original population. A similar opinion 'in favour of the evolutionary transformations in development of diapause' is expressed for Crustacea as well (Alekseev & Starobogatov, 1996), as studies on their ordinal taxa revealed the dominance of apotypic traits of seasonal control. I adhere to the same opinion that seasonal adaptations in arthropods (and in the Acari particularly) emerged not from a non-diapause state, but involve successive modifications of dormant responses that have existed already in their ancestors. Unfortunately, it is as yet not possible to assess which type of dormancy was characteristic for these ancestors, but it is possible to suggest (in contrast to views of Tyshchenko, 1973, and Tauber et al., 1986) that the primary dormant states were of passive, consecutive, and non-specific nature, acquiring connections with photoperiodic responses during successive steps in the evolution of seasonal adaptations. Therefore, the study of simple forms of dormancy (as quiescence) in arthropods is of great interest for the problem under consideration.

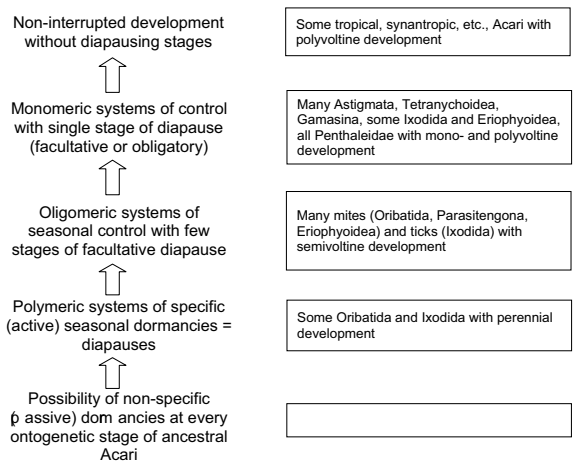

Figure 1 Evolutionary changes in systems of seasonal control of life cycles in Acari. Left: successive steps of evolutionary transformation in systems of seasonal control (SSC) in the Acari. Right: representatives of the Acari with respective SSC type.

My interpretation of the origin and evolution of seasonal adaptations in the Acari, based on results from the analysis of ixodid tick life cycles (Belozerov, 1991) may be considered as a preliminary scenario for the evolution of seasonal adaptations in the Acari. It is still necessary to acquire more data on biology and taxonomy of Acari to obtain a more comprehensive perspective for the evolution of life cycles and systems of seasonal control in these chelicerate arthropods. Three major types of biological studies may provide insight into the evolution of diapause: artificial selection experiments, analyses of adaptive changes among colonizing species, and comparative taxonomic analyses of seasonal adaptations. The latter approach is a powerful tool for evolutionary studies of insect seasonal cycles (Tauber et al., 1986). The value of this approach (not only for insects, but also for other mandibulate and chelicerate arthropods) derives from the fact that the distribution of dormant stages is conservative and species-specific. Former attempts to analyse the evolution of seasonal adaptations (Tyshchenko, 1973, 1983; Tauber et al., 1986), though very important, have been based on analyses of traits involved in diapause control (e.g., photoperiodism) that are too variable and too labile.

Conclusion

Adaptations enabling seasonal control of development in acarines, insects, and other arthropods probably emerged at the earliest steps of life on Earth, and evolved afterwards on the basis of these ancestral devices in various taxa of animals and plants according to their organization, life style, geographic distribution, and environmental changes (Belozerov, 2007). The evolution of seasonal adaptations in the Acari (in accordance to our previous data on ixodid ticks and the current analysis of data available for acariform and parasitiform mites) has proceeded through transformation from ancestral polymeric systems of seasonal control (with numerous dormant stages) to oligomeric and monomeric systems (with a reduced number of diapausing stages), by means of transformation of some diapausing stages into transitory non-diapause stages enabling the acceleration of development. The ontogenetic position of diapausing stages and their number is determined in the Acari by phylogenesis and by adaptations to environmental changes. A better understanding of the origin and evolution of seasonal adaptations in the Acari requires various studies combining biology, taxonomy, and phylogeny. In my view two topics for ecophysiological investigations on acarine seasonality stand out as particularly promising: (1) the relationship between the distribution of diapause and that of quiescence in 'K-selected' Acari, and (2) the role of quiescence as a consecutive dormancy in life cycle control of Acari and its synchronization with seasonality of environmental factors (see also Belozerov, 2008b, 2009a,b).

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Embryonic diapause and cold hardiness of *Ixodes ricinus* eggs (Acari: Ixodidae)

Hans Dautel* & Wilhelm Knülle

Institute of Applied Zoology/Animal Ecology, Freie Universität Berlin, Haderslebener Str. 9, 12163 Berlin, Germany.
*Present adress: IS Insect Services GmbH, Haderslebener Str. 9, 12163 Berlin, Germany. E-mail: dautel@insectservices.de

Ixodes ricinus is medically the most important tick species in temperate Europe. The present study was undertaken to assess the effect of photoperiod and low-temperature exposure on termination of embryonic diapause and on cold hardiness in *I. ricinus* eggs. Engorged *I. ricinus* females (n = 9) were kept at 90% r.h., 15 °C longday (L17:D7), or 15 °C shortday (L14:D10), or at an outdoor site providing natural temperatures and daylength. Eggs of defined ages were continuously kept under these conditions or subjected to changes in photoperiod and temperature, and the effect on diapause incidence was observed. At 15 °C, non-diapause and diapause eggs hatched after 70-120 and 140-240 days, respectively. Interestingly, most egg masses did not show an all or nothing reaction but a certain percentage of diapause eggs. Cold exposure of eggs to 4 °C for 6 weeks completely terminated diapause, whereas the effect of photoperiod was negligible. Also exposure to temperatures between -10 and -20 °C for 24 h terminated diapause. At the outdoor site, eggs were laid in winter, from mid October to early April. All eggs hatched between mid June and early July. The supercooling point of these eggs was around -28 °C between November and January and rose to -27 °C in April. At constant 15 °C, the supercooling point rose from -30 °C in young eggs to -24 °C in physiologically older eggs. The lower lethal temperature in diapause and non-diapause eggs was -21.6 and -18.0 °C, respectively, but the difference was not significant.

Key words: *Ixodes ricinus*, diapause, cold hardiness, egg

Ticks can display four kinds of diapause (Belozerov, 1996): (a) behavioural diapause, expressed as (i) a temporary resilience of unfed ticks to initiate host-seeking, or (ii) a delay of feeding in ticks parasitizing a host in winter, and (b) developmental diapause, manifested by (iii) a delay of development in eggs or engorged immatures, or (iv) a delay of oviposition in fed females. The latter is also called reproductive or ovipositional diapause (Sonenshine, 1988). In *Ixodes ricinus*, the occurrence of behavioural diapause is a matter of debate (Gray, 1987, 2002; Randolph et al., 2002), whereas developmental diapause in engorged larvae and nymphs is well documented (Belozerov, 1982; Belozerov et al., 2002).

Diapause in eggs, also called embryonic diapause is only known from a few tick species, including *I. ricinus* (Belozerov, 1982). According to Belozerov (1974), diapause is induced by a shortday photoperiod in the unfed female. Ticks fed in late summer or autumn then lay eggs that enter diapause and overwinter. In the course of our studies on cold hardiness of *I. ricinus* (Dautel & Knülle, 1996, 1997), we also investigated the egg stage. The primary aim of the study was to elucidate the degree of cold hardiness in diapause and non-diapause eggs (Denlinger, 1991) as well as to find out, in a first attempt, whether photoperiod or low temperatures might terminate embryonic diapause.

MATERIAL AND METHODS

Ticks

Adult *I. ricinus* ticks were collected in a Berlin forest on 20 September 1993 and subsequently fed on a rabbit. All engorged females had detached by 29 September and were subsequently kept individually in gauze-covered vials inside desiccators providing 90% r.h., based to Winston & Bates (1960), and placed either at (i) 15 °C shortday (SD) (L10:D14; n = 3 females), or (ii) 15 °C longday (LD) (L17:D7; n = 3 females), or (iii) at an outdoor site (n = 3 females) protected by direct sunlight and providing good conditions for development of *I. ricinus* (Dautel & Knülle, 1997). Weekly minimum and maximum temperatures at that site were recorded by a minimax thermometer.

Treatment of eggs

After onset of oviposition, egglaying females were carefully removed from their egg-mass and transferred to an empty vial at weekly intervals. Thus, the maximum age difference between eggs in a vial was 7 days. Eggs either remained under the respective conditions or were subjected to other treatments, e.g., transferred to one of various combinations of temperature or daylength within their vials. Direct handling of the eggs was avoided whenever possible in order to minimize mortality. Eggs were inspected weekly under a binocular and the number of hatched and unhatched eggs was estimated. Eggs from the outdoor site were not inspected at subzero temperatures. At the end of the experiments, all eggs and larvae were removed from their vials and they were counted. When the sucercooling point was determined, all eggs were kept at 90% r.h., also during the cold treatments.

Influence of temperature and photoperiod on termination of diapause

Eggs of 15-30 days since oviposition and kept at 15 °C LD or SD, were transferred to 4 °C and darkness for 2 or 6 weeks. Thereafter, the eggs were transferred to either 15 °C SD or 15 °C LD, and the period until hatching was determined.

Determination of cold hardiness

The lower lethal temperature LT_{50}, the temperature at which 50% of the eggs had died) was determined in eggs kept at

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Table 1 Course of oviposition (mean number of eggs/day) of female *Ixodes ricinus* at 15 °C longday (LD) or shortday (SD).

	Tick no.					
Days after start of oviposition	1	2	3	4	5	6
5-11	73.3	81.7	34.4	78.4	46.1	62.3
12-18	75.3	65.1	12.7	65.3	37.3	42.9
19-25	37.7	54.5	8.3	19.3	40.4	34.7
26-32	18.3	20.9	0	16.3	10.3	10.0
Daylength	**SD**	**SD**	**SD**	**LD**	**LD**	**LD**
Total no. of eggs	1852	1927	613	1549	1267	1193
Female body mass (mg)	268.9	221.5	79.4	213.3	156.7	126.3
No. eggs/mg female body mass	6.89	8.70	7.72	7.26	8.09	9.45

Table 2 Oviposition of a female *Ixodes ricinus* and hatching of the eggs at the outdoor site during the winter 1993/1994.

Period of oviposition	Temp. (°C) (min/max)	No. eggs	Mean no. eggs/day	% eggs hatched 06/08	06/21	06/29	07/06	% egg mortality
10/15-10/26	7.5/14.0	719*	59.9	-	-	-	-	-
10/26-11/02	7.0/9.5	263	37.6	0	50	100	-	2.7
11/03-11/16	0.5/10.0	606**	43.3	-	-	-	-	-
11/17-11/23	-1.0/4.5	0	0	-	-	-	-	-
11/24-12/13	-1.0/12.0	422	22.2	0	50	100		0.9
12/14-01/04	3.0/9.0	288	13.1	0	0	100		5.9
01/05-01/11	4.0/9.0	121	17.3	0	0	90	100	0.8
01/12-01/18	3.0/10.0	81	11.6	0	0	100		0.0
01/19-02/21	-7.0/9.0	319	5.2	0	0	70	100	23.5
03/22-04/12	-3.0/14.0	239	17.1	0	0	25	100	20.9
Total		3058	17.1	0	19.8	83.4	100	8.4

*: eggs used for determination of LT_{50}, **: eggs used for determination of T_{50}.

15 °C SD or LD, as well as in eggs from the outdoor site in December 1993. Eggs (n = 79-299 per treatment) were exposed to certain subzero temperatures for 24 h. Temperatures inside the desiccators containing the eggs were regularly monitored by Ni/NiCr thermocouples. The cooling (and warming) rate was between 0.08-0.3 °C/min. Eggs from the outdoor site were at an age of 14-21 days. After cold exposure, these eggs were acclimated at 4 °C for 24 h and then kept at 15 °C and darkness until hatching. Eggs kept at 15 °C LD or SD were 15-30 days old and were kept at their respective conditions after cold exposure.

The lethal time T_{50}, (the time period after which 50% of the eggs had died) was determined in eggs from the outdoor site (age: 14-21 days) in December. Batches of eggs were cooled at 0.08 °C/min and kept at -10.1±0.2 °C for 1, 8, 15, 30, and 60 days. Thereafter, the eggs were warmed to 4 °C for 24 h and then kept at 15 °C and darkness until hatching.

Determination of the supercooling point (SCP)

SCP measurement was performed by attaching groups of eggs (n = 4-6 eggs) to Ni/NiCr thermocouples by means of paraffin oil. Thermocouples and eggs were kept inside an aluminium tube immersed in a bath of 90% ethanol which was cooled by an immersion cooler. Cold exposure started at room temperature resulting in a nonlinear cooling rate of about 0.3 °C/min between 0 and -20 °C. The SCP was determined as the lowest temperature recorded prior to the release of latent heat.

Statistical analysis

LT_{50} and T_{50} were calculated by Probit analysis. Differences in mean supercooling points between eggs of different age classes were evaluated by the Mann-Whitney U-test. Supercooling points of eggs from the outdoor site determined at different moments in winter and spring were compared by one-way ANOVA and the post hoc Scheffé-test.

RESULTS

Oviposition

Table 1 shows the course of oviposition in those ticks kept at 15 °C SD or LD. Depending on body mass after repletion, females laid between 613 and 1,927 eggs, equivalent to 6.9-9.5 eggs/mg body mass. The daily output of eggs was highest at the beginning and gradually diminished to the end of oviposition.

Table 2 shows the seasonal course of oviposition of a single female from the outdoor site as well as that of the hatching of larvae. The female, weighing 435 mg after repletion, laid a total of 3,058 eggs, equivalent to 7.0 eggs/mg body mass. Oviposition started in mid October and continued throughout the winter until late March/early April. Oviposition was interrupted, when temperatures constantly fell below 4-5 °C. There was a phase with such low temperatures in November, and a longer period in February, where even maximum temperatures did not rise above -1 °C for more than a week. Mortality of the overwintering eggs was 0-5.9% in eggs laid between October and January, and rose to >20% in eggs laid between mid January and April. Hatching of the eggs started about mid June and was completed in early July.

Supercooling capacity

In eggs kept at 15 °C LD or SD, the mean supercooling point was close to -30 °C in eggs less than a week old (Table 3). This value rose significantly to about -27 °C and -25 °C at an age of 3-4 weeks and 9-10 weeks, respectively, both under LD or SD conditions.

Eggs from the outdoor site showed a fairly uniform mean SCP of -28 °C from November through January (all eggs used for SCP determination were from a batch laid in early November). There was a statistically significant, although very low increase to -27 °C in April (Table 4).

Cold hardiness

Lower lethal temperature

Table 5 shows the egg mortality after 24 h exposure to different subzero temperatures. The calculated LT_{50} values (+ 95% confidential interval) were -14.5 °C (-2.5 – -21.4) in eggs from the outdoor site, -21.6 °C (-17.7 – -26.5) in eggs kept at 15 °C SD, and –18.0 °C (-3.2 – -30.3) in eggs kept at 15 °C LD. Mortality rates were quite variable resulting in large confidence intervals, and thus, no significant differences were detectable between the LT_{50} values of eggs from the different sites.

Table 3 Supercooling point of *Ixodes ricinus* eggs of different ages kept at either 15 °C shortday (SD) or longday (LD). The supercooling points differ significantly between age groups (Mann-Whitney U-test; P<0.05).

Daylength	Egg age (days)	Mean±SD (min – max)	n
SD	1-7	-30.3±1.5 (-26.3 – -32.0)	34
	22-28	-26.9±0.7 (-24.7 – -28.2)	27
	64-70	-24.4±1.2 (-22.3 – -27.1)	25
LD	1-7	-31.3±1.8 (-26.7 – -33.7)	43
	22-28	-26.8±1.5 (-20.0 – -29.3)	35
	64-70	-25.8±1.2 (-22.3 – -27.1)	27

Table 4 Supercooling point of *Ixodes ricinus* eggs from the outdoor site determined on various moments throughout winter 1993.

Date	Mean±SD (min – max)	n
11/23	-28.4±2.2a (-23.2 – -32.0)	22
12/11	-28.5±1.5a (-23.9 – -31.0)	18
01/13	-28.4±0.6a (-27.2 – -30.1)	27
04/17	-27.2±1.0b (-24.4 – -28.6)	21

Means followed by different letters differ significantly (Scheffé-test, P<0.05)

Table 5 Mortality (%) of eggs from different sites (outdoor site, 15 °C shortday, or 15 °C longday), exposed to constant subzero temperatures for 24 h. Between brackets: no. eggs.

Exposure temp. (°C)	Outdoors	15 °C SD	15 °C LD
-5.5	5.6 (92)	0 (189)	9.4 (167)
-10.1	5.9 (150)	13. 2 (145)	16.2 (79)
-15.4	85.3 (152)	11.8 (206)	64.7 (203)
-20.0	71.8 (175)	-	-
-24.0	98.5 (79)	52.9 (299)	61.1 (236)
-35.0	100 (71)	100 (239)	100 (165)

Lethal time

Exposure of eggs from the outdoor site (n = 606 eggs) at -10.1 °C resulted in 6.0 and 2.9% mortality after 1- and 8-day exposure, respectively. Exposure for 15, 30, and 60 days always resulted in 100% mortality. The calculated T_{50} of the eggs is 10.8 days.

Occurrence of diapause

Development of eggs at constant 15 °C showed a bimodal pattern. A first cohort of eggs from a given batch typically hatched within a period of 10-15 weeks after oviposition, whereas a second cohort hatched between 24 and 34 weeks post oviposition (Table 6). The delayed development of the latter is regarded as diapause (development takes >140 days), whereas eggs developing within 15 weeks (<120 days at 15 °C) are regarded non-diapausing. Only one of the six egg batches developed uniformly without any diapause eggs (tick no. 6, Table 6). All other batches showed a proportion of diapause and non-diapause eggs, that remained constant within a given batch, irrespective whether the eggs were laid at the beginning or at the end of oviposition. The only exception was an egg batch kept at LD (Table 6, tick no. 5), where 50% of the eggs laid at the beginning had entered diapause, but none of the eggs laid at the end. Variation in percentages of diapause eggs were observed both at SD and LD.

Parts of the egg batches kept at SD or LD were exposed to 4 °C and darkness for 2 or 6 weeks and therafter kept at the same photoperiod, or the photoperiod was changed. The results are shown in Table 7. After a 2-week cold exposure, eggs showed the same high or low percentages of diapause eggs as in the control under constant conditions. A change from SD to LD and vice versa apparently did not alter diapause incidence. A cold exposure for 6 weeks, however, always resulted in 0% diapause, irrespective of the photoperiod before or after cold treatment.

Table 6 Duration of embryonic development (days) until emergence of larvae from *Ixodes ricinus* egg batches kept at constant 15 °C shortday (SD) or 15 °C longday (LD). Eggs hatching later than 140 days after oviposition are regarded to have been in diapause.

Tick no.	Experimental condition	n	Time until egg hatch		Mortality (%)	Diapause (%)
			Non-diapause (min-max)	Diapause (min-max)		
1	15 °C/SD	931	70-108	167-187	14.9	40
2		500	77-102	180-228	6.0	95
3		290	77-109	167-228	7.9	95
4	15 °C/LD	862	77-109	190-250	17.6	95
5		309	70-90	142-240	17.5	50-0*
6		185	70-118	-	8.6	0

*At the beginning of oviposition 50% of the eggs entered diapause, but at the end none.

Table 7 Diapause incidence of eggs after (cold) exposure to 4 °C for 2 or 6 weeks with and without change of photoperiod. SD: shortday; LD: longday. Tick numbers are the same as in Table 6.

Tick no.	Experimental condition	n	Mortality (%)	Diapause (%)	
				treatment	control
3	SD 2 weeks 4 °C SD	143	3.8	100	95
3	SD 2 weeks 4 °C LD	89	16.9	80	95
5	LD 2 weeks 4 °C LD	261	3.8	0	50-0
6	LD 2 weeks 4 °C SD	300	5.7	0	0
2	SD 6 weeks 4 °C SD	456	1.8	0	95
1	SD 6 weeks 4 °C LD	532	14.1	0	40
6	LD 6 weeks 4 °C LD	353	36.5	0	0
4	LD 6 weeks 4 °C SD	394	3.6	0	95

Table 8 Developmental time of eggs surviving a 24-h (cold) exposure to subzero temperatures. Eggs originated from two females kept at SD (tick no. 2) or at LD (tick no. 5). Tick numbers are the same as in Table 6.

Tick no.	Exposure temp. (°C)	Developmental time (days) (min-max)	Cold treatment Diapause (%)	n	Control Diapause (%)	n
5	-5.5	96-160	30	151	0/50	309
	-10.1	85-113	0	66	0/50	309
	-15.4	85-103	0	71	0/50	309
	-24.0	130-160	(100)	91	0/50	309
2	-5.5	96-169	80	189	95	500
	-10.1	85-105	0	125	95	500
	-15.4	96-108	0	181	95	500
	-24.0	115-133	?	138	95	500

The development of eggs surviving 24-h cold treatment in the course of LT_{50} determination also showed some interesting patterns (Table 8). After exposure to -5.5 °C, eggs kept at SD conditions showed a diapause incidence of 80%, similar to that of the control (eggs from the same batch kept under constant SD) where 95% diapause was observed. However, exposure to lower subzero temperature resulted in complete non-diapause development. Only in eggs surviving exposure to -24 °C, developmental time was intermediate between diapause and non-diapause development. Similar results were also obtained for the eggs kept at LD. Exposure to -5.5 °C resulted in a diapause incidence similar to the control, whereas exposure to -24 °C resulted in intermediate and diapauses type developmental times. There was no diapause in the eggs at the remaining temperatures.

DISCUSSION

Egg development and diapause

Numbers of eggs laid by ticks largely depend on the body mass of replete females. Compared to data from Gray (1981), ticks in the present study showed a relatively high conversion rate of body mass into eggs, suggesting that the conditions for oviposition were quite good. The fact that development of the eggs at 15 °C was either completed within 70-120 (mostly 70-100) days or delayed until 140-240 days after oviposition, strongly suggests that those eggs exhibiting delayed development had entered a state of diapause. *Ixodes ricinus* is one of the few tick species known to show facultative diapause in the egg stage (Belozerov, 1982). Interestingly, the percentage of diapause eggs within a given batch was mostly not 100% or 0% but in between, which was also observed by Belozerov (1974). This diapause incidence was remarkably constant, i.e., the same in eggs laid at the beginning vs. at the end of the oviposition period. In only one egg batch diapause incidence declined in the course of oviposition under LD.

According to Belozerov (1974), induction of diapause takes place in the unfed female in response to a short-day photoperiod before feeding. This is supported by the present results. Ticks had been exposed to the natural (i.e., declining) day length and thus had been exposed to a number of short days before feeding in late September. Because not all egg batches showed a high diapause incidence, feeding may have taken place at a season where diapause was induced in just a part of the population. However, as diapause incidence declined in the course of oviposition under LD in a single batch, it cannot be excluded that photoperiod may have some influence also on engorged females after feeding. This deserves further investigation.

Diapause development (sensu Tauber et al., 1986) is greatly accelerated by low temperatures in *I. ricinus* eggs. According to our results, a 6-week, but not a 2-week exposure to 4 °C suffices to complete diapause. Such acceleration of diapause development is quite common among arthropods (Tauber et al., 1986, Hodek & Hodkova, 1988), and in nature it commonly results in termination of diapause and change to a quiescent state already at the end of the year, i.e., before mid winter. Interestingly, also a short-term exposure to temperatures of -10 °C and below resulted in non-diapause development. As certain shock events, like treatment with chemicals, heat, or cold shock, can indeed terminate diapause in several arthropod species (Danks, 1987), this may have also been the case with *I. ricinus* eggs. In eggs exposed to -24 °C, diapause was probably also terminated. The fact that development was retarded compared to non-diapausing eggs may have been caused by cold-induced injuries suffered at these low temperatures.

At the outdoor site, egglaying in *I. ricinus* was observed throughout the winter, a remarkable feature not often seen in arthropods. The results suggest that the female laid eggs whenever temperatures were above ca. 5 °C and intermittently stopped oviposition at lower temperatures. Mortality in the eggs was quite low. Also embryonic development in this species proceeds at low temperatures compared to other tick species (Dautel & Knülle, 1998). Visible development in the eggs even takes place at temperatures between 6 and 10 °C (Czapsa, 1967) and can be completed at constant 10 °C (cited in Randolph et al., 2002). Although the seasonal period of larval hatching was quite narrow, the results clearly show that larvae emerged earlier in eggs laid earlier in winter, and vice versa. This may indicate that eggs laid in early winter underwent some development already at mild winter temperatures.

Supercooling capacity and cold hardiness

Eggs of *I. ricinus* showed a high supercooling capacity. With increasing physiological age, the supercooling point rose from -30 to -24 °C. Winter acclimatized eggs showed a quite stable SCP of -28 °C from November through January. However, in April it was slightly elevated. This might be indicative that development had already started before April.

Altogether, eggs are the stage with the lowest SCP in *I. ricinus* (Dautel & Knülle, 1997). However, the SCP does not correspond with a similar degree of cold tolerance, as was also shown for some other mite species (Veerman, 1992; Morewood, 1993). The SCP thus is not indicative of cold hardiness in *I. ricinus* eggs.

Nevertheless, the long term exposure showed that eggs survive a temperature of 10 °C for about 11 days and 50% of the eggs survived a 24-h exposure to temperatures between

-14.5 and -21.6 °C, depending on maintenance conditions. As it is unknown whether the eggs from the outdoor site were in a state of diapause at the time of cold exposure and as there were no statistical differences detectable between the LT_{50} of predominately diapausing and non-diapausing eggs, no clear conclusions can be drawn as to whether or not diapause is associated with a higher degree of cold hardiness in *I. ricinus* eggs.

In the present study, cold hardiness of the eggs was determined during an early phase of embryonic development (15-30 days after oviposition). Egg diapause in *I. ricinus* arrests development at mild autumn temperatures, conserving the eggs in a yet unknown phase for overwintering. In future studies, it seems worthwile to compare cold hardiness of diapause and non-diapause eggs also in later phases of embryonic development.

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Phoresy revisited

A.M. Camerik
School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Wits, 2050, Johannesburg, South Africa. E-mail: Anne.Camerik@wits.ac.za

Based on Farish & Axtell's (1971) definition of phoresy and using Euzet & Combes (1980) terminology for the classification of host-parasite relationships, Athias-Binche (1994) proposed a classification of eco-physiological phoretic behaviour in mites. However, in the light of recent publications on mite-host relationships and of my own observations, Farish & Axtell's (1971) definition of phoresy has to be revised and, consequently, Athias-Binche's (1994) classification adapted.

Key words: Phoresy, mite-host relationships, ecological and eco-physiological categories, evolution

Lesne's (1896) definition of phoresy referred to a *temporary*, initially loose, interspecific association between certain free-living organisms – a transport vector (= host) and its passenger – which ended when the traveller disembarked as it arrived at a suitable location. The primary outcome of phoresy was dispersal. However, the author envisaged that this association over many generations could have evolved into a more permanent affiliation and finally became parasitic.

In 1917, Deegener coined the word *symphorium* for phoretic associations. In this definition, he, like Lesne (1896), excluded parasitic and symbiotic relationships but did not include the *temporary* character of the phoretic association. He incorporated in his definition more *permanent* associations like barnacles on whales and sharks, anemones on crabs, and sessile protozoans on snails and insects.

The issue became confused, when van Someren & McMahon (1950) and Noble & Noble (1964), according to Farish & Axtell (1971), began to use Lesne's term *phoresy* in the sense in which Deegener defined *symphorium*. Farish & Axtell (1971) therefore proposed to re-introduce Lesne's original understanding of phoresy and limit its use to strictly *temporary* associations. They defined phoresy as 'a phenomenon in which one animal actively seeks out and attaches to the outer surface of another animal for a limited time during which the attached animal (termed the phoretic) ceases both feeding and ontogenesis, such attachment presumably resulting in dispersal from areas unsuited for further development, either of the individual or its progeny'.

The physiological aspect of the phoretic relationship in this definition was indeed limited to a strictly temporary, non-parasitic association. However, already in 1931 Vitzthum, and later Lindquist (1983), noted how difficult it was to distinguish between phoresy, pseudo-, and real parasitism amongst free-living mites. The phoretic, according to Athias-Binche (1994), even when entering a quiescent stage during the journey, disturbs its host already by its sheer presence. Phoresy, according to this author, could therefore be considered to be a particular form of parasitism.

This notion was further corroborated by the results of Houck & Cohen's (1995) experiments with the astigmatic mite *Hemisarcoptes cooremani* Thomas and the coccinellid *Chilocorus cacti* (Coleoptera). This species-specific phoretic mite (phoretomorph or phoriont) rendered ambiguous the physiological aspect of a phoretic relationship being limited to a non-parasitic and non-symbiotic state. The phoretomorph lacked chelicerae, a mouth, and seemed to have a non-functional gut. However, during transport on the beetle the mite's phoretomorph acquired at least tritiated water directly from its host's haemolymph into its functional hindgut. Furthermore, the beetle's haemolymph was a prerequisite for further development as hypopes not in contact with *C. cacti* died without completing their life cycle. Consequently the two attributes, feeding from its host and subsequently using the nourishment to promote its own development, belong to a parasitic way of life.

Furthermore, the authors believed that the nymphal stage of some astigmatic lineages co-evolved with their common associates to such a degree that the mites developed an alternative deutonymphal stase, called the hypopus (hypopode or hypope). This stase could therefore be considered a link between free-living ancestors and a future strict parasitic way of life. Parasitism, they reasoned, comes to conclusion when the hypopes would moult into adults that continue to feed on their host. This pathway has not yet been completed in this species.

It seemed, however, also possible that some free-living mites could develop into parasites without this kind of evolutionary pathway. In 1996 Polak described the unspecialised homeomorph of the mesostigmatic mite *Macrocheles sub-*

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badius (Berlese), phoretic on *Drosophila nigrospiracula* (Diptera). During transport the traveller pierced the fly's integument and ingested its haemolymph. Moreover, the mite was not only phoretic on flies, but also attached to scarab beetles and rodents. *M. subbadius*, as GW Krantz stated (pers. comm.), 'seems to have taken a shortcut to parasitism, without the expected *evolutionary fanfare...*'.

Summarizing, restriction of the phoriont-phoront (traveler-host) association to the time during which transport takes place, limits the scope of the association. Nevertheless, even when this is restricted to the transport interval, phoresy excluding physiological connections is not guaranteed.

PHORESY REDEFINED

I propose to redefine phoresy as a function of the evolutionary phoretomorph in the sense in which Lesne (1896) first defined it and propose to include a modified version of Athias-Binche's (1994) eco-biological and eco-physiological phoretic categories. This leads to the following:

Phoresy is a dynamic interspecific, *temporary* relationship whereby the phoretic (traveler, phoretomorph, phoriont) attaches to the host (carrier, phoront) for the duration of migration from one habitat to another, with the primary outcome being dispersal.

The migration is facultative or obligatory; the physical associations could represent some stage along the evolutionary axis between *euryxeny* to *steno-* or *oioxeny* (= strict host-specificity), and their physiological relationships at any juncture between an *allotrophic* or *predatory* and *parasitic* or *parasitoid* life style.

Phoresy *includes* at its origin an unspecialized phoretic homeomorph, but *excludes* at the other extreme those relationships in which the transition from phoriont to parasite is completed and its primary outcome, namely dispersal, no longer exists.

TYPES OF PHORESY

Ecological phoretic relationships

Athias-Binche (1994) described ecological types of phoretic associations as a function of the *frequency* with which migration occurs. Related to the increasing frequency of migration, the types are classified from *occasional* or *accidental* to *facultative* and *obligate* (Fig. 1). Phoresy is termed *occasional* or *accidental* when free-living edaphic, not strictly phoretic mites accidentally meet another animal and mount for 'a ride'. *Facultative* phoresy is environmentally induced and encountered mainly in unpredictable, transient habitats, while *obligate* phoresy mostly occurs in predictable, seasonal or cyclical environments.

Athias-Binche's parameter 'frequency of migrations' has become ambiguous with Polak's (1996) *M. subbadius*. As an opportunist, this mite presumably migrates *frequently*, at least more than once in its life. Such a frequent traveller would not fit in Athias-Binche's first category. On the other hand, as a polyphagous predator, 'taking a ride' whenever a suitable host comes its way, it should be classified under this category.

Athias-Binche (1994) reserved the term *obligate* phoresy for groups of mites inhabiting long-term habitats, like decaying logs. Migration in these populations is induced seasonally and determined by cyclical recurrent climatic factors. According to Knülle (1991), phoretomorph formation in these populations was anticipated through season-related cues like photoperiod or seasonal drought.

Athias-Binche (1994) classified *Pediculaster* under the category *facultative* phoresy, since Gurney & Hussey (1967) and Cross & Kaliszewski (1988) were able to delay phoretomorph formation in *Pediculaster* by continuously transplanting the population to fresh fungus cultures. Knülle (1987), experimenting with *Lepidoglyphus destructor* (Schrank) (Glyciphagidae), explained that delaying phoretomorph formation was possible because the development of the phenotypically different heteromorph was genetically programmed, yet environmentally induced.

In general, it may be presumed that in long-term as well as short-term temporary habitats, phoresy induced by fluctuations in the environment is a function of these changes and as such is obligate and not facultative. I therefore suggest that phoretic categories be expressed in terms related to fluctuations or changes in the environment rather than to frequency of migration. The advantage of this would be that, using Athias-Binche's (1994) categories, the term *obligate* would encompass mite migration induced by a deteriorating habitat (Gurney & Hussey, 1967; Karg, 1967; Farish & Axtell, 1971; Cross & Kaliszewski, 1988), migration in response to factors inherent to the host (Krantz & Mellot, 1972; Schwarz & Müller, 1992; Schwarz et al., 1998), and environmental factors affecting host emigration and consequently mite migration (Zhang, 1998). Within this category one could introduce the parameter *frequency of migration*.

In short-term temporary habitats, as in a dung pad or a water-filled tree-hole, each new generation moves on via a phoretomorph within a relatively short 'migration window'. The frequency of migration in such a habitat is high, and directly linked to a deteriorating environment.

In a decaying tree log or a savannah pan, a long-term temporary habitat, the comparatively low frequency of migration also occurs via a phoretomorph. In these habitats though, not every new generation travels. Phoretomorph formation is induced by environmental fluctuations, linked to seasonal factors, such as photoperiod, temperature, humidity. In both short-term and long-term habitats, phoretic migration is a regular and recurrent (cyclical) event. Migrations unrelated to environmental induction would be termed facultative. These would then be characterised by their unpredictability.

In this classification system, host-specificity is excluded, as it is not a function of environmental fluctuations but of the degree of phoretic adaptations that have taken place in the course of the phoretic-phoront's common evolution. The proposed modifications of Athias-Binche's classification system are summarised in Figure 2.

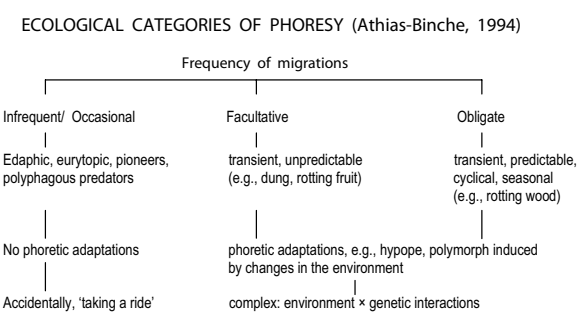

Figure 1 Ecological categories of phoresy of mites (Acari) as a function of migration frequency (Athias-Binche, 1994).

Physiological phoretic relationships

Athias-Binche (1994) applied Euzet & Combes's (1980) terminology for parasitic to phoretic assemblages. The relationships between phoretic partners, termed *phoretic specificity,* could progress from loose, occasional, *euryxenous*, via *eco-ethological,* to *steno-* and *oioxenous.* An assemblage was called 'euryxenous' when the phoriont potentially used a wide range of carriers, and 'eco-ethological' when the phoretic partners shared the same habitat and when the host's activities were linked to the mite population's survival. Athias-Binche (1994) explicitly excluded phylogenetic relationships from the eco-ethological category.

As the host-phoretic relationships became increasingly more stringent and the phoretic exhibited preferences for a host family or genus, the assemblage was called stenoxenous. Oioxenous or monospecific was reserved for associations where the phoriont consistently chose a single host species, or a closely related species.

The term *specificity* in parasitic relationships usually refers to a monospecific liaison between host and parasite. However, phoretic relationships are usually more dynamic and, depending on the availability of their preferred host, the phoretic could be found attached to alternative hosts (own observation). This usually occurred only towards the close of the migration window. I therefore suggest the use of the term *host preference* instead of *host specificity*.

I also suggest the inclusion of phylogenetic relationships in the category 'eco-ethological'. In a dung environment, which necessitates frequent recurrent migrations, the phoretic preference remains within the dung fauna. This preference is usually restricted to one or a few different insect orders. Because of this restriction a common evolutionary history between host and phoretic may be expected. This assumption is supported for heterostigmatic mites by Magowski (1995). He described an 85 million years old fossil assemblage in amber, in which a heterostigmatic mite, possibly a female pygmephoroid (its external morphology suggests an affinity to the taxonomically confused *Pediculaster* complex), was found associated with its caeculid (Diptera) host.

The two evolutionary axes, ecological categories and the physiological phoretic relationships are synthesised in Figure 3.

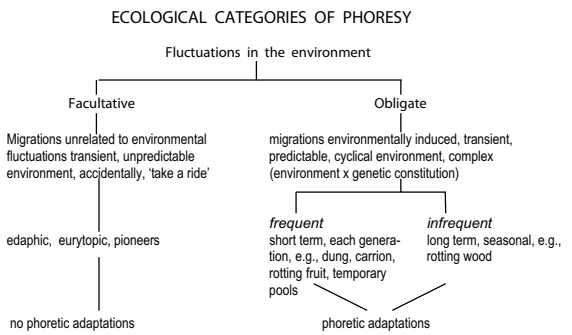

Figure 2 Ecological categories of phoresy of mites (Acari) as a function of environmental fluctuations.

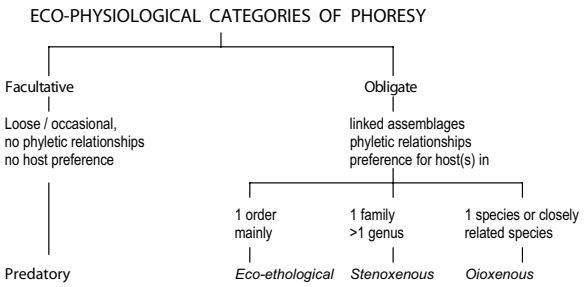

Figure 3 Synthesis of the ecological and physiological categories of phoresy of mites (Acari).

Phoretic feeding relationships

The feeding relationships between mites and their hosts could, according to Athias-Binche (1994), have evolved from a casual (*allotrophic* or *predatory*) via *commensal* and *mutualistic* to either *parasitoid* or *parasitic* type.

According to Cross (1965), Vitzthum stated that mated but non-gravid females of *Pigmephorus* (sic) – now *Pediculaster* – *mesembrinae* (Canestrini) would 'ride upon adult flies and drop off at the oviposition sites where they (would) attack developing larvae' (1931) – however, this observation has never been confirmed since and doubted by several researchers (EE Lindquist and EA Cross, pers. comm.). If Vitzthum's observation was correct, these larvae were developing immatures of other than those of the host species, and thus the host/phoretic relationship did not involve feeding of the host larvae and can be classified as *eco-ethological* and *predatory*. Were the larvae immatures of the host species, the association could be classified as *eco-ethological* and *parasitoid*. However, as Lindquist noted (pers. comm.), one has to question whether Vitzthum (1931) presumed or actually observed such a feeding relationship as, according to present day authors, *P. mesembrinae* is fungi- and not larvivorous (Gurney & Hussey, 1967; Kosir, 1975; Clift & Toffolon, 1981; own observations).

That the feeding relationships are not always clear-cut and do not have to progress in parallel along various axes is illustrated by the previously discussed phoretic feeding relationships of *H. cooremani* and the beetle *C. cacti* presented by Houck & Cohen (1995) and of *M. subbadius* and its various hosts, described by Polak (1996). However, the proposed modifications and extensions in Athias Binche's system may prove to be a useful tool to classify phoretic relationships.

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Pediculaster–host relationships (Acari: Siteroptidae)

A.M. Camerik
School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Wits, 2050, Johannesburg, South Africa. E-mail: Anne.Camerik@wits.ac.za

Pediculaster species are mites that are associated mainly with Diptera. They occupy very specific attachment sites on their host and have a specialised morph for dispersal. This suggests a long-standing relationship with their hosts. Based on a modified version of Athias-Binche's (1994) eco-physiological phoretic categories, the *Pediculaster* species of this study are obligate phoretics in their natural habitat. Using a statistically determined host preference index, I propose to put *P. morelliae, P. gautengensis*, and *P. gracilis* into the eco-ethological category, and *P. norrbomialis* into the stenoxenous category.

Key words: *Pediculaster*, phoresy, host-preference index, quantifying eco-physiological categories

Fifty-eight of the 95 hitherto described *Pediculaster* species are associated with animals (61%). Of these, 41 species (43%) are phoretic on various Diptera. No hosts have been recorded for the remaining 37 species. *Pediculaster* preferring Diptera have specific attachment sites on their hosts, which suggests a long-standing co-evolution with their hosts and therefore also a certain degree of host-specificity. According to Athias Binche (1994) less host-specific mites tend to be less selective with respect to the attachment site on their carrier.

Besides attachment site specificity, phoretics have developed a range of adaptations in order to find their hosts, to attach to them, and to make dispersal successful. A phoretic life style involves not only morphological, but also behavioural, physiological, chemical, and thermal adaptations. Some of these adaptations in *Pediculaster* are discussed below.

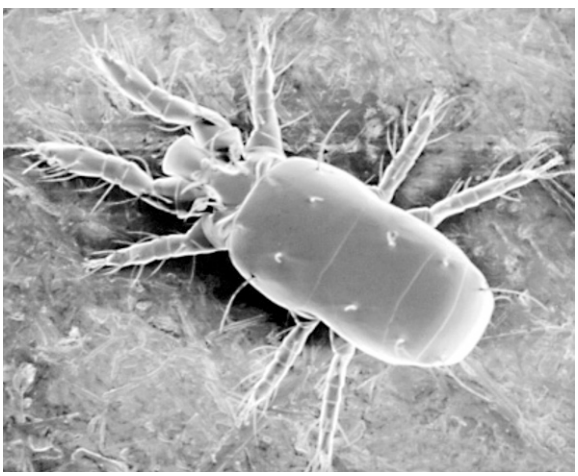

Figure 1 *Pediculaster* sp., phoretomorph. Photo: E. de Lillo.

Morphological adaptations – polymorphy and physical adaptations

Athias-Binche (1994) distinguished two forms of polymorphy: heteromorphy and demographic polymorphy. Heteromorphic mites have an instar morphologically different from the general body plan of the other instars, as in astigmatic hypopes. In demographically polymorphic mites the same instar has two different morphs (stases), one sedentary, and the other phoretic (the phoriont). The polymorphic phoretic instar, whether as a nymph or an adult female, is thought to be an adaptation to its host and has evolved over a very long time in which the association has become increasingly more specific (Athias-Binche, 1994). *Pediculaster* phorionts are in several ways morphologically adapted to their mode of transport. In contrast to the sedentary females, the phoretics (Fig. 1) are fitted with a tough, sclerotized exoskeleton, with a powerful single claw that fits in a V-shaped counter-piece on the robust fused tibiotarsus of leg I (Fig. 2), and with gripping pads against the claws of the second and third limb (Figs. 3 and 4).

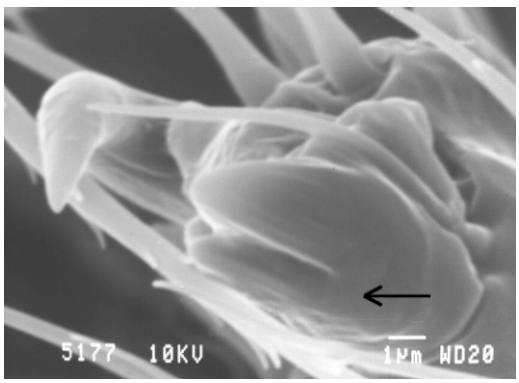

Figure 2 V-shaped counter piece of claw of leg I.

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Behavioural adaptations

In contrast to the sedentary stase, the phoretics frequently halt and make questing movements with their first pair of legs (Fig. 5), probing the environment for tactile, sensory, thermal, or chemical clues. *Pediculaster* phoretics are equipped with a variety of sensory receptors on leg I (Fig. 6) like tactile setae, sensory eupathidia, and solenidia, specialised for chemo-reception (Evans, 1992).

Life-cycle synchronization

Since the dipteran hosts live in dung, mites have to emigrate when the dung quality declines. Life-cycle synchronization of mite and their carrier is therefore crucial for *Pediculaster* to survive. *Pediculaster* phoretics are attracted to maturing fly pupae and are then ready to mount the adult fly on eclosion (AM Camerik, pers. obs.; Greenberg, 1960).

Dispersal strategy

Pediculaster phorionts exhibit an efficient dispersal strategy. Dispersal in mites, according to Mitchell (1970), is progressively successful with an increasing number of founders and a higher probability that these founders reach a new resource. Mated *Pediculaster* phoretic females, each capable of founding a new population, are dispersed. No males or larvae are carried. A host-specific association with their carriers greatly increases the probability that the mites will reach a new habitat.

The present study aims to describe *Pediculaster*-host relationships and to quantitatively classify these according to a modified version of Athias-Binche's (1994) eco- physiological categories of phoresy (Camerik, 2009). I hypothesise that the higher the host-preference indexes for a *Pediculaster* phoriont, the better the chance of success for the next generation to migrate to a fresh habitat and found new populations.

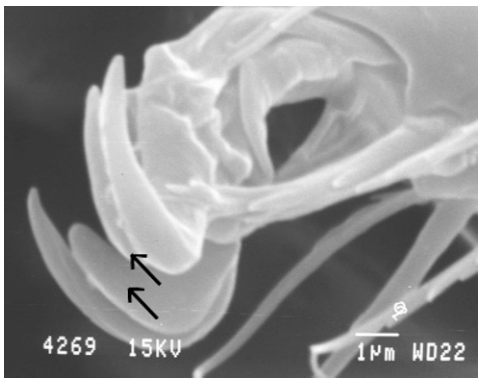

Figure 3 Gripping pads (arrows) against claw of leg II.

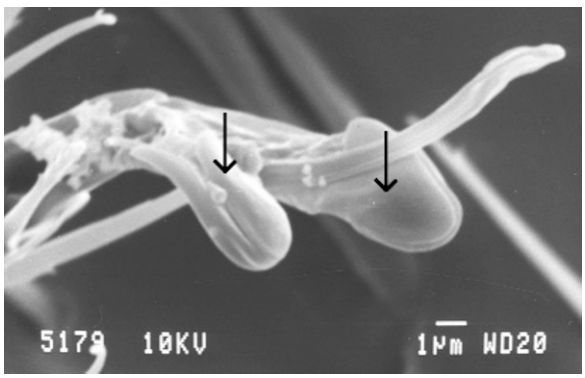

Figure 4 Gripping pads (arrows) against claws of leg III.

MATERIAL AND METHODS

Freshly defecated dung for the two experiments referred to in this paper was collected over 3 days at Innesfree Farm close to Johannesburg, South Africa, as follows. Cow and horse dung, collected separately in a meadow where cows and horses grazed, were taken to the laboratory. The cow dung was homogenized and the horse dung separately mixed on a platform, taking care that the boluses were kept intact. Subsequently 32 units of 1 kg (about the size of a normal dung pad in the field) of each dung type were placed in plastic bags and deep-frozen in order to kill any arthropods that might already have invaded the collected dung.

After 72 h at -19 °C, the bags were removed from the deep-freeze and the dung allowed to thaw for a day at about 23 °C ambient temperature. Then they were taken to the same farm meadow, where the bags were removed and the dung dropped onto a wire mat and taken to a grid with numbered sections, surrounded by an electrical fence to keep the cattle and horses out of the experimental area (Fig. 7). Within the grid the experimental pads were deposited, alter-

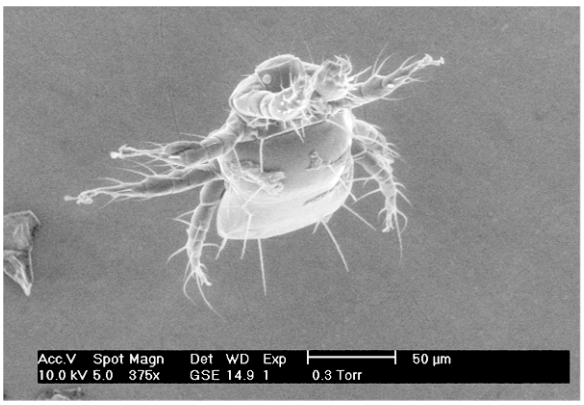

Figure 5 *Pediculaster* sp. questing behaviour.

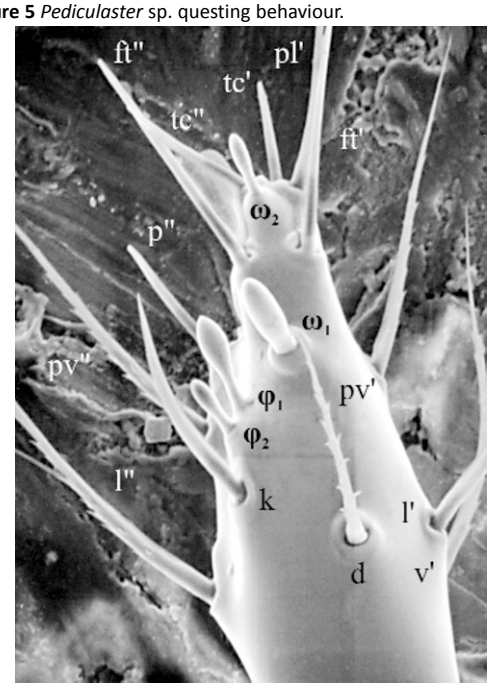

Figure 6 Tibiotarsus of leg I (photo E. de Lillo). $\omega_1\omega_2$, solenidia (chemo/olfactory receptors); $\varphi_1\varphi_2$, solenidia (chemoreceptors); l", pv", pl', pv', l',v',d, tectiles (mechanosensitive receptors); k, famulus (function still unknown); ft',ft", tc', tc", p", eupathidia (gustatory/mechanosensory receptors).

nately cow and horse dung, and left there exposed for eight consecutive days to allow the immigration of Diptera and Coleoptera to be completed. This time span was considered to be the period when the moisture content was sufficiently high to allow arthropods to exploit their dung habitat under normal weather conditions. Note that in the cow dung, arthropod activity resulted in breaking down part of the pad, while birds often scattered the horse dung boluses (arrows in Figs. 8 and 9, respectively). Two pads of each dung type were kept in emergence boxes in the laboratory as a control.

Each day during the 8 days, two dung pads of each kind were randomly removed from the grid, each one separately placed into a numbered emergence box (Fig. 7) and transported to the laboratory. On the shelves in the laboratory the collecting bottles, mounted on the boxes (Fig. 10), faced fluorescent light mounted on the opposite wall. Emerging arthropods were attracted to the light and immediately preserved in 70% ethanol at the bottom of the collecting bottle. Temperature and relative humidity in the laboratory were kept constant at 31.5±3 °C and 50-55 % r.h.

The collecting bottles were emptied each day and the contents kept in numbered and dated vials. All *Pediculaster* specimens in the vials were separately mounted in Hoyer's and their pad number, host's name, date of pad collection, and host emergence was noted. Several new species had to be described first and subsequently the collected data were analyzed.

Pediculaster specimens, their carriers, and the attachment sites were observed under a dissecting microscope. *Pediculaster* specimens separated from their carriers were counted and mite attachment sites noted. The lactophenol cleared specimens were mounted in Hoyer's and identified. Hosts were sent to relevant specialists for identification. In the analyses, the term 'attached' (or its synonym) refers to mite specimens attached to their hosts, and includes detached specimens from vials in which only one possible host category was present.

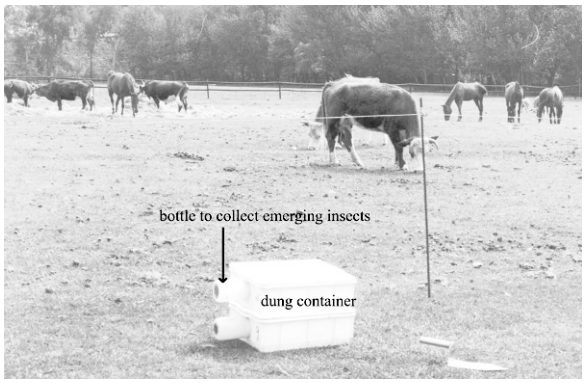

Figure 7 Emergence boxes containing dung, with attached collecting bottles ready for transport to the laboratory. Notice the electrical fence just behind the boxes to keep cattle and horses out of the experiment area.

Figure 8 Cow dung pad affected by arthropod activity (arrow).

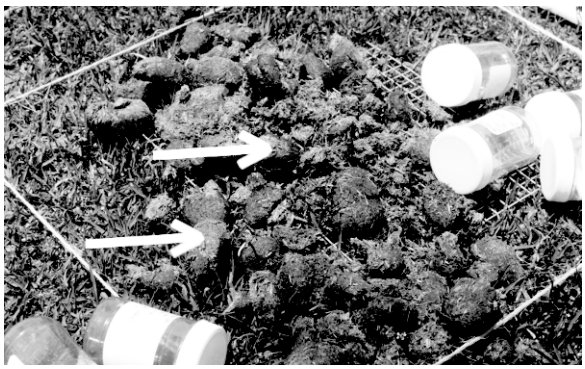

Figure 9 Horse dung boluses scattered by birds.

Statistical analysis

The data set for *Pediculaster* species was presumed to be a random sample of the total population and was held to be appropriate in calculating the distribution of the species across different hosts according to the Shannon-Weaver Index (SWI) (Zar, 1984):

$$H' = \frac{n \log n - \sum_{i=0}^{k} f_i \log f_i}{n},$$

where k represents the number of host categories, n the mite sample size, and f_i the number of attached mites, observed on host category i. The diversity index, H', disregards the number of host specimens. The SWI therefore allows one to include detached mite specimens from vials where only one possible host category was found. This increased the sample size of some migratory mite populations considerably, which in turn decreased the bias of H'. Bowman et al. (1971) showed that H' underestimates the diversity of a sampled population, and that the bias decreases with increased sample size. The minimum sample size for a species to be considered for classification in the present study was arbitrarily set at n = 15.

The maximum diversity possible for any set of data consists of k categories: $H'_{max} = \log k$. The observed diversity J', also called evenness or homogeneity, can be expressed as a fraction of H_{max}: $J' = H' / H'_{max}$. Its complement, 1-J', is a measure for heterogeneity or dominance (Zar, 1984). In order to express host preference in a proportional fashion, I propose the 'Host preference index', S': S' = 1-J'.

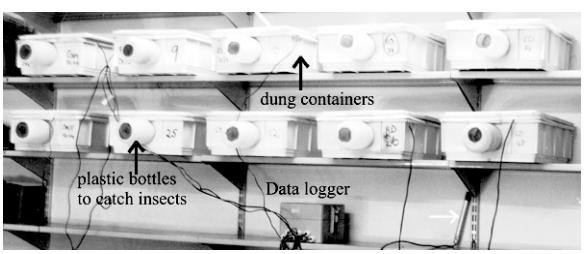

Figure 10 Emergence boxes containing dung, with attached collecting bottles on shelves in the laboratory. Temperature and humidity in laboratory are controlled.

Table 1 Total number of *Pediculaster* specimens collected over 30 days from their different hosts emerging from cow dung, September (spring) 1991. k, category (1-10); sp. n., new (undescribed) species; n, sample size; H', Shannon-Weaver Index; H'_{max}, log k; J', homogeneity; S' (= 1-J'), host preference.

Host species					Total number of *Pediculaster* specimens per species					
Order	Family	Genus	Species	k	*copridis*	*norrbomialis*	*australis*	*gracilis*	*gautengensis*	*morelliae*
Diptera	Muscidae	indet		1	1	10	0	0	89	14
	Sphaeroceridae	*Coproica*	sp. n.	2	0	0	0	0	29	7
		Elachisoma	near *braacki*	3	0	0	0	0	0	2
			gravis	4	0	0	0	0	1	13
		Norrbomia	*marginatis*	5	0	225	8	0	183	1
		indet		6	0	992	1	57	13	21
	Tachinidae	*Imitomyia*	sp.	7	0	0	0	0	23	8
Hymenoptera	Cynipidae	indet		8	0	0	0	63	0	0
Coleoptera	Scarabaeidae	*Aphodius*	sp.	9	0	1	1	2	45	4
	Staphylinidae	indet		10	0	0	0	0	1	0
				n	1	1,228	10	122	384	70
				H'	0.00	0.23	0.28	0.33	0.63	0.78
				H'_{max}	1.00	1.00	1.00	1.00	1.00	1.00
				J'	0.00	0.23	0.28	0.33	0.63	0.78
				S'	1.00	0.77	0.72	0.67	0.37	0.22

Zar (1984) remarked that k underestimates the number of categories in a population, then J' is biased and overestimates evenness, and S' in turn underestimates host preference in phoretic relationships. When H' = H'_{max} and J' = 1 (1-J' = 0), *Pediculaster* species are evenly dispersed amongst their hosts, and the phoretic relationship is called *eco-ethological.* If, on the other hand, H' and subsequently J' are near zero (S' approaches 1), the phoretic will be maximally host specific and classified as *oioxenous*.

RESULTS

Attachment sites

Typical *Pediculaster* attachment sites on dipteran hosts were the conjunctival membranes in the coxal region around leg insertions and sternites (Fig. 11), in the gular region, between tergites (Fig. 12), and on the probosces of sphaerocerid flies. The phorionts attached themselves to host setae mostly by clasping the setae between the pads and claws of legs II and III (Camerik, 1996; Figs. 3 and 4). In the present study mites were never seen attached to their hosts using the large tibiotarsal claw of their first legs, a mode of attachment reported, amongst others, for pyemotid adults (Kinn, 1971), the heterostigmatic mite *Arthyreacarus pleiotretus* (Lindquist et al., 1990), and *Scutacarus deserticolus* (Ebermann, 1991).

Mites were distributed symmetrically on their hosts. When the mite load was low, the first locations on the host to be occupied were the setae between the legs on the conjunctival membrane, below the protrusion of the exoskeleton, posterior to the third pair of legs. As the load increased, the other sites were occupied in the order given above. The heaviest infestation was observed on an individual of *Norrbomia marginatis* (Diptera: Sphaeroceridae) that was carrying 111 *Pediculaster* specimens (AM Camerik, unpubl.).

Pediculaster was found in low numbers on various parts of the exoskeleton of Coleoptera, e.g., attached to setae between coxae, mouthparts, elytrae, antennae, legs, and wings. In general, these attachment sites seemed very atypical. The highest infestation observed was four *P.* cf. *morelliae* on one *Aphodius* specimen.

Pediculaster - host relationships

An overview of all *Pediculaster* species that were in the process of emigration from the dung of horses and cows, their distribution over different host species, and the statistical analysis of these data is presented in Tables 1-3. For statistical analysis only collections of 50 or more *Pediculaster* specimens per species were considered viable.

Pediculaster norrbomialis was mainly attached to the fly *N. marginatis*. Sixty-nine % of the migratory spring population and 47% of the summer population were collected attached to their hosts. This species displayed a consistent distribution pattern across their hosts in both seasons (S' = 0.77 and 0.79, respectively; Tables 1 and 2).

About 50% of the migratory spring population and 72.5% of the summer population of *Pediculaster gautengensis* were found affixed to their hosts. This species appeared to be opportunistic with regard to its choice of host (S' = 0.37 in

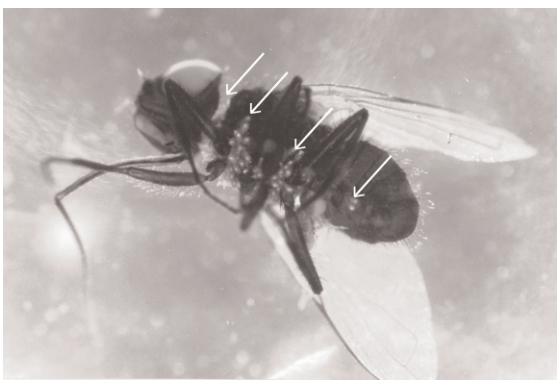

Figure 11 Typical attachment sites on ventral side of host fly.

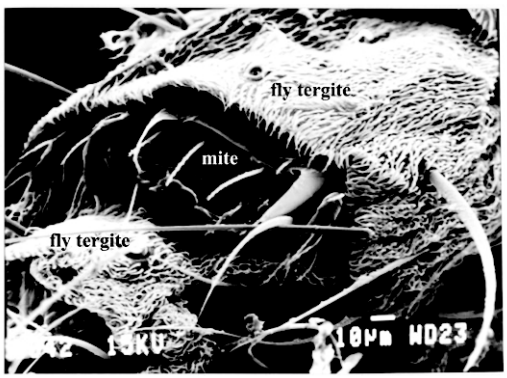

Figure 12 Fly with *Pediculaster* mites under the tergites.

Table 2 The total number of *Pediculaster* specimens collected over 30 days from their different hosts emerging from cow dung, January (summer) 1992. k, category (1-18); n, sample size; H', Shannon-Weaver Index; H'_{max}, log k; J', homogeneity; S' (= 1-J'), host preference.

Host species						Total number of *Pediculaster* specimens per species		
Order	Family		Genus	Species	k	*luriei*	*norrbomialis*	*gautengensis*
Diptera			indet		1	3	30	17
	Muscidae	Muscinae	*Musca*	*confiscata*	2	9	12	60
				lasiophthalma	3	0	4	536
		indet			4	0	0	63
		non-Muscinae		sp. 1	5	0	0	11
				sp. 2	6	0	0	27
	Phoridae	indet			7	0	0	2
	Sphaeroceridae		indet		8	0	2	3
			Coproica	sp. n.	9	0	0	1
			Elachisoma	*afrotropicum*	10	0	0	22
			Norrbomia	*marginatis*	11	0	270	27
	Sepsidae		Sepsis	sp.	12	0	0	9
	Tachinidae		*Imitomyia*	sp.	13	0	0	9
		indet			14	0	0	34
Coleoptera	Scarabaeidae		*Aphodius*	sp.	15	0	1	12
	Staphylinidae		indet		16	0	0	10
	indet				17	0	1	4
Heteroptera	indet				18	0	0	1
					n	12	320	848
					H'	0.24	0.27	0.76
					H'_{max}	1.26	1.26	1.26
					J'	0.19	0.21	0.54
					S'	0.81	0.79	0.46

Table 3 Total number of *Pediculaster morelliae* specimens per host species collected over 30 days from their different hosts emerging from horse dung in spring (September) 1991 and January (summer) 1992. k, category (1-2); n, sample size; H', Shannon-Weaver Index; H'_{max}, log k; J', homogeneity; S' (= 1-J'), host preference.

Hosts				Spring 1991		Summer 1992	
Order	Family	Genus	Species	k		k	
Diptera	Sphaeroceridae	*Norrbomialis*	*marginatis*	1	5		
		Elachisoma	*afrotropicum*			1	59
	Tachinidae	*Imitomyia*	sp.	2	4		
Coleoptera	Scarabaeidae	*Aphodius*	*pseudolividus*			2	4
				n	9		63
				H'	0.30		0.1
				H'_{max}	0.30		0.30
				J'	0.99		0.34
				S'	0.01		0.66

spring, 0.40 in summer; Tables 1 and 2). Its preference for certain Diptera appeared to be related to the chronological order of host emergence (Camerik, 1996). Any fly host that was first available was mounted by the mites and used to move to a fresh dung pad. Both *P. norrbomialis* and *P. gautengensis* were the most abundant species in spring as well as in summer.

Pediculaster morelliae changed its host and dung preference according to season (Tables 1 and 3). The mites emigrated in spring from cow and horse dung and in summer from horse dung only. In cow dung (spring), the mites were distributed fairly evenly (S' = 0.22) across *Norrbomia* spp., *Elachisoma gravis, Coproica* spp. (Sphaeroceridae), and *Imitomyia* sp. (Tachinidae). In horse dung in spring, the mites were equally distributed between Sphaeroceridae and Tachinidae (S' = 0.01), but in summer they occurred more frequently on the sphaerocerid fly, *Elachisoma afrotropicum* (S' = 0.66; Table 3).

DISCUSSION

It has to be borne in mind that the Shannon-Weaver Index (SWI) represents a descriptive and relative statistic and that the outcome has to be interpreted within the phoretic context. The cyclic recurrence of the phoretomorphic phenotype, epigenetically induced by the habitat (Camerik et al., 2006), is a feature of all *Pediculaster* species, which makes them obligate phoretic. Within this category *P. norrbomialis*, with a host preference index (S') between 0.77 and 0.79, consistently preferred Sphaeroceridae, and, amongst these, mainly the fly *N. marginatis*. This phoretic relationship tends towards host-specific preference. But since the mites' choice of alternative hosts (e.g., Muscidae and *Aphodius*) changed from less than 1% of the total number collected in spring to 15% in summer, when fewer Sphaeroceridae and more Muscidae were available, *P. norrbomialis* may not yet have developed a strict host-specific preference but a flexible relationship with its preferred hosts.

In contrast to *P. norrbomialis*, *P. gautengensis* (S' = 0.37 and 0.46, in spring and summer, respectively) attached consistently to a variety of hosts: Diptera, Coleoptera, as well as Heteroptera. However, flies were the most preferred host. The mites chose mainly Sphaeroceridae in spring and Muscidae in summer, as these increased in number.

Pediculaster morelliae had a multiplicity of hosts and its phoretic behaviour seemed to vary with the season and the dung type. This species migrated in spring from cow dung on flies of three families (Muscidae, Sphaeroceridae, and

Tachinidae) (S' = 0.22), and on Sphaeroceridae and Tachinidae from horse dung (S' = 0.01). In horse dung in summer, however, the mites were mainly attached to the fly *E. afrotropicum*. On one occasion, a few were also found attached to a beetle. This phoretic host choice behaviour, restricted mainly to Diptera, is similar to that of *P. gautengensis*. *Pediculaster gracilis* was quite evenly spread over two different orders, Diptera and Hymenoptera. A few specimens were also attached to *Aphodius* sp. (Coleoptera).

The fact that *Pediculaster* has a specialised morph for phoretic dispersal indicates a long-standing relationship with its hosts. The mites are associated mainly with Diptera on which they occupy very specific sites of attachment. This suggests a high degree of host specificity. Therefore, all *Pediculaster* species of this study are, according to their eco-physiological phoretic categories, expected to be obligate phoretics in their natural habitat.

Using the revised eco-physiological classification (Camerik, 2009) and a statistically determined host preference index, I propose to put *P. morelliae, P. gautengensis* and *P. gracilis* into the eco-ethological, and *P. norrbomialis* into the stenoxenous category.

The proposed hypothesis, that *P. norrbomialis* with the highest host-preference index was the most successful for the next generation to migrate to a fresh habitat and to found a new population, is applicable to the spring and summer experiments of *Pediculaster* in cow dung, followed by *P. gracilis, P. gautengensis,* and *P. morelliae*, in order of decreasing success.

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Generalist and specialist strategies in macrochelid mites (Acari: Mesostigmata) phoretically associated with dung beetles (Coleoptera: Scarabaeidae)

Jérôme Niogret[1,2], Jean-Pierre Lumaret[1] & Michel Bertrand[1]

[1]UMR 5175 CEFE, Ecologie des Arthropodes, Laboratoire de Zoogéographie, Université Montpellier 3, route de Mende, F-34199 Montpellier Cedex 5, France. [2]Current address: Subtropical Horticulture Research Station, USDA-ARS, 13601 Old Cutler Road, Miami, FL 33158, USA. E-mail: jeromeniogret@yahoo.fr, jerome.niogret@ars.usda.gov

Macrochelid mites have phoretic associations with coprophilous arthropods, thereby promoting dispersal and colonization of new substrates. Contrasting strategies were observed. Opportunistic mites like *Macrocheles perglaber* exploit a large range of carriers, whereas specialists like *M. saceri* occur only on roller dung beetles (genus *Scarabaeus*). The opportunistic species live inside dung pats, whereas specialists live in the pedotrophic nests of *Scarabaeus* beetles. These modes of life are expected to have consequences for host choice. We performed olfactometer tests and morphology comparisons to assess the adaptations of opportunists and specialists to their carrier hosts. The opportunist *M. perglaber* was shown to discriminate between various qualities of dung. Opportunists are thought to use this ability when their carrier buries a new dung pat and they have to decide whether to leave their carrier to live in a good-quality dung pat or to stay until the carrier finds another dung pat of good quality. The specialist *M. saceri* did not discriminate between qualities of dung; specialists always stay on the body of their host, whatever the dung quality. These life-style features are thought to describe essential differences between generalist and specialist macrochelids. Also morphometric parameters differed among three specialist and three generalist species. Relative to the generalists, specialists had a larger body and longer PI legs (= first leg pair); both characters are hypothesized to be an advantage in active searching for carrier hosts. Availability of carrier hosts is more likely limiting to specialists than to generalists. Small size has the advantage of reaching more prey living inside the dense dung material.

Key words: *Macrocheles perglaber*, *Macrocheles saceri*, olfactometer, morphometry, host selection, phoresy, coprophily

Association with flying insects is the key feature that enables phoretic macrochelid mites to survive in transient microhabitats, such as dung pats. They are adapted to these ephemeral conditions in that they have a short generation time and reproduce by arrhenotokous parthenogenesis, which enables a single female to found a new colony on a virgin substrate (Costa, 1969). Phoresy of fertilized macrochelid females by associating with coprophilous insects is an adaptive response to rapidly find dung pats that are patchily distributed in pastures. Phoresy can be defined as the commensal association between poorly mobile organisms and large and mobile carriers, promoting dispersion of the less mobile organisms (Farish & Axtell, 1971).

Macrocheles mites live in dung and carrion. They occupy a key position in the dung community as they control some populations of cattle-associated pests. They feed on fly eggs and newly emerged larvae and they contribute to reducing both free and parasitic nematode populations (Tyndale-Biscoe & Wallace, 1981; Krantz, 1983). They predominantly reproduce with haplodiploid sex determinism (Filipponi, 1964; Cicolani, 1992). Only fertilized females are phoretic (Costa, 1969). *Macrocheles perglaber* Filipponi & Pegazzano can be considered as the most representative model of an opportunistic species: it is abundant, widely distributed (cosmopolitan) and found to be carried both by many dung beetles species (small and large beetles belonging to various guilds: dwellers, tunnelers, and rollers) and by many coprophilous flies (Walter & Krantz, 1986). On the contrary, *Macrocheles saceri* Costa is a specialist mite exclusively found on a few *Scarabaeus* species in the western part of the Mediterranean Basin, roller beetles that rapidly make a ball from a dung pat, which is rolled away inside pedotrophic nests (Cambefort & Hanski, 1991). These beetles are available to mites staying in the dung pat for a very short time.

Coprophilous macrochelid mites differ in the preferences for their carriers. Generalist or opportunistic mites (e.g., *M. perglaber*) are carried by large to minute host flies or scarab beetles. The choice of hosts can vary with the annual cycle. Often they choose carriers that are the most abundant in any particular season, and also the larger species as these can carry more mites on their body (Glida & Bertrand, 2002). Carriers of opportunists are therefore often tunneler and dweller beetles, remaining in dung pats for several days and abundant all along the year. Specialist mites (e.g., *M. saceri*) are strongly linked to one preferential host species or few related hosts, often belonging to a single genus. They are never collected from 'accidental' hosts.

We hypothesize that opportunists and specialists differ in their searching behaviour for carrier hosts. Some parameters seem essential, such as efficient host recognition, to find a carrier host in the shortest time and to select the right carrier host, because dung beetle species differ in behaviour (rollers, dwellers, and tunnelers) and these differences bring (dis)advantages depending on the life style of the mite. The aim of this paper is to investigate the factors implicated in host-finding behaviour and to examine them in the light of opportunistic and specialist dispersal strategies.

MATERIALS AND METHODS

Sampling method and laboratory breeding

Dung beetles were collected in Southern France and Northern Morocco using pitfall traps of standard design (Lobo et al., 1988; Veiga et al., 1989) baited with fresh cattle dung. Only four dung beetle species were considered: three rollers (*Scarabaeus sacer*, *S. cicatricosus*, and *S. laticollis*) and one tunneler (*Bubas bubalus*). Phoretic mites were collected from the ventral body of the beetles. They were

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Table 1 Two-choice tests with *Macrocheles* spp. mites between two beetle species, i.e., *Bubas* sp. and *Scarabaeus* spp. (n = 35 mites per test).

Mites	Beetle species		Choice		χ^2	P
	1	2	1	2		
M. perglaber	*B. bubalus*	*S. laticollis*	27	8	10.31	<0.01
M. saceri	*S. sacer*	*S. cicatricosus*	24	11	4.82	<0.05
	S. sacer	*S. laticollis*	24	11	4.82	<0.05
	S. cicatricosus	*S. laticollis*	26	9	8.25	<0.01
	S. sacer	*B. bubalus*	28	7	12.60	<0.001
	S. laticollis	*B. bubalus*	25	10	0.43	<0.05

reared in small cylindrical plastic boxes (84 mm high, 54 mm diameter) which received a small piece of cattle manure weekly. Live nematodes and a mixture of re-hydrated dry mosquitoes and fly larvae were provided as food. Cultures were kept at 20±2 °C and 75% r.h.

Two-choice tests

The phoretic behaviour of fertilized *M. perglaber* and *M. saceri* females was tested to verify whether semiochemicals are used as kairomones in the phoretic association between dung beetles and mites, and whether these may explain the degree of attractiveness observed towards various carrier species. Experiments began with 2-month-old laboratory colonies (>6 generations). Before each test mites were kept individually in a small clean plastic container (10 × 10 × 5 mm) for 1 h.

Two live and non-conspecific beetles were offered as alternative carriers. These were prevented to move by transverse bars at the opposite sides of a rectangular plastic box (85 × 55 × 45 mm) at 20±2 °C. One mite was placed in the central compartment by the aid of a fine brush. When the mite oriented, climbed up onto the beetle body, and stayed put on this host, its choice was registered. Every five consecutive tests, the boxes were washed with ethanol 90% to eliminate eventual traces of any semiochemicals left by mites.

Olfactometer studies

The olfactory attractiveness of dung and dung beetles was studied in a Y-tube olfactometer (arm length: 90 mm, trunk: 35 mm, tube diameter: 6 mm). Tests were conducted in a climatized room at constant temperature (20±2 °C). A neon tube provided steady illumination (1,280 lux) (lightmeter CA 811, Chauvin Arnoux®, Physics line). Beetles or dung of various rates of moisture were placed in a polyurethane chamber (40 mm high, 45 mm diameter), with an airtight lid connected to an olfactometer arm with a Teflon tube (9 mm diameter). Each arm was provided with a small interception chamber, 50 mm from the point of introduction of the mite, allowing to stop the mite before it reached the odour source. Air from an inlet pump (air vacuum pressure station; Labover®, France) was passed through a flow-meter into two separate odour source tubes. Airflow into the olfactometer was set at 660 ml min^{-1}. Mites that reached the interception chamber were recorded as having made a choice. Females that did not reach the chamber within 15 min were recorded as 'no response'. Only naïve females were tested. To avoid bias, the odour sources were interchanged after five consecutive tests.

Response time was measured as the time spent by the mite in the olfactometer to reach the interception chamber, in comparison with a blank used as control. Three sets of dung were tested: 1) fresh (>83% r.h.), 2) medium fresh (70-80%), or 3) semi-dry (40-50%). Each set corresponded to dung of <30 h, 2-3 days, and >6 days old, respectively.

Body mite morphometry

A 480 Motic digital Camera driven with Motic Image Plus® 2.0 software was used to measure the dorsal shield dimensions of mites, and the length of the first pair of legs (legs I) and the pedipalps. Three generalist species – *M. perglaber*, *M. glaber*, and *M. vernalis* – and one specialist species – *M. saceri* – were measured. We also included the measurements of the specialists *M. parapisentii* and *M. cristati* published by Costa (1967) to balance the comparison between specialists and generalists.

Statistical analysis

The results of two-choice experiments were analyzed with χ^2 tests using Yates correction (Statistica® 6.0 package). Mites that made no choice were excluded from the analysis. The response time and the morphometric comparisons were analyzed using a Mann-Whitney U-test (Statview® 5.0 package).

RESULTS

Experiments with the opportunist *Macrocheles perglaber*

Two-choice tests

In two-choice tests with *S. sacer*, *S. laticollis*, *S. cicatricosus*, and *B. bubalus*, *M. perglaber* significantly preferred *B. bubalus* (Table 1), its dominant carrier under field conditions (Glida & Bertrand, 2002). All beetle species were attractive to this mite (data not shown).

Olfactometer tests

In two-choice tests, *M. perglaber* was always significantly attracted, by odour from cattle dung and from beetles, when clean air was offered as an alternative (data not shown). When beetles and dung were offered simultaneously, no significant preference was observed between *B. bubalus* vs. fresh or semi-dry dung, but medium fresh dung was more attractive than *B. bubalus* (Table 2). The roller beetle *S. laticollis* (a less efficient carrier under field conditions) was always non-attractive for *M. perglaber*: mites were either not more attracted by *S. laticollis* than by semi-dry dung, or they were more attracted by fresh and medium fresh dung (Table 2).

Response time

The time spent by *M. perglaber* in the olfactometer varied according to dung beetles used as odour sources. The mite was more reactive to *B. bubalus* than to *S. laticollis* (158±28 vs. 314±62 s, respectively), but the difference was not significant (U-test: z = -1.56, P>0.05; n = 22).

Experiments with the specialist *Macrocheles saceri*

Two-choice tests

Macrocheles saceri was significantly more attracted by *S. sacer* than by *S. cicatricosus*, *S. laticollis*, or *B. bubalus* (Table 1). When offered simultaneously, *S. cicatricosus* was signifi-

Table 2 Two-choice olfactometer tests with *Macrocheles* spp. mites between semiochemicals from a dung beetle (*Bubas* sp. or *Scarabaeus* sp.) vs. dung with varying moisture content: fresh (FD), medium fresh (MD), or semi-dry dung (SDD) (n = 30-48 mites per test).

Mites	Odour source		Choice		χ^2	P
	1 (beetle)	2 (dung)	1	2		
M. perglaber	*B. bubalus*	FD	17	20	0.24	ns
	B. bubalus	MD	6	24	10.80	<0.001
	B. bubalus	SDD	22	26	0.33	ns
	S. laticollis	FD	9	21	4.80	<0.05
	S. laticollis	MD	11	29	8.10	<0.01
	S. laticollis	SDD	22	26	0.33	ns
M. saceri	*S. sacer*	FD	19	11	2.13	ns
	S. sacer	MD	20	10	3.33	ns
	S. sacer	SDD	18	12	1.20	ns

ns, not significant (P>0.05).

Table 3 Comparative sizes (in µm) of *Macrocheles* mites according to species and strategy.

Strategy	Species	Body length (BL)	Body width	Length leg I (PI)	Length pedipalp (Pp)	PI/BL	Pp/BL
Specialist	*M. saceri*	908 ± 12	513 ± 8	676 ± 15	393 ± 8	0.75 ± 0.02	0.44 ± 0.01
	*M. cristati**	978 ± 31.3	545 ± 16.5	/	/	/	/
	*M. parapisentii**	965 ± 30.2	551 ± 11.4	/	/	/	/
	mean	917 ± 11	518 ± 8	676 ± 54	393 ± 68	0.75 ± 0.02	0.44 ± 0.01
Generalist	*M. vernalis*	726 ± 21	400 ± 8	490 ± 8	312 ± 6	0.68 ± 0.02	0.43 ± 0.05
	M. glaber	756 ± 11	433 ± 14	558 ± 6	323 ± 8	0.74 ± 0.01	0.43 ± 0.03
	M. perglaber	705 ± 22	419 ± 3	459 ± 19	312 ± 21	0.70 ± 0.02	0.40 ± 0.06
	mean	732 ± 11	416 ± 7	507 ± 11	316 ± 6	0.69 ± 0.06	0.44 ± 0.01

*, data from Costa (1967).

cantly more attractive than *S. laticollis*. *Scarabaeus laticollis* was more attractive than *B. bubalus*, which was never found carrying this mite species under field conditions.

Olfactometer tests

Although more mites were attracted towards *S. sacer* than towards dung, regardless its moisture content, the differences were not significant (Table 2).

Response time

The time spent by *M. saceri* to find a carrier from a distance depended on the dung beetle species used for the test. The rollers *S. sacer* and *S. cicatricosus* were found significantly faster (149±20 and 161±31 s, respectively) than *S. laticollis* (309±58 s) (U-test: z = -2.74 and -2.67, respectively; P<0.01).

Morphometric analysis

Opportunistic mites (*M. glaber*, *M. perglaber*, *M. vernalis*) and specialized mites (*M. saceri*, *M. parapisentii*, *M. cristati*) differed in body size and also in length of their appendages (first leg pair [PI] and palps), even when taken relative to body length (Table 3, Fig. 1). Specialists had a broader and longer body than opportunists (U-test: z = -4.72 and -4.61, respectively; P<0.0001). Legs PI and palps was longer in specialist mites (z = -4.47, P<0.0001, and z = -3.77 and P<0.0002, respectively), and the ratio PI: body length was higher in specialists than in opportunists (z = -2.35, P<0.05). However, the ratio of palp length to total length was not significantly different (z = -1.018, P>0.05). Thus, specialist mites had longer PI legs relative to their body size than opportunists.

DISCUSSION

Olfactometer tests showed that phoretic macrochelid mites can recognize the right carrier via chemical compounds. This recognition ability has been investigated by Niogret et al. (2006) for the case of the specialist *M. saceri* in relation to several roller beetles as carrier hosts. Here, we did the same for the opportunist *M. perglaber*. Our results showed that

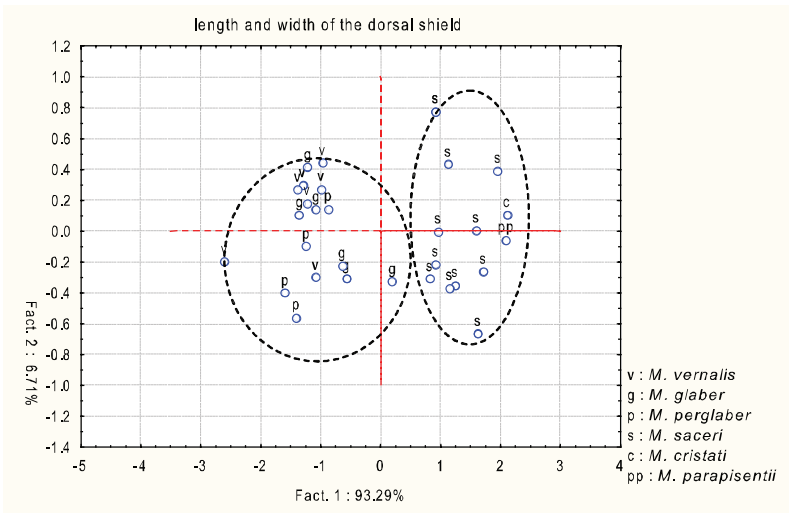

Figure 1 Body length and width of three specialist mites: *Macrocheles saceri* (s), *M. parapisentii* (p), and *M. cristati* (c), and of three generalist mites: *M. vernalis* (v), *M. glaber* (g), and *M. perglaber* (p).

the most common beetle inhabiting dung together with this opportunist mite (*B. bubalus*; Glida & Bertrand 2002) was also the most attractive beetle under laboratory conditions, and *Bubas* beetles were also more attractive than *Scarabaeus* species. However, the specialist *M. saceri* showed preferences for *Scarabaeus* species only, with *S. sacer* more attractive than *S. cicatricosus* and with *S. laticollis* being less attractive than the other two (Niogret et al., 2006). This preference order reflected the associations observed in the field. The tunneler *B. bubalus*, which does not occur in the same sites as *M. saceri*, revealed to be the least attractive carrier for this mite. Apart from demonstrating specific recognition of the carrier host, our experiments also showed differences between specialists and generalists in the way their behaviour is affected by the quality of the dung. *Macrocheles perglaber* was attracted either by beetles or by dung, depending on dung quality. This could explain why they move from the carrier to fresh dung, and do the reverse when the dung has dried out. However, under field conditions *M. saceri* mites always stay on the body of their host (or at least very close) whatever the moisture content of the dung.

Morphometric comparison showed that specialist mites are larger than generalists, and that the ratio PI leg length: body length is higher for specialists. Olfactory and gustatory receptors are concentrated on the palps and the tarsi of the first pair of legs (Coons & Axtell, 1973). As PI legs are involved in odour recognition, their relatively greater length may promote sensitivity to detect olfactory cues (Niogret et al., 2004). Our observations showed that macrochelid species differ in their host-finding behaviour. The opportunists did not simply mount on any carrier, they discriminate between potential hosts. We found that *M. perglaber* preferred *Bubas* to *Scarabaeus* spp., but some individuals were found to be carried by other coprophilous insects, e.g., flies (*Musca domestica*, *Stomoxys calcitrans*), albeit to a lesser extent (Krantz, 1983; Walter & Krantz, 1986).

Specialists, such as *M. saceri*, were shown to discriminate their hosts at species level and detected even the subtle differences between *S. sacer* and *S. cicatricosus*. This ability to discriminate was supported by observations on their choices for carrier hosts in the field. However, in the laboratory *M. saceri* was significantly attracted to *S. laticollis*, even though this roller beetle has never been found carrying this specialist species under field conditions. Alternately, *B. bubalus* was found not to be attractive for *M. saceri*, whereas this tunneler was the preferred carrier for opportunistic macrochelids. Indeed, opportunist and specialist macrochelids have separate ecological niches as they use different carrier hosts for phoresy. Tunneler beetles revealed to be very attractive for generalists, as these insects spend a long time inside dung pats (several hours or days) contrary to rollers which make their ball in just a few minutes and move away. Thus, specialists require particular adaptations (morphological or physiological) to find their short-stay carriers and they develop inside their pedotrophic nest.

Olfactometer experiments showed that generalist and specialist mites respond differentially to the presence and moisture content of dung. In most cases, *M. perglaber* was attracted by dung cues, but when moisture conditions became unfavourable the mites preferred to move on their carrier host. Dung of 2-3 days old was the most attractive substrate, and this may well correspond to the optimal conditions for macrochelid mite development (Niogret et al., 2004). In the presence of a non-host, not belonging to the guild of tunnelers, most generalist mites preferred the dung substrate. Such a generalist strategy is possible due to the great availability of potential carriers that visit the dung. The phoretic behaviour of mites is elicited when a beetle happens to be within a few centimetres of them. Mites are not really actively searching for carriers, probably because generally host availability is not a limiting factor in dung pats. This leaves them more time to seek their prey actively (nematodes and fly larvae) and to oviposit. Morphological adaptations are probably related to this relatively carrier-independent life in the dung pats. The intense sclerotization of the integument relative to specialist mites, which develop in the climate-buffered nests of the rollers, may be an adaptation to resist water loss when the dung desiccates. Moreover, as mites are numerous in the pats, intra- and interspecific competition with other coprophilous mites and insects is high and drought resistance may give them a competitive advantage (Athias-Binche, 1994; Combes, 1995). PI legs are proportionally smaller in generalists and this may cause them to move more slowly than specialists. However, a high walking speed is not necessary for a generalist, because food is abundant and the availability of their carriers is not limiting for a long time in the dung pat.

Specialists require another strategy for survival. Under field conditions, *M. saceri* was closely associated with *Scarabaeus* beetles, leaving their carrier only when the pedotrophic nest is dug and the dung ball is buried. Before this, mites stay on the body of their carrier or very close during the time the beetle makes a dung ball, irrespective of the moisture content of the dung pat. In laboratory experiments, *M. saceri* showed delay significantly shorter when the available carrier was a roller beetle (*S. sacer* and *S. cicatricosus*). Any advantages are linked with this strategy. As the majority of the mites carried by rollers were specialists, competition with opportunistic species is low in pedotrophic nests. Sivinski (1983) showed that in most cases fauna active inside the nest at the surface of the dung ball (mites, dipterans, nematodes), was very poor when compared with fauna inside the dung pat. This considerably reduces competition for specialists and provides them with rather constant microclimatic conditions for development. This may account for the lower degree of sclerotization of their integument. The phoretic behaviour of specialist mites is characterized by an active search for the host. Specialists were found to be larger than generalists, with legs (but not palps) proportionally longer, relative to body length. This may allow them to be more mobile than smaller species, increasing their possibility to find transient hosts which are not available for a long time in the dung pat. In macrochelids, the first pair of legs has no direct role in locomotion, but is involved in olfactory reception (Coons & Axtell, 1973; Niogret et al., 2004); the pedipalps have a role in chemotactic recognition (gustatory reception). Species with long PI legs equipped with sensors may be better to locate scarce hosts from a distance. This may explain why *M. saceri* detected *Scarabaeus* presence faster than *M. perglaber*.

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Development of microsatellite markers for *Tetranychus kanzawai* (Acari: Tetranychidae) and analysis of spatio-temporal gene flow among populations on different host plants

Shinya Nishimura[1,†], Norihide Hinomoto[2,*] & Akio Takafuji[1]

[1]Laboratory of Ecological Information, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan; [†]Current address: Agricultural Chemical Research Laboratory, Sumitomo Chemical Co., Takarazuka, Hyogo 665-0051, Japan; and [2]National Institute of Agrobiological Sciences, Tsukuba, Ibaraki 305-8634, Japan. E-mail: hinomoto@affrc.go.jp

We developed seven microsatellite markers for *Tetranychus kanzawai* (Acari: Tetranychidae) and examined how well the isolated markers conformed to Mendelian laws. All microsatellite markers fit the expected 1:1 disomic segregation ratio and hence were inherited in a Mendelian fashion. Using these markers, we investigated spatio-temporal gene flow among populations of *T. kanzawai* on three host plants, *Hydrangea macrophylla*, *Akebia quinata*, and *Clerodendrum trichotomum*. Temporal genetic variation showed that population differentiation was slightly reduced from April to May but greatly increased from May to October. In the analysis of spatial genetic variation, no isolation by distance was detected among the three host-plant populations. Gene flow between populations on *H. macrophylla* and those on other host plants was relatively restricted, whereas the populations on *A. quinata* and *C. trichotomum* were almost panmictic. Genetic differentiation among the populations was attributed to the effect of host plants rather than to the effect of geographic distance among populations, suggesting the possibility of sympatric host race formation in *T. kanzawai*.

Key words: *Tetranychus kanzawai*, microsatellite markers, gene flow, genetic variation, host race, sympatric speciation

The Kanzawa spider mite, *Tetranychus kanzawai* Kishida (Acari: Tetranychidae), is a highly polyphagous herbivore that injures both wild and cultivated plants (Gotoh et al., 1999; Morishita & Takafuji, 1999). Variations in host preference, host adaptability (Kuwahara, 1982; Gomi & Gotoh, 1996; Gotoh et al., 1999; Takafuji et al., 2001), and diapause characteristics (Takafuji & Morishita, 2001; Takafuji et al., 2001) have been recognized among *T. kanzawai* populations. Gomi & Gotoh (1996) and Gotoh et al. (1999) performed crossing experiments among populations collected from different host plants and recognized two distinct strains (K and T) that were reproductively incompatible and differed in host plant preference. The K strain was later described as *T. parakanzawai* Ehara (Ehara, 1999), although molecular phylogenetic trees and crossing experiments by Hinomoto & Takafuji (2001) suggested that the strains were still undergoing speciation. Conversely, Navajas et al. (2001) revealed by DNA sequence analyses and crossing experiments that *T. hydrangeae* Pritchard et Baker, which had been considered a distinct *Tetranychus* species associated with the genus *Hydrangea*, is a synonym of *T. kanzawai*. These host-related differences suggest the existence of host races in *T. kanzawai*.

A host race is a population partly reproductively isolated from other conspecific populations as a direct consequence of adaptation to a specific host (Diehl & Bush, 1984). According to Drès & Mallet (2002), host races in plant-feeding insects represent an intermediate stage of the sympatric speciation process, which extends from polymorphism within a species to differentiation of genuine species. Variations within *T. kanzawai* related to host plants seem to indicate host races, suggesting that sympatric speciation is occurring in this species. To elucidate whether host races exist in this species, fine-scale gene flow among populations must be examined using genetic markers.

Microsatellites are highly polymorphic DNA markers that consist of a variable number of tandem repeats of short nucleotide sequences (Goldstein & Schlötterer, 1999). Because of this high level of polymorphism, microsatellites are considered to be among the most powerful tools for the study of population genetics. Recently, a faster and easier protocol of microsatellite isolation was proposed by Zane et al. (2002), and microsatellite markers have been developed in several mite species (Delaye et al., 1998; Osakabe et al., 2000; Evans et al., 2003; Solignac et al., 2003; Bailly et al., 2004; Hinomoto & Maeda, 2005).

Here, we introduce microsatellite markers developed for *T. kanzawai* and show the usefulness of the markers in population genetic studies. Before using the isolated markers, we examined their conformity to Mendelian laws and analyzed the linkage among the loci. We then used the markers to investigate genetic differentiation and the degree of gene flow among sympatric populations of *T. kanzawai* occurring on different host plants. We analyzed the variation of allele frequencies both spatially and temporally, thereby examining host race formation and sympatric speciation in this species.

MATERIALS AND METHODS

Development of microsatellite markers

To isolate microsatellite regions from the *T. kanzawai* genome, we used a method described by Schlötterer (1998), with some modifications. Here we describe the method briefly; full details are provided by Nishimura et al. (2003).

Total DNA was extracted from 40 adult females of a laboratory strain, which had been collected in 1993 at Kanaya in Shizuoka Prefecture, Japan, and thereafter kept under laboratory conditions. The DNA was digested with restriction enzymes and ligated with SauL linker (Schlötterer, 1998) or

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Table 1 Core sequences, fluorescent dyes used to label the 3' end of primers, primer sequences, and the concentration of each primer in PCR amplification for seven *Tetranychus kanzawai* microsatellite markers.

Locus name	Core repeat*	Fluorescent dyes	5' to 3' primer sequences	Concentration in PCR (pmol/μL)	Accession Number
TkMS002	$(AG)_{29}$	HEX	ATGAAGAAACTGAAAGTTTTGGTC	0.11	AB107759
		-	GCAACAGGGTTTCTAGATTTTGAT	0.11	
TkMS006	$(AG)_{18}$	HEX	AGCGCGTTTACAGCTTTCAG	0.18	AB107760
		-	ACCAGGCCTATTGCAACTTC	0.18	
TkMS010	$(GA)_{23}$	6-FAM	TTACGGGCTTGATAGTGACC	0.07	AB107761
		-	AAATGACCTCCGCTCTTCCT	0.07	
TkMS011	$(AG)_{41}$	HEX	GCACTCACTCATCTTCCAGTG	0.10	AB107762
		-	TCTCGTCTCCTCTCCATTCTG	0.10	
TkMS013	$(GA)_{23}$	6-FAM	TGATGAAGATGATAACGGGTAA	0.11	AB107763
		-	TTGCCTGAAAATGACAACCA	0.11	
TkMS014	$(CT)_{53}$	TAMRA	CTTGCCTTTGTTTTCTTCATCC	0.17	AB107764
		-	CTAAGGGAACATTCATGATTAGA	0.17	
TkMS015	$(TC)_{39}$	6-FAM	GATGGATCAACATTGAACAGATTTACT	0.20	AB107765
		-	TCCTACACTTGACATAAAATCAATCAC	0.20	

*From sequenced clone.

SNX linker (Hamilton et al., 1999). The restriction enzyme *Mbo*I was used for ligation with SauL, and *Nhe*I was used with *Alu*I, *Csp*45I, *Hinf*I, or *Msp*I for ligation with SNX.

After ligation, PCR was performed with the linker-specific primers, and the fragments were hybridized on a nylon membrane with two oligonucleotides, $(TC)_{16}$ and $(AC)_{16}$. The hybridized fragments containing microsatellite sequences were removed from the membranes with washing buffer. The fragments were precipitated with ethanol, dried, and reamplified by PCR with the linker-specific primers.

The DNA sequences of the reamplified fragments were determined by using TA cloning and direct sequencing methods with a Taq Dye Deoxy Terminator Cycle Sequencing Kit using an ABI Prism 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). When microsatellite sequences were detected in the fragments, primers were designed from the flanking region using PRIMER3 software (Rozen & Skaletsky, 1998).

Microsatellite sequences were found in 21 of the 37 clones sequenced (56.7%), and primer pairs designed on seven sequences were consistently amplified fragments. Therefore, we used the seven loci (DDBJ accession nos. AB107759–AB107765) for further analysis (Table 1). On the basis of their size ranges, we divided the seven microsatellite loci into two groups: (1) *TkMS002*, *TkMS011*, and *TkMS015*; and (2) *TkMS006*, *TkMS010*, *TkMS013*, and *TkMS014*. Each primer group was used separately for multiplex PCR. One of the primers of each locus was labeled by fluorescent dyes to distinguish several loci in a single electrophoresis gel.

Segregation and linkage analyses

To analyze the segregation ratio and linkage among microsatellite loci, we conducted crossing experiments using 14 strains of *T. kanzawai*, which had been kept under laboratory conditions until use for crossing experiments (from 1 month to 9 years; see Table 1 in Nishimura et al., 2003). In each experiment, we used three strains randomly chosen from the 14 strains. We transferred a teleiochrysalid female onto a detached bean leaf disc (ca. 4 cm^2) with an adult male from another strain for copulation. Ten days later, individual F1 females at the teleiochrysalis stage were transferred to a new leaf disc with an adult male from another randomly selected strain. After 5 days, when the F1 female had laid sufficient eggs, both sexes were removed and used for DNA extraction.

To investigate the segregation ratio in F2 female progeny, we extracted DNA from F2 females 10 days after removal of their parents. For segregation analysis of male progeny and linkage analysis among microsatellite loci, individual F2 females at the teleiochrysalis stage were transferred alone to a new leaf disc. One and 2 weeks after introduction of the female, we extracted DNA from each F2 female and her arrhenotokous sons, respectively.

For DNA extraction, each mite was individually placed into a 1.5-ml microcentrifuge tube. Males were homogenized with 15 μl of Prepman Ultra Reagent (Applied Biosystems), by crushing with a pipette tip (females with 30 μl). The following procedure was conducted according to the manufacturer's instructions. For amplification of microsatellite loci, we used primers labeled with one of the three fluorescent dyes 6-FAM, HEX, or TAMRA. Multiplex PCR amplification and fragment analysis using ABI Prism 377 DNA Sequencer and GeneScan ver. 3.1.2 (Applied Biosystems) were then conducted.

For segregation analysis, a χ^2-test was performed to confirm that all the microsatellite markers isolated were inherited in a Mendelian fashion. Because mites reproduce by arrhenotoky, only one segregation ratio (1:1) is expected in both male and female progeny. We performed a χ^2-test for goodness of fit between the expected and observed genotype frequencies for each locus.

To estimate the significance of linkages among microsatellite loci, a χ^2-test was performed. The χ^2 value for linkage analysis (χ_L^2) and the recombination fraction (r) were calculated using the following equations:

$$\chi_L^2 = (a_1 - a_2 - a_3 + a_4)^2/n \quad (1)$$

and

$$r = (a_2 + a_3)/n, \quad (2)$$

where a_1, a_2, a_3, and a_4 are the genotype frequencies of A_1B_1, A_1B_2, A_2B_1, and A_2B_2, respectively, and $n = a_1 + a_2 + a_3 + a_4$. Here, A and B represent two loci analyzed, and the subscripts 1 and 2 indicate alleles of maternal and paternal origin, respectively. The smaller the recombination fraction between two loci, the more closely they are linked.

Genetic differentiation within and among field populations on different host plants

We studied genetic variation within and among populations of *T. kanzawai* using populations collected at Inami (33.8°N,

Table 2 Sampling data of *Tetranychus kanzawai* populations at Inami, Wakayama, Japan.

Location code	Site no.	Geographic coordinates	Host plant	Population code[1] 25 April	23 May	3 October
A	1	33°48'40.8"N-135°13'46.6"E	Chocolate vine (*Akebia quinata*)	A_A	A_M	A_O
B	1	33°48'39.3"N-135°13'46.3"E	Chocolate vine (*A. quinata*)	-	B_M	B_O
C	1	33°48'38.3"N-135°13'47.7"E	Chocolate vine (*A. quinata*)	-	C_M	C_O
D	1	33°48'38.7"N-135°13'45.3"E	Glory bower (*Clerodendrum trichotomum*)	-	D_M	-
E	1	33°48'38.3"N-135°13'45.2"E	Glory bower (*C. trichotomum*)	-	E_M	-
F	1	33°48'38.1"N-135°13'47.6"E	Glory bower (*C. trichotomum*)	F_A	F_M	F_O
G	1	33°48'39.3"N-135°13'46.0"E	Hydrangea (*Hydrangea macrophylla*)	G_A	-	-
H	1	33°48'38.9"N-135°13'45.4"E	Hydrangea (*H. macrophylla*)	H_A	-	-
I	2	33°48'23.6"N-135°13'31.4"E	Chocolate vine (*A. quinata*)	I_A	-	-
J	2	33°48'23.8"N-135°13'31.0"E	Glory bower (*C. trichotomum*)	J_A	J_M	J_O
K	3	33°49'00.9"N-135°13'04.6"E	Glory bower (*C. trichotomum*)	K_A	-	-
L	4	33°48'50.0"N-135°12'12.8"E	Chocolate vine (*A. quinata*)	L_A	L_M	-
M	4	33°48'49.9"N-135°12'12.4"E	Glory bower (*C. trichotomum*)	M_A	M_M	-

[1] Population codes indicate location (A-M) and sampling date (subscript A, 25 April; M, 23 May; O, 3 October 2002).

135.2°E), in Wakayama Prefecture, Japan. We collected mite samples from *Akebia quinata* (Lardizabalaceae), *Clerodendrum trichotomum* (Verbenaceae), and *Hydrangea macrophylla* (Hydrangeaceae), at 13 locations in four sites (Fig. 1), three times between April and October 2002 (for sample details, see Table 2). For each sample, we examined the allele frequencies of the six microsatellite loci. The detailed procedures of multiplex PCR and fragment analysis are described by Nishimura et al. (2005). At the same study site, we also examined the seasonal occurrence of the mite population density eight times from April to December 2002 by counting the number of adult females on 20 leaves at each location.

The genetic diversity of each population was estimated by using the following parameters: mean number of alleles per locus, allelic richness (El Mousadik & Petit, 1996), and expected and observed heterozygosities. The expected number of heterozygotes was calculated after Levene's (1949) correction using GENEPOP ver. 3.3 (Raymond & Rousset, 1995). The difference between expected and observed heterozygosities is a measure of deviation from Hardy-Weinberg equilibrium, which is best expressed by F_{IS} (Weir & Cockerham, 1984). Allelic richness and multilocus F_{IS} were computed using FSTAT ver. 2.9.3 (Goudet, 1995).

We quantified genetic differentiation between populations using pairwise F_{ST} values (Weir & Cockerham, 1984). We tested the deviation of pairwise F_{ST} values from zero by randomizing multilocus genotypes with FSTAT ver. 2.9.3 (Goudet, 1995).

To elucidate the spatial genetic variation in allele frequency, the pairwise F_{ST} values among populations collected on the same date were subjected to analysis of covariance (ANCOVA) to test the effects of geographic distance and host plant. When the effect of host plant was significant, the Tukey-Kramer HSD test was used for multiple comparisons as a post hoc test.

To reveal the temporal trends in genetic differentiation, we analyzed the pairwise F_{ST} among the nine populations collected from locations A, F, and J (see Fig. 1) at three sampling times. The pairwise F_{ST} values between populations collected on the same date were examined by ANOVA with Scheffé's post hoc test to reveal any temporal effect.

RESULTS

Segregation and linkage analyses

For the segregation ratio in the mite progeny, no significant deviation was found between expected and observed genotype frequencies in either female or male progeny (data not shown). The result indicates that all microsatellite markers fit the expected 1:1 disomic segregation ratio and hence were inherited in a Mendelian fashion. All progeny had the alleles present in their parents. Females had one (homozygous) or two (heterozygous) alleles per locus, whereas males had only one allele for each locus, which is in agreement with the arrhenotokous mode of reproduction in spider mites.

In the linkage analysis, significant pairwise linkage was detected in three pairs of microsatellite loci: *TkMS002*/*TkMS006*, *TkMS010*/*TkMS013*, and *TkMS014*/*TkMS015* (Table 3). Among the pairs, *TkMS014*/*TkMS015* was the most closely linked, followed by *TkMS002*/*TkMS006* and *TkMS010*/*TkMS013*, as expressed by the pairwise recombinant fraction (r = 0.110, 0.333, and 0.389, respectively; see Table 3).

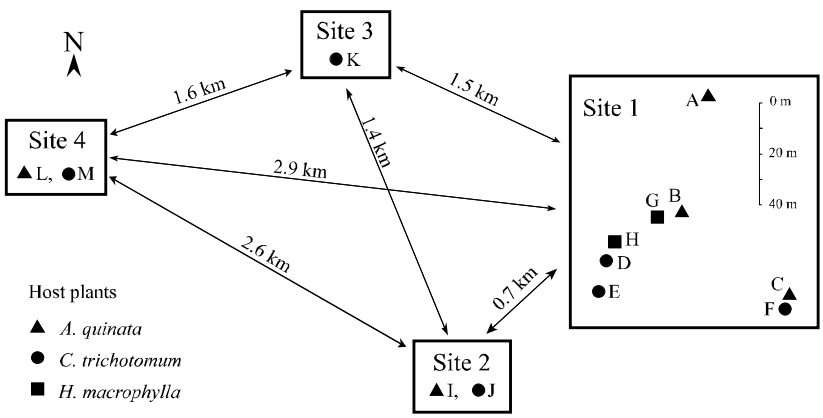

Figure 1 Map of the study sites. Letters correspond to the location codes in Table 2. Symbols indicate host plants of *Tetranychus kanzawai*: *Akebia quinata* (triangles), *Clerodendrum trichotomum* (circles), and *Hydrangea macrophylla* (squares).

Table 3 Goodness-of-fit χ^2-test to estimate the significance of linkage between pairs of microsatellite loci (below the diagonal) and pairwise recombinant fraction (above).

Locus name	*TkMS002*	*TkMS006*	*TkMS010*	*TkMS011*	*TkMS013*	*TkMS014*	*TkMS015*
TkMS002	-	0.333	0.541	0.481	0.485	0.485	0.490
TkMS006	**34.37**	-	0.505	0.455	0.463	0.502	0.518
TkMS010	3.75	2.84	-	0.514	0.389	0.458	0.475
TkMS011	3.09	6.26	9.92	-	0.454	0.533	0.516
TkMS013	5.00	12.09	**29.77**	10.35	-	0.558	0.540
TkMS014	1.66	1.98	7.86	5.03	6.83	-	0.110
TkMS015	1.50	6.07	5.55	3.10	5.92	**171.30**	-

Bold numbers indicate significant pairwise linkage (P<0.05). χ^2 values were calculated by adding all χ^2 values of crosses per pair between microsatellite loci, and the degrees of freedom were equal to 2n–1, where n is the number of crosses.

Genetic differentiation within and among field populations on different host plants

The genetic diversity within each population is summarized in Table 4. The data indicated that the populations were highly diverse, with many alleles per locus and high allelic richness. In all populations, the observed heterozygosities were lower than those expected, but the values of F_{IS} in four populations (A_A, D_M, E_M, and B_O; see Table 2) were not significantly different from zero.

In the spatial analysis of the nine populations collected in April, the populations on *H. macrophylla* were genetically distinct from the others, because the pairwise F_{ST} values in the following three combinations of host plant were significantly higher than the other combinations: *A. quinata – H. macrophylla*, *C. trichotomum – H. macrophylla*, and *H. macrophylla – H. macrophylla* (Fig. 2a). In the analysis of the populations collected in May, ANCOVA showed that the pairwise F_{ST} did not significantly differ owing to the effect of either host plant (F_2 = 0.29, P>0.05; Fig. 2b) or geographic distance (F_1 = 0.19, P>0.05). Thus, frequent gene exchange occurred among populations, regardless of host plant species and geographic distance among the populations. In populations collected in October, however, ANCOVA showed that the pairwise F_{ST} values were significantly affected by both host plant (F_2 = 5.70, P<0.01) and geographic distance (F_1 = 6.96, P<0.05). Among the combinations of host plants, the pairwise F_{ST} value of *A. quinata – A. quinata* was significantly lower than the others (Fig. 2c). Geographic distance and pairwise F_{ST}values were negatively correlated (data not shown). However, when we removed population F_O from the October analysis, both effects were no longer significant (ANCOVA: F_1 = 1.70, P>0.05; F_1 = 1.68, P>0.05, respectively). Therefore, the negative correlation between geographic distance and pairwise F_{ST} and the significant effect of host plant on pairwise F_{ST} were both caused by population F_O, which was geographically close to the other populations but genetically distant from them.

We found a seasonal change in the pairwise F_{ST} values, which decreased slightly during the period from April to May and then increased markedly from May to October (Fig. 3). This result indicated that allele frequencies changed greatly and population differentiation became significantly larger during the summer season.

DISCUSSION

We successfully isolated seven microsatellite markers that were sufficiently polymorphic to allow detection of fine-scale genetic differences among populations. With traditional methods of microsatellite isolation, positive clones usually represent between 0.04 and 12% (Zane et al., 2002); seven of the 37 clones that we sequenced (18.9%) were positive, indicating that the method of microsatellite isolation used here was highly effective. Although Navajas et al. (1998) showed that microsatellite sequences are relatively rare in mite genomes compared with vertebrate genomes, we showed that this weakness could be overcome by using a microsatellite-rich library. The effectiveness of the method should promote great advances in acarine population genetics in the future.

Using the isolated microsatellite markers, we analyzed the genetic structure both spatially and temporally among *T.*

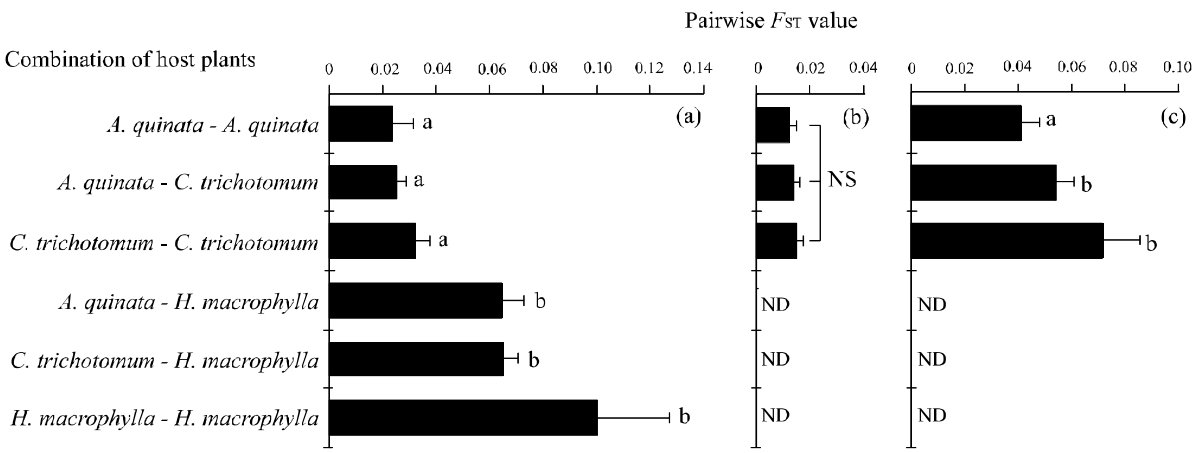

Figure 2 Effect of host plant on the genetic differentiation (expressed as F_{ST}) among populations collected on (A) 25 April, (B) 23 May, and (C) 3 October. Different letters indicate significant differences among combinations of host plants (Tukey-Kramer HSD test: P<0.05), and 'NS' indicates no significant difference among host plants. 'ND' indicates that no data were available, because no mites were collected from *Hydrangea macrophylla* on 23 May and 3 October. Error bars indicate standard errors.

Table 4 Mean (± SE) number of alleles per locus, allelic richness, and observed and expected heterozygosities averaged over microsatellite loci and the multilocus F_{IS} of each population.

Population code[a]	No. adult females used	No. alleles per locus	Allelic richness	Heterozygosity Observed	Expected	F_{IS}
A_A	10	9.17±0.75	8.44±0.65	0.806±0.053	0.855±0.036	0.061
F_A	26	14.67±1.80	8.29±0.84	0.695±0.054	0.845±0.041	0.181*
G_A	13	9.83±0.95	7.78±0.55	0.679±0.046	0.847±0.022	0.205*
H_A	35	12.67±1.73	7.02±0.63	0.757±0.052	0.826±0.032	0.083*
I_A	26	11.67±1.26	8.77±0.82	0.705±0.042	0.876±0.024	0.201*
J_A	40	16.17±1.92	8.37±0.67	0.690±0.063	0.862±0.026	0.202*
K_A	35	14.17±1.49	7.86±0.51	0.661±0.048	0.846±0.022	0.221*
L_A	40	19.50±2.96	9.18±0.87	0.763±0.068	0.882±0.031	0.136*
M_A	10	10.17±0.70	9.68±0.66	0.678±0.087	0.920±0.012	0.275*
A_M	16	13.33±1.58	9.62±0.74	0.705±0.028	0.903±0.029	0.225*
B_M	28	15.00±2.02	8.43±0.93	0.750±0.050	0.866±0.033	0.136*
C_M	18	12.33±1.73	8.82±0.88	0.583±0.075	0.874±0.036	0.340*
D_M	25	16.50±2.13	9.67±0.86	0.875±0.032	0.909±0.026	0.038
E_M	14	10.50±0.92	8.45±0.69	0.852±0.027	0.891±0.025	0.045
F_M	10	8.83±1.25	8.03±1.07	0.767±0.097	0.838±0.064	0.090*
J_M	40	17.67±2.81	9.14±0.99	0.743±0.032	0.889±0.030	0.166*
L_M	24	15.83±1.82	9.43±0.63	0.742±0.067	0.905±0.014	0.183*
M_M	40	17.33±2.03	9.05±0.78	0.733±0.047	0.882±0.031	0.171*
A_O	15	9.17±1.35	7.15±0.88	0.629±0.097	0.830±0.039	0.248*
B_O	19	9.50±1.12	6.81±0.58	0.779±0.048	0.820±0.023	0.052
C_O	28	15.33±1.78	8.69±0.91	0.621±0.042	0.855±0.047	0.278*
F_O	18	8.50±1.43	6.27±0.86	0.673±0.106	0.763±0.077	0.122*
J_O	17	13.17±1.08	9.12±0.74	0.694±0.046	0.875±0.039	0.212*

[a]Population codes indicate location (A-M) and sampling date (subscript A, 25 April; M, 23 May; O, 3 October 2002).
*Significantly larger than zero (Randomization test after Bonferroni correction: P<0.05).

kanzawai populations that occurred sympatrically on different host plants. In the spatial analysis, gene flow between populations on *H. macrophylla* and those on the other hosts was restricted, whereas the populations collected from *A. quinata* and *C. trichotomum* were almost panmictic (Fig. 2a). Various isocoumarin derivatives such as hydrangenol and phyllodulcin are produced by plants of the genus *Hydrangea*, and these compounds contribute to defense against herbivorous insects and pathogenic fungi (Nozawa et al., 1981; Hashimoto et al., 1987; Ujihara et al., 1995). Gotoh & Gomi (2000) suggested that defensive secondary compounds in *H. macrophylla* curtail the developmental performance of *T. kanzawai* on this host. Thus, the defensive compounds of *H. macrophylla* and the relative detoxification ability among populations of *T. kanzawai* may jointly cause the genetic distinctness of populations on *H. macrophylla*.

In the temporal analysis, pairwise F_{ST} values slightly decreased from April to May but increased markedly from May to October (Fig. 3). These results can be attributed to the seasonal occurrence pattern of *T. kanzawai* at the study site (Fig. 3). High population density from April to May might have caused dispersal of *T. kanzawai*, thereby resulting in frequent gene exchange among populations. Thus, genetic differentiation would be reduced among populations in May. Thereafter, the population density abruptly declines and remains extremely low during midsummer, owing to heavy predation pressure (Morishita, 2000). From May to October the allele frequency of each population changed greatly and the population differentiation became significantly larger, probably because of bottleneck effects.

Hinomoto & Takafuji (1994, 1995) revealed the hierarchical genetic structure and small breeding units of *T. urticae* populations using an allozyme marker, showing that the mite populations were established via a process of colonization, reproduction, and dispersal. In such a situation, bottlenecks and genetic drift will frequently arise in the populations. Several studies have revealed host plant specialization in *T. urticae* (Fry, 1990; Gotoh et al., 1993), and genetic differentiation related to host plants has also been revealed by allozyme analyses and crossing experiments (Tsagkarakou et

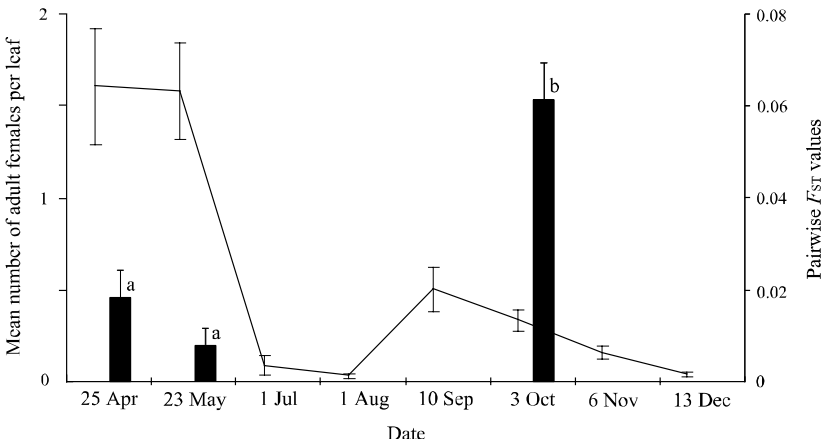

Figure 3 Seasonal occurrence of *Tetranychus kanzawai* (lines, left axis) and temporal fluctuation of genetic differentiation (expressed as F_{ST}) among populations collected from locations A, F, and J on the same date (bars, right axis). Different letters indicate significant differences in F_{ST} values among sampling dates (Scheffé's test: P<0.05). Error bars indicate standard errors.

al., 1998; Navajas et al., 2000). Our results showed that the population differentiation in *T. kanzawai* was caused by the effects of host plant rather than geographic distance, indicating that the populations often encounter local differentiation events triggered by genetic drift accompanied by their adaptation to host plants. Several studies have suggested the existence of host races and the possibility of sympatric speciation via host shift in these polyphagous spider mites (Fry, 1990; Gotoh et al., 1993, 1999; Gomi & Gotoh, 1996; Tsagkarakou et al., 1998; Navajas et al., 2000; Takafuji & Morishita, 2001; Takafuji et al., 2001). To fully understand the process of speciation in spider mites, comprehensive studies based on morphology, molecular phylogeny, and population genetics will be needed.

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Demographic and reproductive parameters of *Polyphagotarsonemus latus* in *Carica papaya*

Martha de Coss, Gabriel Otero-Colina[1], Jorge E. Peña[2] & Ricardo Magallanes

Universidad Autónoma de Chiapas, Facultad de Ciencias Agrícolas, Chiapas, Mexico, [1]Colegio de Postgraduados Campus Montecillo, Km 36.5 Carr. Mexico - Texcoco, Montecillo, 56230 Edo. de México. *E-mail: gotero@colpos.mx; and [2]University of Florida, Homestead, FL, USA

Cohorts of specimens of *Polyphagotarsonemus latus* were bred on seedlings of papaya trees cv. Maradol under controlled environmental conditions. This was done to determine the effect of temperature and relative humidity on population and reproductive parameters of *P. latus*, as well as the suitability of papaya as a host. Developmental times of *P. latus* at 25, 28, and 31 °C and 55, 67, and 97% r.h. were compared. Egg-to-adult developmental time varied between 2.81 and 4.93 days, which is considered normal when compared to development on other host plants. The intrinsic rate of increase (r_m) varied from 0.03 to 0.4, depending on relative humidity and temperature. Papaya is considered a suitable host, but temperature and humidity outside the most favorable values considerably reduce the potential of the mite to colonize it.

Key words: *Carica papaya*, life table, fecundity table, Tarsonemidae.

Polyphagotarsonemus latus (Banks) is an important plant pest in warm and humid areas. It is considered the most important factor reducing papaya yield in the island of Réunion (Aubert et al., 1990). In Chiapas (Soconusco Region, Mexico), *P. latus* has been found on various wild and agricultural host plants, but on papaya it is not found frequently and colonies apparently are not long-lasting (De Coss, 1999, 2006). This work was carried out to determine the effect of temperature and relative humidity on development and reproduction of *P. latus* on papaya as a host. The results were used to calculate demographic parameters, especially the intrinsic rate of increase (r_m), and to verify whether papaya cv. Maradol is susceptible to *P. latus.*

MATERIALS AND METHODS

Polyphagotarsonemus latus was initially collected from hot pepper, *Capsicum annuum* L. var. Jalapeño, and then maintained in a greenhouse on bean, *Phaseolus vulgaris* L. var. Jamapa, at 28±1 °C and 67.5±2.5% r.h. Stock bean plants were sown weekly and infested on a weekly basis. Seedlings of 7-day-old papaya cv. Maradol, at the stage of two true leaves, were transplanted into pots containing organic soil and compost of *Eisenia foetida* (Savgny). Then these seedlings were placed in climatized chambers under controlled temperature and relative humidity, with a photoperiod of L11.5:D12.5. Initially the chambers were calibrated at 25±1, 28±1, or 31±1 °C, and held at 67.5±2.5% r.h. Thereafter, the climatic chambers were calibrated at 28±1 °C and 55.0±2.0, 67.5±2.5, or 97.5±2.5% r.h. with new papaya seedlings.

A first test was aimed at estimating survival and developmental times of the life stages of *P. latus*. A single female, presumably mated, was placed on each papaya plant. The female was removed 2 h later, after she had oviposited, and a single egg was left. Time to egg eclosion, duration of egg, larva, nymphochrysalis, and adult were daily observed at 7:00, 13:00, and 19:00 h until the mites died. A second test was aimed at estimating adult survival and reproduction. A male and a female were placed on each plant and they were incubated under the combinations of temperature and r.h. given above. The number of eggs, percentage of egg eclosion, and female survival were recorded daily at 7:00, 13:00, and 19:00 h. In both tests, cohorts comprised 10-15 mites. Estimates of developmental time, survival, and reproduction were combined to construct a life table and a fecundity table, following the statistical demographic analysis of Carey (1993). Developmental times for the immature stages were analyzed following the program 'Menu' (Olivares, 1994), using a completely random design.

RESULTS AND DISCUSSION

Developmental time

There were no significant differences in the time to egg eclosion at 25±1, 28±1 and 31±1 °C, all at 67.5±2.5% r.h. (Table 1). Developmental times for larvae, nymphochrysalis, and egg-to-adult were significantly longer at 25±1 °C. Poikilothermic organisms usually have an increasing developmental rate as temperature increases, in a linear relationship within a certain temperature range (Arnold, 1959). Thus, the significantly longer developmental times at 25±1 °C are as expected. By contrast, no significant differences were found in developmental times between 28±1 °C and 31±1 °C. It is suggested that 31 °C is close to the upper limit of the temperature range where *P. latus* can thrive. Silva et al. (1998) obtained similar results when they bred *P. latus* on sweet pepper. By contrast, Vieira & Chiavegato (1998) observed that this mite needed more time (4.1±0.1 days) for egg-to-adult development at 28.5±0.3 °C and 71±2.6% r.h., when cotton cv. IAC-20 was used as a host.

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Table 1 Mean (± SE) developmental times (days) of immature stages of *Polyphagotarsonemus latus* on *Carica papaya* at various temperatures (with constant humidity) and various relative humidities (with constant temperature).

Temperature (°C)	25±1	28±1	31±1
Relative humidity (%)	67.5±2.5	67.5±2.5	67.5±2.5
Egg	1.48±0.19a	1.48±0.15a	1.7±0.13a
Larva	1.27±0.15a	0.68±0.40b	0.68±0.06b
Nymphochrysalis	2.18±0.22a	0.65±0.10b	0.73±0.06b
Total	4.93±0.57a	2.81±0.33b	3.11±0.25b
Relative humidity (%)	55±2	67.5±2.5	97.5±2.5
Temperature (°C)	28±1	28±1	28±1
Egg	1.14±0.03b	1.48±0.15ab	1.9±0.12a
Larva	0.94±0.03a	0.68±0.13b	0.7±0.06b
Nymphochrysalis	1.0±0.16a	0.65±0.07b	0.97±0.003a
Total	3.04±0.22ab	2.81±0.33b	3.57±0.19a

Means within a row followed by the same letter are not significantly different (Tukey's HSD, P>0.05).

A less clear picture was observed when comparing developmental time at different r.h. because there was no linear relationship. However, as a trend, developmental times of immature stages of *P. latus* at 67.5±2.5% r.h. were equal or shorter than those at more extreme humidities (Table 1). Thus, the combination of 28 °C and 67.5% r.h. is postulated as (near) optimum, for the development of *P. latus*.

Adult longevity

At different temperatures, mortality of adult mites was evenly distributed over the successive days (Figure 1); so the curves of mortality obeyed a type II shape after Krebs (1985). At 28±1 °C the mean life span was significantly longer (Tukey, P<0.05). At 67.5±2.5% r.h., the curve of mortality is again of type II, but at 55.0±2.0 and 97.5±2.5% r.h., the curves of mortality approached to type III (after Krebs, 1985), indicating a higher rate of mortality in young adults, presumably in the pre-reproductive phase (Figure 2). Conditions resulting in the longest life span were 28 °C and 67.5% r.h., just the mean values used in this study. Hence, it is postulated that these values are (near) optimal conditions for adult survival.

Demographic parameters

With a given humidity (67.5±2.5% r.h.) and variable temperature, the highest values of demographic parameters are obtained at 28±1 °C, except generation time, which is longer at 25±1 °C (Table 2). At 31±1 °C, life expectancy was lowest, indicating a negative effect of high temperature. The intrinsic rate of population increase (r_m) showed much variation. At 28±1 °C it was highest (0.40 females/female/day), similar to that found by Silva et al. (1998) on sweet pepper (var. Melrose), but considerably lower than the value found by Ramos (1985) on *Citrus* sp. (0.93 females/female/day). However, all the values of r_m estimated in this study were positive, indicating populations capable of growth.

With a given temperature (28±1 °C) and variable humidity, the the highest values of e_0, R_0, r_m, and b, as well as the lowest developmental rate were obtained at 67.5±2.5% r.h. (Table 2). However, generation time was shortest at 55±2% r.h. Once again, 28 °C and 67.5% r.h. proved the most favorable combination for *P. latus* development, survival, and reproduction.

Reproductive parameters

From the test at three temperatures, the highest gross and net fecundities were obtained at 28±1 °C, with a daily production of 2.17 eggs. At 25±1 °C the mean age of first reproduction was highest and the period of oviposition was shortest, indicating low and late reproduction (Table 3). Breeding *P. latus* at 28±1 °C, Vieira & Chiavegato (1998) estimated a fecundity of 4.5±0.9; at 31±1 °C they obtained the highest daily egg production, the lowest age at first reproduction, and the shortest oviposition period.

The Tukey test to evaluate the effect of RH on reproduction of *P. latus* showed that gross and net fecundities and daily egg production were highest at 67.5±2.5% r.h. However, mean age to reproduction and preoviposition were lower at 55±2% r.h. (Table 3).

According to Silva et al. (1998), the highest fecundity is attained at 25 °C, with a mean of 27.9 eggs per female in sweet pepper in Brazil. Jones & Brown (1983) reported an optimal temperature for reproduction on lime (*Citrus limon* L.) in California (USA) of 23.7 °C. Our own data are somewhat different from these published values, because the most favorable temperature seemed to be 28±1 °C. There is no way to determine whether these differences depend on the presence of local biotypes or from a host-related effect.

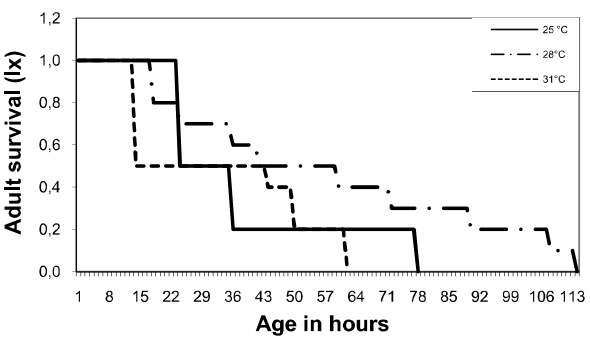

Figure 1 Survival (l_x) of adult *Polyphagotarsonemus latus* on *Carica papaya* at 67.5±2.5% RH and different temperatures.

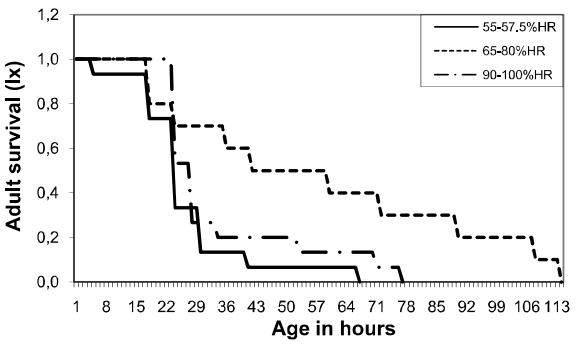

Figure 2 Survival (l_x) of adult *Polyphagotarsonemus latus* on *Carica papaya* at 28±1 °C and different relative humidities.

Table 2 Demographic parameters of *Polyphagotarsonemus latus* on *Carica papaya* at various temperatures (with constant humidity) and various relative humidities (with constant temperature).

Temperature (°C)	25±1	28±1	31±1
Relative humidity (%)	67.5±2.5	67.5±2.5	67.5±2.5
R_0	1.3	7.64	2.4
GT (days)	7.4	5.0	4.5
r_m	0.03	0.40	0.20
e_0 (days)	1.6	2.4	1.4
λ	1.0	1.5	1.2
b	0.96	4.4	4.1
d	0.92	4.06	3.5
DT (days)	17.6	1.73	3.9
Relative humidity (%)	55±2	67.5±2.5	97.5±2.5
Temperature (°C)	28±1	28±1	28±1
R_0	1.4	7.64	1.9
GT (days)	4.2	5.0	5.7
r_m	0.09	0.4	0.12
e_0 (days)	1.4	2.4	0.96
λ	1.01	1.5	1.0
b	2.30	4.4	4.4
d	2.21	4.06	4.3
DT (days)	8.0	1.73	5.8

R_0, net rate of reproduction; GT, generation time; r_m, intrinsic rate of population increase; e_0, life expectancy; λ, finite rate of multiplication; b, birth rate; d, death rate; DT, duplication time.

Table 3 Reproductive parameters (± SE) of *Polyphagotarsonemus latus* on *Carica papaya* at various temperatures (with constant humidity) and various relative humidities (with constant temperature).

Temperature (°C)	25±1	28±1	31±1
Relative humidity (%)	67.5±2.5	67.5±2.5	67.5±2.5
Gross fecundity (total no. eggs)	9.5±0.45b	17.2±1.28a	10.3±0.65b
Net fecundity (eggs/female)	1.3±0.82b	8.1±3.54a	2.4±0.97b
Daily egg laying (eggs/female/day)	0.65±0.67c	2.17±0.33b	2.87±0.18a
Mean age of first reproduction (days)	2.05±0.42ab	1.64±0.33b	1.58±0.13b
Period of oviposition (days)	1.21±0.34a	0.81±0.11a	0.29±0b
Relative humidity (%)	55±2	65-80	95-100
Temperature (°C)	28±1	28±1	28±1
Gross fecundity (total no. eggs)	7.73±0.4b	17.2±0.85a	15.5±0.45a
Net fecundity (eggs/female)	1.44±0.16b	7.6±0.27a	1.98±0.21b
Daily egg laying (eggs/female/day)	1.01±0.21b	2.17±0.18a	1.24±0.13b
Mean age of first reproduction (days)	1.01±0.3b	1.64±0.33a	1.5±0.6ab
Period of oviposition (days)	0.71±0b	0.81±0.11a	0.97±0.02a

Means within a row followed by the same letter are not significantly different (Tukey's HSD, P>0.05).

Conclusion

Polyphagotarsonemus latus is abundant in tropical climates and in crops grown under glass in temperate climates (Gerson, 1992). Data obtained in the present study agree with the known distribution of this mite, because it thrives under warm conditions. However, a constant very high temperature of 31 °C seems to harm the mite, and a reasonably high temperature of 25 °C results in relatively slow development, low reproduction and, hence, a reduced r_m compared to 28 °C.

Relative humidity has an important effect on development, survival, and reproduction of *P. latus*. The optimal humidity was about 67.5% r.h., which is fairly high and consistent with the fact that this mite occurs naturally in the humid tropical lowlands. The optimal temperature and humidity conditions are close to the mean values found in the region Soconusco (Chiapas), Mexico. This explains why the mite can easily establish and thrive in that region.

Under optimal conditions on papaya trees, *P. latus* shows an r_m value comparable to those exhibited by very injurious pests (Sabelis, 1985). However, it is remarkable that temperature and humidity lower and higher than the optimal values cause steep drops of life span and r_m. Aubert et al. (1981) and De Coss (1999, 2006) observed that *P. latus* does not form permanent colonies on papaya trees, but disappears after a short period on papaya leaves. Possibly, this is caused by catastrophic population declines resulting from climatic factors.

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Effect of temperature on the life history of the old world date mite, *Oligonychus afrasiaticus* (Acari: Tetranychidae)

Ibrahim J. Al-Jboory & Taha M. Al-Suaide[1]

University of Baghdad, College of Agriculture, Plant Protection Department, Baghdad, Iraq. Present address: PO Box 17399, Amman 11195, Jordan. E-mail: ijboory@yahoo.com; and [1]Ministry of Science and Technology, Jadrea, Baghdad, Iraq

Oligonychus afrasiaticus is considered one of the major pests threatening the production of dates in Iraq. It causes between 50-80% yield loss in years of dry, dusty, stormy weather. The biology of this mite was studied at four constant temperatures (20, 25, 30, and 35 °C), 50-60% relative humidity, and L16:D8 photoperiod. No development was observed at 15 and 40 °C. Incubation period peaked at 20 °C (7.6 days) and reached a minimum at 35 °C (2.7 days), development of larva, protonymph, and deutonymph took 5.3, 4.3, and 4.5 days at 20 °C, and 1.9, 1.3, and 1.4 days at 35 °C, respectively. Number of eggs/female was 12.5 at 20 °C and 27.2 and 35 °C. Longevity of female and male was 33.2 and 29.1 days at 20 °C, and 11.5 and 10.1 days at 35 °C, respectively. The results were used to establish a life table of this mite.

Key words: Old world date mite, *Oligonychus afrasiaticus*, Iraq, date palm

The production of dates in Iraq is threatened by many pests (Abdul-Hussein, 1963). The old world date mite (Ghobar mite) *Oligonychus afrasiaticus* (McGregor) is one of the major pests causing 50-80% yield loss in years of dry, dusty and stormy weather in Iraq (Al-Jboory, 1999). Observations have been done on the biology of this mite in Iraq and in Tunisia. These revealed that the fecundity ranged between 30-100 eggs/female at 35 °C and 50% r.h., incubation period 3 days, larval period 2 days, nymphal period 4-7 days, and generation time 8-14 days (Abdul-Hussein, 1985; Al-Haidari et al., 1982, 1986; Al-Jboory, 1999; Dhouibi, 2000). Studies on the life history of the tea mite, *Oligonychus coffeae* (Nietner) showed a trend similar to that of the old world date mite (Das & Das, 1967). High temperature reduces longevity and increases female fecundity in some tetranychid species (Nickel, 1960; Das & Das, 1967; Tanigoshi et al., 1975; Carey & Bradley, 1982). Al-Haidari et al. (1982) concluded that 30 °C was the optimum temperature for the development of old world date mites.

This paper is one of three publications on this mite investigating the effect of various temperatures and a constant relative humidity on the life history of *O. afrasiaticus.*

MATERIALS AND METHODS

Various mite stages were collected from an infested date palm tree, *Phoenix dactylifera* L., and transferred to date palm seedlings (1-9 weeks old) kept at 29-33 °C in the laboratory. Two substrates for rearing have been used, date palm leaflets and immature dates.

Date palm leaflet. Four circular holes of 2 cm diameter were drilled in the cover of a plastic Petri dish plus a small hole close to them for injection of water, to keep the dish sufficiently humid (Fig. 1). A piece of sponge, 9 cm in diameter and 1.5 cm thick, was placed in the Petri dish, covered by filter paper. A piece of palm leaflet (5.5 × 2.5 cm) is placed on the top of the filter paper underneath two of the holes drilled. The cover was fixed by small pins at the edges of the dish. The leaflets were replaced from time to time whenever they turned yellowish. A piece of moistened cotton was placed over the edge of the leaflet in such a way that it causes the leaflet to be slanted, thereby enabling the mite to secrete and deposit its silk under an angle. After copulation, a single egg was transferred to each replicate (n = 30). The Petri dishes were incubated under constant temperature (15-40 °C, at 5-degree intervals), 50-60% r.h., and L16:D8, to assess progress in development.

Immature dates (Khalal stage). Plastic containers with a cover of 10 cm in diameter and 3.5 cm deep were used for mite rearing (Fig. 2). Two circular holes of 2 cm diameter were drilled in each cover and the container was filled with moistened sand. An immature yellow stage date (Khalal stage) was inserted in each hole and 2/3 of the fruit was exposed. A ring of Vaseline oil applied to the date's base served to prevent the mites from escaping. A single egg was transferred to each fruit and incubated at 35 °C, 50-60% r.h., and L16:D8 (n = 30).

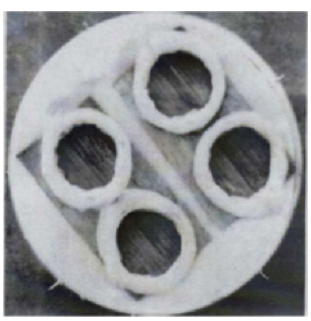

Figure 1 Rearing of *Oligonychus afrasiaticus* on date palm leaflet.

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In the laboratory the life cycle, fecundity, pre- and post-oviposition periods, and longevity of female and male mites were assessed. Also, the offspring sex ratio was determined by following the eggs laid by each female at each temperature, and counting the females and males resulting from these eggs. Sex ratio was calculated among all offspring that reached maturity.

A completely randomized design was used and the data were analyzed by ANOVA followed by Duncan's Multiple Range Tests (Duncan, 1955; Steel & Torrie, 1960).

RESULTS AND DISCUSSION

Overall, the duration of all active and quiescent stages decreased with increasing temperature (Table 1). Egg incubation took 7.6 days at 20 °C, significantly longer than at 25, 30, or 35 °C. Freshly laid eggs, 0.5-1 day old, were negatively affected at 15 and 40 °C. They shriveled and died, or reached the pre-larval stage and then failed to hatch and died. A temperature as high as 36 °C was also harmful for the desert mite *Tetranychus desertorum* Banks, especially at low humidity where it led to decreased hatchability (Carey & Bradley, 1982).

The average developmental time of the active larval stage was 3.8 days at 20 °C, significantly longer than at the other temperatures. In general, the quiescent larva lasted shorter than the active larva (viz., about half as long), in agreement with results reported for the two-spotted spider mite *Tetranychus urticae* Koch, the strawberry mite *T. turkestani* (Ugarov & Nikolskii), and the Pacific mite *T. pacificus* McGregor on cotton (Carey & Bradley, 1982).

Active and quiescent protonymph stages period were 2.8 and 1.5 days at 20 °C respectively and this results doesn't show any significant differences at 25 and 30 °C (Table 1). The average time spent in the active and quiescent protonymphal stage peaked at 4.3 days at 20 °C, significantly longer than at the other temperatures. The response of the deutonymhal stage to temperature was similar to that of the protonymph.

Development from egg to adult at 20 °C required 21.5 days, significantly longer than at the other temperatures (Table 1). Egg-to-adult development was significantly fastest at 35 °C. Also Dhouibi (2000) found that the optimal temperature for development of *O. afrasiaticus* was 35 °C. The longest period for development of males and females was at 20 °C (19.9 and 22.4 days, respectively), significantly longer than at the other temperatures (Table 2). Also males of the grass mite *O. pratensis* (Banks) develop 1-2 days faster than the females (Tan & Ward, 1977).

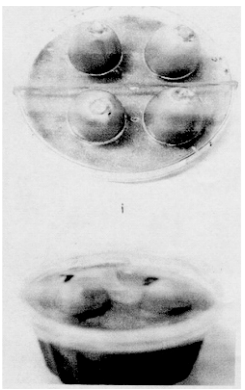

Figure 2 Rearing of *Oligonychus afrasiaticus* on immature date fruit (yellow Khalal stage).

Table 1 Effect of constant temperature on mean (±SE) developmental time (days) of immature *Oligonychus afrasiaticus*.

Temperature (°C)	Egg incubation	Larva			Protonymph			Deutonymph			Egg-adult
		Active	Quiescent	Total	Active	Quiescent	Total	Active	Quiescent	Total	
20	7.6±0.71a	3.76±0.5a	1.52±0.42a	5.29±0.68a	2.76±0.53a	1.55±0.41a	4.31±0.65a	2.88±0.61a	1.62±0.41a	4.5±0.62a	21.55±1.54a
25	5.23±0.68b	2.3±0.81b	1.18±0.29a	3.4±0.86b	1.94±0.57ab	1±0.17ab	2.94±0.75b	2±0.79ab	1.03±0.27ab	3.3±0.85ab	14.33±1.77b
30	3.9±0.66bc	2.5±0.5b	0.95±0.22a	3±0.52b	1.6±0.33ab	0.93±0.25ab	2.53±0.39bc	1.87±0.34ab	0.83±0.24ab	2.7±0.4bc	12.1±1.24b
35	2.67±0.47c	1.28±0.4b	0.68±0.24a	1.95±0.44b	0.68±0.2b	0.6±0.2b	1.28±0.25c	0.83±0.24b	0.58±0.19b	1.42±0.25c	7.28±0.71c

Means within a column followed by different letters are significantly different (Duncan's multiple range test, P<0.05).

Table 2 Effect of constant temperature on mean (±SE) egg-to-adult developmental time (days) and longevity (days) of adult male and female *Oligonychus afrasiaticus*.

Temperature (°C)	Male		Female	
	Egg-to-adult	Longevity	Egg-to-adult	Longevity
20	19.93±0.68a	29.14±1.96a	22.36±1.16a	33.23±2.61a
25	13.14±0.99b	18.71±2.19b	15.35±1.63b	20±2.72b
30	11.4±1.36b	11.4±1.36c	12.45±1.01b	14.3±1.74bc
35	6.57±0.32c	10.14±1.64c	7.64±0.64c	11.55±2.02c

Means within a column followed by different letters are significantly different (Duncan's multiple range test, P<0.05).

Table 3 Effect of constant temperature on mean (±SE) pre-oviposition period (days) and oviposition rate (no. eggs/female) of *Oligonychus afrasiaticus*.

Temperature (°C)	Pre-oviposition period	Oviposition rate
20	3.27±0.5a	12.54±1.12b
25	1.9±0.37b	17.5±2.17b
30	1.55±0.35b	19.5±2.92b
35	0.68±0.24b	27.2±5.05a

Means within a column followed by different letters are significantly different (Duncan's multiple range test, P<0.05).

Table 4 Offspring sex ratio of *Oligonychus afrasiaticus* at various constant temperatures.

Temperature (°C)	Total no. eggs	No. males	No. females	Male : female
20	153	37	116	3.14 : 1
25	163	46	117	2.54 : 1
30	181	56	125	2.23 : 1
35	253	94	159	1.69 : 1

Table 5 Effect of different food and rearing methods on mean (±SE) developmental time (days) of *Oligonychus afrasiaticus* at 35 °C.

Developmental stage	Yellow Khalal	Leaflet
Egg	1.87±0.39	2.67±0.47
Larva	2.08±0.40	1.95±0.44
Protonymph	1.38±0.28	1.28±0.25
Deutonymph	1.5±0.18	1.42±0.25
Egg-to-adult	6.77±0.67	7.28±0.71

Means within a column did not differ significantly (Duncan's multiple range test, P>0.05).

Females and males live shorter with increasing temperature; at 20 °C they lived 29-33 days, but at 35 °C this was only 10-12 days (Table 2). In general, males lived 1.5-4 days shorter than females. This agrees with the findings for the red tea mite *O. coffeae* (Palevsky et al., 2003).

The longest pre-oviposition period was 3.3 days at 20 °C, significantly longer than at higher temperatures (Table 3). The number of eggs per female increased from 12.5 at 20 °C to 27.2 at 35 °C. Temperature also affected the sex ratio of *O. afrasiaticus*: the ratio of females to males ranged from 3.1 (20 °C) to 1.7 (35 °C) (Table 4). Thus, the proportion of males in the offspring increased with increasing temperature. Palevsky et al. (2003) stated that the sex ratio of *O. afrasiaticus* was highly female-based, usually being above 0.85 (proportion of females).

There were no significant differences in the period needed for life cycle completion between the two rearing methods (Table 5), suggesting that palm leaflet and immature fruit did not differ as a food source for *O. afrasiaticus*.

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Ecological Acarology: Associations with Insects

Habitat selection in the bug *Pyrrhocoris apterus*: Does it minimize the risk of being parasitized by the ectoparasitic mite *Hemipteroseius adleri*?

Rostislav Zemek & Radomír Socha

Biology Centre of Academy of Sciences of the Czech Republic, Institute of Entomology, Branišovská 31, 370 05 České Budějovice, Czech Republic. E-mail: rosta@acarus.entu.cas.cz

The firebug *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae) is a polyphage whose European populations mainly feed on the seeds of trees (*Tilia cordata*, *T. platyphylos*, *Robinia pseudoacacia*) or plants such as mallows (Malvaceae). It is frequently found on the ground near host trees or at the base of their trunks. During summer, however, we observed many individuals to climb up the trees. Movement of the bugs to upper parts of the trees and their occurrence in canopies have not been reported so far and there is also no clear explanation for this behavior. We formulated the hypothesis that *P. apterus* switches habitat to find optimal abiotic conditions for its development and to avoid the ectoparasitic mite *Hemipteroseius adleri* (Otopheidomenidae). To test this hypothesis we collected samples of adult males and females of the firebug from linden tree trunks at >1 m above the ground (tree habitat) and from the ground near these trees (ground habitat). The insects were individually preserved in vials with ethanol and examined using a microscope. Eggs, larvae, nymphs, and adults of *H. adleri* found on each specimen were counted. The results revealed that the bugs dwelling on linden trees were nearly devoid of parasitic mites (only 5% were parasitized), whereas 29% of those collected on the ground were parasitized. The mean numbers of *H. adleri* found on the respective groups were clearly different. We also tested whether *P. apterus* is more likely to be infested by *H. adleri* during its final larval ecdysis. Laboratory experiments showed no differences between the infestation by the mite in adult bugs and adults emerged from the last instars. We conclude that selection of a tree habitat in *P. apterus* might contribute to reducing the risk of being parasitized by *H. adleri*.

Key words: *Hemipteroseius adleri*, *Pyrrhocoris apterus*, insect parasites, avoidance behavior, Otopheidomenidae

The firebug, *Pyrrhocoris apterus* (L.) (Heteroptera: Pyrrhocoridae), is widely distributed in the Palaearctic region. It can have two generations per year in the Czech Republic during warmer years and in warmer places (Socha & Šula, 1992). It produces flightless individuals of long-winged and short-winged morphs (Socha, 1993) whose reproduction and wing length pattern are controlled by photoperiod and temperature (Hodek, 1968; Honěk, 1976; Socha, 2001). The two wing morphs of this bug differ in their life-history strategies, including several physiological and behavioral traits, e.g., the length of pre-oviposition period (Honěk, 1985; Socha & Šula, 1996), feeding and digestive enzyme activities (Socha et al., 1997, 1998), walking and dispersal activity (Socha & Zemek, 2000a, 2003), and mating success (Socha & Zemek, 2004; Socha, 2004, 2006).

The wing polymorphism in *P. apterus* represents a case where flightlessness has secondarily evolved (Socha & Zemek, 2000b) and where the mode of movement changed from flight to walking in individuals of both wing morphs (Socha & Zemek, 2000a), despite the fact that flightless long-winged adults of this species maintain developed flight muscles at the time of ambulatory dispersal (Socha & Šula, 2006). Walking is a common mode of dispersal not only in this wing-dimorphic bug, but also in some other insect species, e.g., apterous and alate morphs in aphids (Hodgson, 1991). Persistence of non-functional macropterism in *P. apterus* is conjectured to be related to the prevention of immediate oviposition in early-moulted macropterous females and establishment of a second generation, which would otherwise be abolished near the end of the season (Honěk, 1995).

In central Europe, both larvae and adults of *P. apterus* are frequently found on the ground in the vicinity or at the bases of linden trees (*Tilia* spp.) whose seeds serve as food. However, we observed that during the hot summer months many last-instar larvae and adults of this species congregate on the higher parts of the linden trees (*Tilia cordata* Mill. and *T. platyphylos* Scop.). Movement of the larvae and adults to the upper parts of trees was not reported before for this species. It might allow the bugs to find new linden seeds as fresh food in the late summer and avoid overheating of their bodies by solar radiation. This assumption is supported by findings that behavioral regulation of body temperature often has crucial significance in insect life and that various insects, including the bug *P. apterus*, regulate body temperature by seeking places with optimal temperatures (Cloudsley-Thompson, 1962; Heath & Wilkin, 1970; Humphreys, 1974; Honěk & Šrámková, 1976). However, the other possible reason for last-instar larvae and adults of *P. apterus* to climb up the trees is to avoid the mite *Hemipteroseius adleri* Costa that was reported to be a parasite of this bug (Lewandowski & Szafranek, 2005). In this case, the upward movement of last-instar larvae and newly emerged adults of *P. apterus* could eliminate or at least decrease the probability of their contact with bugs already infested with these mites which mostly occur on the ground in the vicinity of trees. Testing of this hypothesis is the main objective of the present study.

MATERIALS AND METHODS

Field study

To assess ambulatory migration up into the trees and the degree of infestation by *H. adleri*, a wild *P. apterus* population was studied in a large park close to České Budějovice, South Bohemia (49°N; Czech Republic) during August 2005. The colonies under study occupied *T. cordata* trees and their vicinity. Bugs occurring on the trunk of a selected linden tree were located using a binocular and the height above ground

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at which they were found was measured using a hand-held laser meter DISTO classic (Leica Geosystems).

To test the hypothesis that *P. apterus* switches habitat to avoid parasitization by mites, the samples of adult males and females of this bug were collected at several places in the park. They were divided into two groups: the *tree habitat* group, i.e., bugs collected from linden tree trunks at a height exceeding 1 m above the ground, and the *ground habitat* group, i.e., bugs collected from the ground in the vicinity of these trees. The insects were individually preserved in Eppendorf vials with 75% ethanol and later examined using a dissection microscope. The numbers of eggs, larvae, nymphs, and adults of mites found on each specimen were recorded. Temporal slide mounts were prepared from the adult mites using lactic acid and mites were identified according to the key by Wainstein (1972).

Laboratory experiments

Laboratory experiments were carried out to test whether *H. adleri* can more easily parasitize immobile moulting stages of *P. apterus*. For this purpose we used a mite-free laboratory stock culture of *P. apterus* maintained at the Institute of Entomology (České Budějovice). One juvenile female of the last (fifth) instar before final larval ecdysis or one adult female of *P. apterus* were transfered into a Petri dish and two adult females of *H. adleri* were added. Two linden seeds were provided as food and water was supplied in a glass vial plugged with cotton wool. The dishes were sealed with Parafilm and placed into a climatized cabinet at 26±1 °C and 18L:6D photoperiod. The bugs were inspected after 5 days when all juveniles moulted into adults. The parasitic mites found under their wings were recorded.

RESULTS AND DISCUSSION

We observed that both juveniles and adults of *P. apterus* occupied the whole linden tree trunk and we found them also on twigs (Figs. 1 and 2). According to our knowledge, the many papers dealing with *P. apterus* report that this species occurs on the ground in the vicinity of linden trees or on the base of their trunks, but no occupation of canopies has been reported. Movement of *P. apterus* to tree canopies is not an unusual phenomenon as we found these bugs frequently on linden but also other trees at various localities during summer and autumn.

The parasitic mites found on the bugs examined were identified as *H. adleri*. About 29% of bugs collected on the ground were parasitized, whereas the bugs collected on linden tree trunks were nearly devoid of parasitic mites (only 5% parasitized). Lewandowski & Szafranek (2005) reported that the prevalence of infested *P. apterus* collected in the vicinity of *R. pseudoacacia* trees fluctuated during the year from 2 to 100%. The difference between the percentages of infested *P. apterus* females in the ground and tree groups was highly significant, but the same difference between the percentages of male bugs was not significant (Table 1).

Table 1 Percentage of males and females of *Pyrrhocoris apterus* infested by *Hemipteroseius adleri* at ground and tree habitats.

Sex	Ground		Tree		P-value
	%	n	%	n	
Females	33.3	39	0	40	<0.0001
Males	24.3	37	10	40	0.13

Fisher's exact test (two-tailed).

Mean numbers of mites per infested bug differed between groups (Fig. 3) with the highest number found in females collected on the ground. The differences were statistically significant (one-way ANOVA: $F_{2,23} = 4.43$, $P = 0.024$). According to Lewandowski & Szafranek (2005), the maximum number of mites (all stages) found on one insect was 178, whereas in our study this was 81 mites for a female collected on the ground.

Laboratory experiments revealed that artificial infestation was successful in 45.5% of adult *P. apterus* females (n = 33) and in 43.6% of females (n = 39) which moulted from the last instar to the adult stage during the experiments. Since

Figure 2 *Pyrrhocoris apterus* on a *Tilia cordata* trunk: colony of bugs on trunk several meters above the ground (top left), adult bugs on leaves (top right), bugs in bark crevices (bottom left), and bug completing final larval ecdysis (bottom right).

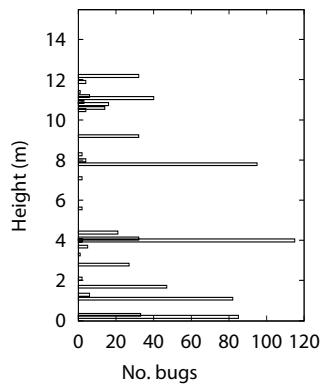

Figure 1 Distribution of *Pyrrhocoris apterus* on a *Tilia cordata* trunk. The height indicates the location of bugs on the tree measured from the ground.

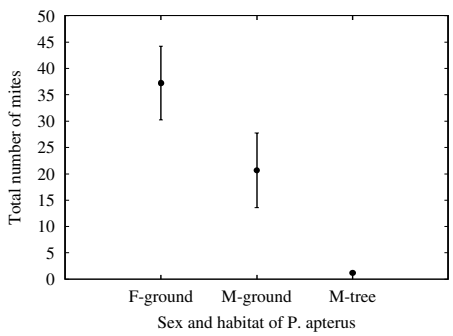

Figure 3 Mean number of *Hemipteroseius adleri* per infested individual of *Pyrrhocoris apterus*. F = females, M = males. The category F-tree is omitted because all females collected on trees were uninfested. Vertical bars indicate standard error of the mean.

the difference is not statistically significant (Fisher's exact test, P = 1.0), we do not reject the null hypothesis that there is the same probability of being parasitized by *H. adleri* during adulthood and during final larval ecdysis of the bug. Thus, even if the ecdysis takes several hours including sclerotization during which the bug is more vulnerable, adults do not seem to be more protected or able to defend themselves against mite infestation.

We conclude that by selection of a tree habitat during summer *P. apterus* incurs a lower risk of parasitization by *H. adleri*. Further research is currently on-going where we test how parasitic mites spread within *P. apterus* populations.

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Mites associated with concealed and open nests of *Apis cerana indica* in Kerala, South India

M.A. Haq, K. Sumangala[1] & N. Ramani
Division of Acarology, Department of Zoology, University of Calicut, Malappuram, India-673635.
E-mail: haqzas@yahoo.co.in; and [1]School of Engineering and Applied Sciences, Rai University, Bangalore, India-83

Apis cerana indica, the common Indian honeybee, constructs nests in dark places, like hollow tree trunks, soil crevices, shaded roofs, subterranean holes, etc. In contrast to these concealed nests, also some open nests were found in the area of Malabar. Here, parallel structures of 6-8 combs were observed hanging from branches of *Anacardium occidentale* and *Bambusa gigantea* trees. An open nest may have advantages to the bees in that they suffer less disturbance from ectoparasitic and phoretic mites. Moreover, they are less resticted in nesting space than when their nests are concealed in hollow trees, termitaria, soil cracks, etc. It may also have advantages for apiculture. Beekeepers and apicultural researchers can regularly observe colony organization, mobilization, propagation, parasitic invasion, and parasite resistance. Moreover, open nests, being larger, may produce more honey and facilitate extraction of honey without much destruction of bees and brood cells. A survey of mites was carried out on natural colonies of *A. cerana indica*, covering various districts of Kerala.

Key words: *Apis cerana indica*, phoretic mites, habitat switching

Apis cerana indica is a species of honeybee that is traditionally used for apiculture in India. The species exhibits a broad latitudinal and altitudinal distribution pattern. Accordingly, natural hives of this bee can be encountered throughout South East Asia, including areas at sea level and at altitudes of approximately 1,500 m. The Indian honeybee is a typical cavity nesting type of bee, preferring hidden localities. Natural hives of this bee usually comprise hollow tree trunks, abandoned termitaria or subterranean holes of rodents, etc. The species builds multiple combs arranged parallel to one another, hanging down from the roof of the nesting cavity. Depending on the size of the cavity, the bees build 2-10 combs within a hive, under natural conditions. Bees of this species are not so aggressive and hence suitable for domestication. Bee keepers in India have been exploiting these habits of the species for domesticating the colonies in artificial hives like hollowed-out logs, earthen pots, and wooden boxes provided with chambers and vertical frames.

Apis cerana indica exhibits swarming and absconding behaviour in both natural and artificial hives. Swarming is mainly concerned with colony propagation, while it also helps to ease problems like over crowing and scarcity of food. Peak swarming activity coincides with the early half of the summer season (February, March). This is common in both natural and domesticated hives in south India. However, the species is known to exhibit absconding behaviour. Often this activity is found associated with a colony crisis caused by parasites, pathogens, predators, scarcity of food, etc. During absconding, the bees abandon their hive in search of new habitat. Mites have been established as one of the dominant groups of associating organisms inhabiting bee hives (Eickwort, 1994; Kumar & Kumar, 1995; Sammataro et al., 2000; Sumangala & Haq, 2001).

The extent of the association of mites with bees varies from simple saprophagy to complex parasitism. Ectoparasitism, endoparasitism, brood parasitism, cleptoparasitism, phoresy, and saprophagy represent some of the major aspects of mite-honeybee interaction (Hirst, 1921; Definado-Baker & Baker, 1982; Eickwort, 1994; Sumangala & Haq, 1996, 1999, 2001; Haq, 2001). Of these, ectoparasites and brood parasites constitute the most injurious categories. However, the phoretic and cleptoparasitic mites often create problems to bee mobility and stored foods, particularly during the rainy season when the supply of food is limited in bee hives.

MATERIALS AND METHODS

A survey was carried out on natural colonies of *A. cerana indica*, covering various districts of Kerala. Bees from various colonies were brought to the laboratory and examined under a stereo-microscope for the presence of phoretic mites. The collected mites were preserved, cleared, and mounted in Hoyer's medium and identified. Data were also collected on the impact of these mites on the behaviour of *A. cerana indica* and its possible role in triggering a habitat switch under natural conditions.

RESULTS AND DISCUSSION

Often, high population densities of *Pseudacarapis indoapis* (Lindquist), *Caloglyphus oudemansi* (Zachvatkin), and *Neocypholaelaps stridulans* (Evans) were observed in domestic hives of *A. cerana indica* during the rainy season, from May to July (Table 1). The frequency of absconding among the colonies showed a peak during the same period. A similar increase in population density of the same three species was observed both in the combs and on their bee hosts during the monsoon months, May to July. However, the *P. indoapis* population peaked in August. In the current investigation, 11 out of 120 colonies of *A. cerana indica*, deserted

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Table 1 Population fluctuation of phoretic mites on *Apis cerana indica* and their hives. Numbers are average no. mites on eight combs in a super chamber and, in parentheses, the average no. mites per 10 bees (same colony).

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Pseudacarapis indoapis	11 (2)	12 (4)	15 (6)	16 (6)	20 (9)	25 (14)	28 (15)	29 (18)	21 (8)	19 (7)	13 (6)	10 (5)
Caloglyphus oudemansi	8 (3)	9 (6)	12 (8)	13 (8)	18 (10)	16 (6)	11 (5)	10 (5)	9 (3)	6 (2)	5 (3)	3 (0)
Neocypholaelaps stridulans	15 (3)	19 (4)	23 (6)	28 (9)	32 (10)	38 (8)	31 (6)	22 (5)	18 (4)	12 (2)	9 (3)	6 (1)

their domesticated hives during June to August. However, no incidence of absconding was recorded during the remaining part of the year. This suggests that the phoretic and cleptoparasitic mites induce absconding among colonies, when mite population density crosses a critical level.

While investigating the distribution of natural hives of *A. cerana indica* in Kerala, a peculiar nesting habit was observed in Kannur, Northern Kerala. Unlike the common concealed nests, this colony was found settled on one of the branches of *Anacardium occidentale* (cashew). Parallel constructions of six combs were found hanging down from one of the horizontal branches of the tree (Fig. 1). An extensive survey carried out throughout the state revealed the occurrence of such a nest in two other localities in the Malabar area. These observations suggested that *A. cerana indica* has an alternative nesting strategy to the commonly occurring construction of a concealed nest. The open nest strategy may have the advantage of minimising mite infestation within the nest, because the mite fauna associated with open-nest-making bee species has been shown to be much poorer than that of concealed nesting bees (Eickwort, 1994). Mites in open nests may suffer from exposure of the nest to sun, wind, and rain. Although the bees may also suffer from these abiotic factors, they have the advantage of having less restrictions on nest space available compared to others nesting in tree hollows, termitaria, subterranean holes, etc.

Figure 1 Six parallel combs of an open nest of *Apis cerana indica* on *Anacardium occidentale*.

These observations on a different strategy of nest building may be useful to develop a new method of apiculture by using open nests that meet less restrictions on space and will ultimately produce more honey. Perhaps apicultural practices have been based too much on observations made in concealed nests. In any case, open nests of *A. cerana indica* facilitate direct observation and manipulation of all colony processes, such as colony settlement, growth, propagation, comb construction, honey production, parasitic invasion, swarming tendency, etc. Open nests also facilitate extraction of honey without much destruction of bees and brood cells. However, much of the success of open nests will depend on the risk of predation and the impact of abiotic and biotic factors, including parasites other than mites.

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Ecological Acarology: Invasive Species

Tracking the colonisation history of the invasive species *Varroa destructor*

Maria J. Navajas
Institut National de la Recherche Agronomique, Centre de Biologie et Gestion des Populations, Campus Baillarguet, CS 30016, 34988 Montferrier, France. E-mail: navajas@ensam.inra.fr

The development of molecular tools and the theory of invasion genetics have stimulated interest in the study of the invasion of new environments or new hosts by alien species. This approach has recently been applied to better understand the colonisation history of *Varroa destructor*, an invasive parasitic mite that has dramatically expanded its geographical distribution area after it shifted from its original host, the Asian cavity-nesting *Apis cerana*, to the honey bee *A. mellifera* during the last century. Varroa is currently considered as the major pest of honey bees in most parts of the world. Initial work found little evidence of genetic variability in *V. destructor*. More recent work, taking advantage of the sequencing of the mite's complete mitochondrial (mt) genome, made it possible to define specific PCR primers for amplifying long DNA fragments covering different mitochondrial genes. Mitochondrial sequences (a total of 2,700 nucleotides) display substantial polymorphism. In total 20 haplotypes were identified, representing nucleotide diversity of 0.75%. Interestingly, six of them were detected in mites infesting *A. mellifera* and represented a nucleotide diversity of 0.4%. This contrasts with the remarkable homogeneity of the two haplotypes (known as the Korean and Japan haplotypes) that have invaded *A. mellifera* worldwide. It thus appears that although only two *Varroa* genotypes have managed to colonise *A. mellifera* worldwide (presumably carried by humans), multiple shifts from its natural host *A. cerana* to this species have occurred in Asia. This and other reports of variation in *V. destructor* in relation with the invasion process are reviewed and discussed, including the threatening possibility that additional genotypes might spread into *A. mellifera* in the future.

Key words: Mitochondrial haplotypes, mircoratellites, genetic markers, invasion genetics, *Varroa destructor*

In recent decades, invasive species have appeared as one of the major ecological problems related to the extension of human activities and global changes. These species can have strong environmental and economic impacts and are clearly identified as a threat to ecosystems, habitats, and species (Pimentel, 2000; Pimentel et al., 2005). To judge by the increasing number of publications in this field, they are leading to growing mobilisation of efforts by international research.

As worldwide trade increases, the number of both accidental and international exotic introductions is likely to increase, in such a way that invasive species are recognized as a leading threat. To limit the impact that exotic species have on natural and man-made communities, efforts need to be made in understanding the major mechanisms of the invasion events (Keane & Crawley, 2002; Facon et al., 2006). Whereas ecological research on exotics has received considerable attention (e.g., Sax et al., 2005), evolutionary aspects of invasions have remained relatively unexplored. Still, the evolutionary genetics approach may provide insight into invasion mechanisms (Lee, 2002), and may be used in particular to trace the colonisation routes of the alien species, help to combat them, and limit new invasions. The use of this approach relies on the availability of genetic markers.

Evolutionary genetics has made a substantial contribution to better understanding the colonisation history of *Varroa destructor*, an ectoparasite of the honey bee. This invasive mite first performed a host shift before considerably extending its distribution area in the last century (see below). This invasion and the subsequent rapid expansion of the mite in the honey bee has attracted much attention and prompted numerous studies in the past decades. The biology of *Varroa* and its negative economic impact on apiculture is fairly well known, but the invasion process of this new parasitic mite of the honey bee has only recently started to be better understood. In particular, recent studies on the mite genome enabling the development of new molecular markers (Anderson & Trueman, 2000; Evans, 2000; Navajas et al., 2002; Solignac et al., 2003) has made it possible to address new questions and shed new light on the colonisation routes of the parasite.

I briefly review early studies on genetic variation and species identification issues. I then present in some detail the results of the recent search for new genetic markers which have been applied to estimate genetic variation among *V. destructor* samples worldwide. Rather than an exhaustive review of the genetic variation of the species, I discuss the various features that allow conclusions on *V. destructor* invasion. Finally, I discuss some of the emerging threats that the species represents for beekeeping.

Origin and spread of *Varroa destructor*, an almost cosmopolitan pest of *Apis mellifera*

Varroa destructor has dramatically extended its geographical distribution since it shifted from its original host during the last century (Matheson, 1996). This obligatory ectoparasite of bees of the genus *Apis* was discovered by Oudemans in Sumatra on the Asian cavity-nesting *Apis cerana* (Oudemans, 1904). For beekeeping reasons, the western bee *A. mellifera* was introduced in Asia in 1877 (Saiki & Okada, 1973) and this led to the establishment of a zone of sympatry between the two bee species that had hitherto been allopatric (Ruttner & Maul 1983). However, *Varroa* was only reported on *A. mellifera* for the first time in 1957 in the Philippines (Delfinado, 1963). The progressive invasion of *A. mellifera* colonies in the rest of the world was then accomplished to a considerable extent by 'human-mediated movements'. Two main invasion routes thus emerged: (1) from Japan to Paraguay in 1971 and Brazil in 1972 (De Jong et al., 1982; De Guzman et al., 1998), and then to North America in 1987 (De Guzman et

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al., 1997); (2) from eastern to western Russia, certainly via the trans-Siberian rail facility (Oldroyd, 1999), and then to Bulgaria in 1972 and Germany in 1977 (Griffiths & Bowman, 1981). Spread continued to the rest of Europe and also to the USA, where the two *Varroa* origins from the two world invasion pathways met (De Guzman et al., 1999). An opportunistic host-shift has thus made *V. destructor* an almost cosmopolitan parasite of *A. mellifera.* This mite is currently considered as the major pest of honey bees in most parts of the world because of the losses it causes in apiculture (Sammataro et al., 2000).

On its original host, *V. destructor* is considered to be an insignificant pest. It did not become a concern to beekeepers until it made the cross-species jump to *A. mellifera* (Cobey, 2001). *Apis cerana* has probably coevolved with the parasite and adapted to keep the mite under control by developing hygienic behaviour, and in particular behaviour consisting of the cleaning of worker bee cells before operculation (Rath, 1999).

Varroa destructor infesting *Apis mellifera*: a new species

Varroa mites were first considered as belonging to the same species on both hosts. Recent work based on mitochondrial DNA sequences (mtDNA) has made it possible to revise the systematics of the various close species of the genus *Varroa* (Anderson & Trueman, 2000). The sequencing of a 458 pair bases (pb) mtDNA fragment coding a portion of the gene *COI* made it possible to distinguish between two species, *Varroa destructor* Anderson et Trueman and *Varroa jacobsoni* Oudemans (Acari: Varroidae), that had previously been grouped under the name *V. jacobsoni* (Anderson & Trueman, 2000). Eighteen haplotypes were determined on the basis of sequences of this fragment. Among them, seven belong to *V. destructor* and two of these, the Korean and Japan haplotypes (named after the countries in which they were detected for the first time on *A. cerana*), are the most widely distributed in the world (Anderson & Trueman, 2000; Solignac et al., 2005). Both haplotypes are present in North America (De Guzman et al., 1999). The marked consanguinity of the pseudo-arrhenotokous reproductive mode in *Varroa* (de Ruijter & Pappas, 1983; Martin et al., 1997) may have substantial effects on the genetic variability of *Varroa* populations in the world.

Genetic variation of *Varroa destructor* infesting *Apis mellifera*

Initial searches yielded little evidence of genetic variation of *V. destructor* (Anderson, 2000). The first study on 17 allozyme loci revealed a remarkable absence of polymorphism in the *Varroa* population of Europe sampled on *A. mellifera* (Biasiolo, 1992). Analyses of RAPD markers showed the same absence of polymorphism in populations from Europe and the USA collected on *A. mellifera*, and in Malaysia in mites collected on *A. cerana* (Kraus & Hunt, 1995).

An extensive search for genetic variation among *V. destructor* infesting *A. mellifera* worldwide has only recently been completed. Taking advantage of the sequencing of the complete mitochondrial genome of *V. destructor* (Evans & Lopez, 2002; Navajas et al., 2002), specific PCR primers were defined to amplify six fragments totalling 5,185 nucleotides and covering different mitochondrial genes. The fragments of the mitochondrial genome and the technical details are presented in Solignac et al. (2005). Using these sequences, the diversity was examined in mites originating from an extensive part of the species distribution area (12 geographic regions distributed among four continents). These sequences virtually showed a total absence of polymorphism within each of the two mitochondrial types present in *Varroa* that had invaded *A. mellifera* (Korean and Japan haplotypes). Likewise, the microsatellite loci studied for the same samples showed great genetic homogeneity in each of the two mitochondrial haplotypes, thus revealing that each characterised a 'clone'. The absence of genetic variation, despite the use of highly variable microsatellite markers, was astonishing but interesting. It shows that the presence of each of the two haplotypes corresponds to a single host capture event followed by the rapid spread of the 'clone'. The microsatellite markers also gave a surprising result: the two *Varroa* clones present on domestic bees seem to be almost completely isolated reproductively (Solignac et al., 2005).

Genetic variation of *Varroa destructor* infesting *Apis mellifera* and *Apis cerana* in Asia

The practically clonal structure of the *V. destructor* population infesting *A. mellifera* probably results from a severe bottleneck that probably occurred at the time of the host shift. The absence of a structure of populations indicates that it was not after the host transfer. Also, the dramatic increase in population size of the species may have prevented the erosion of variability: the mite can attain 10-15 generations per year in temperate regions. The hypothesis of a bottleneck that eroded the genetic variability of the species assumes that the latter is variable in its area of origin. This has been shown by Anderson & Trueman (2000). Using a short fragment of the mtDNA, these authors found six haplotypes of *V. destructor* on *A. cerana* in Asia. More recently, the search for genetic variation was performed in greater depth by using sequences covering a much larger portion of the mitochondrial genome (four fragments of a total of 2,700 nucleotides analyzed; Fig. 1), in a large sample of both *A. mellifera* and *A. cerana*. A total of 21 *V. destructor* females originating from Asia were studied. Among them, 14 mites infested *A. cerana* (originating from China: Beijing, Nanchang, Yunnan, and Guangdong; Japan: Tokyo, Machida, and Shikoku; Vietnam: Hanoi; Thailand: Chiang Mai) and seven infested *A. mellifera* (from Taiwan: Taichung; Thailand: Chang Mai; Vietnam: Hanoi; Japan: Tokyo; China: Yunnan; Korea: Seoul; Russia: Vladivostok). Mitochondrial sequences displayed significant genetic diversity with six of a total of 20 haplotypes detected on *A. mellifera* and 14 on *A. cerana*, representing a total nucleotide diversity of 0.75% (0.4% in mites infesting *A. mellifera*).

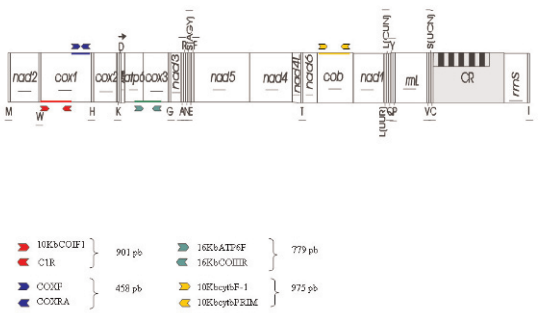

Figure 1 Gene order of the mitochondrial DNA genome of *Varroa destructor*: GenBank accession number AJ493124 (Navajas et al., 2002). The four pairs of PCR primers used to amplify four DNA fragments (see text) are positioned on the molecule and their size indicated.

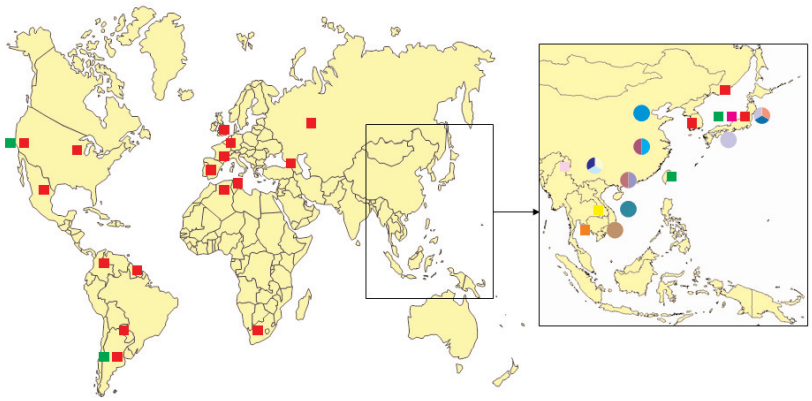

Figure 2 Geographical distribution of the different mitochondrial haplotypes of *Varroa destructor* harboured by *Apis mellifera* (squares) and by *Apis cerana* (circles). Each haplotype is depicted by a colour. Red and green squares stand for the Korean and Japan haplotypes of *V. destructor*, respectively.

The distribution of this variability is shown in Figure 2. This result contrasts with the remarkable homogeneity detected in mites infesting *A. mellifera* in other regions of the world (Solignac et al., 2005). It is to be expected that much more variation in *V. destructor* will be found.

Loss of genetic diversity, founder effects, and host shifts

Colonization events are often accompanied by founder effects, in which the genetic diversity of a colonizing population is reduced compared to the source (Tsutsui et al., 2000). Alternatively, or in addition to this, genetic diversity could be reduced by post-colonisation selection in the novel niche (Lee, 2002). Although *V. destructor* is polymorphic in its area of origin in Asia, mites sampled on *A. mellifera* in other areas worldwide are extremely homogeneous (Solignac et al., 2005). These contrasting results match the hypothesis of a bottleneck at the time of the host shift that reduced genetic variation of *Varroa* on the new host. This reduction probably occurred at the very beginning of the invasion of the Western honey bee, because population size of the parasite subsequently increased in such proportions (annual 12-fold increase in mite numbers; Martin. 1998), that it is hardly compatible with post-colonisation erosion of variability.

It thus appears that although only two *Varroa* genotypes have succeeded in colonizing *A. mellifera* worldwide (presumably carried by humans), multiple shifts from its natural host to this species have occurred in Asia. Thus, we may fear that the future will bring additional genotypes to shift to *A. mellifera* and spread over a larger region.

Invasion pathways

Although there is no clear geographical structure of mitochondrial variation of *V. destructor* in Asia, five of the six haplotypes close to that previously identified as the Japan haplotype have been detected in Japan, suggesting this area to be the potential origin of the Japan type.

Although our results do not allow identification of the precise geographic origin of the invasive mites that have colonized *A. mellifera*, the data do shed light on the invasion process. It is interesting that despite the worldwide presence of the Korean haplotype on *A. mellifera*, this type was found in Asia only in Korea (Seoul) and Russia (Vladivostok). The other *A. mellifera* samples harboured different Korean haplotypes (in Nanchang, Hanoi, and Xishunagbanna).

Whether the mites that have colonised *A. mellifera* possess mtDNA haplotypes all originating from a discrete geographic region close to Korea and Japan, as has been suggested by Warrit et al. (2006), needs to be further investigated. However, the results presented here suggest that the Korean and Japan haplotypes that have so successfully invaded *A. mellifera* worldwide are not the most frequent haplotypes in Asia.

The *Varroa destructor* threat issues

Work on the characterisation of genetic variability of *V. destructor* is far from complete and the detection in Asia of a significant diversity of *Varroa* implies that much more work is needed to gain a full picture of the distribution of the diversity of this mite. High-resolution molecular analysis should give new insights and improve understanding of the process of *Varroa* invasion in *A. mellifera* populations which is thought to have started in the mid last century (Oldroyd, 1999). It also should provide an important basis for addressing new introduction issues and should ultimately form an integral component of attempts to evaluate their impact and the assessment of risk for apiculture. Bee mites have greater dispersal potential than most of the Acari, first through their host and then by humans who move beehives for trade and pollination purposes (Sammataro et al., 2000). As a result, the detection in Asia of new haplotypes of *V. destructor* on *A. mellifera* highlights the permanent possibility that a new *Varroa* type might expand on *A. mellifera* outside Asia and form a new threat to apiculture. These results call for an effort to better understand the nature of the host-parasite interactions that enable the successful parasitism of the bees by the mite. Technical innovations in genomics (e.g., microarray technology) offer many opportunities for exploring genetic architecture and gene expression patterns as for example to understand the bee/mite interactions (Navajas et al., 2008). Likewise, these innovative approaches should help to define the genetic characteristics of populations that have the capacity for range expansions.

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The rice mite *Steneotarsonemus spinki*, an invasive species in the Americas

Denise Navia[1], Renata Santos de Mendonça[1] & Ronald Ochoa[2]

[1]Embrapa Recursos Genéticos e Biotecnologia, Cx. Postal 02372, 70.770-900, Brasília, DF, Brasil.
E-mail: navia@cenargen.embrapa.br; and [2]Systematic Entomology Laboratory, USDA-ARS, BA, PSI, Building 05, BARC-West, 10.300, Baltimore Avenue, Beltsville, MD 20705, USA

The rice mite, *Steneotartsonemus spinki* Smiley, has been considered a serious rice pest in several tropical Asian regions since 1970. However, it was in the Americas that *S. spinki* assumed its largest economic importance. Yield losses in Central America have ranged from 30 to 90%. Until now, *S. spinki* has not been found in other (South or North) American countries. Damage caused by *S. spinki* infestations can be direct, as a consequence of toxin injection during feeding, or indirect, through the spreading of phytopathogens. At first, rice (*Oryza sativa* L.) was considered its only host plant, but in Costa Rica, colonies were observed completing their life cycle on the invasive *Oryza latifolia* Desv., indicating its potential to adapt to other hosts, especially *Oryza* species. There are indications that *S. spinki* can be disseminated via trade of rice seeds or naturally by wind, insects or birds. In Central America integrated management of rice crops, involves mainly cultural methods and resistant varieties. The introduction and establishment of *S. spinki* in South and North America would probably imply a drastic reduction of rice productivity and an immediate increase of agrochemical applications for pest control. Therefore, it is essential to reinforce the use of quarantine measures in order to avoid or to delay the introduction of the mite pest into South and North America. The development of a contingency plan for rice mite management in the Americas is of vital importance.

Key words: *Oryza sativa*, rice, biosecurity, New World, IAS, plant protection

Invasive Alien Species (IAS) refers to species that invade, proliferate, and spread in a new environment in ways that are destructive to ecosystems and to human interests (Convention on Biological Diversity, 2002). They can cause serious damage to agricultural systems and greatly decrease the level of biodiversity in the environment. The impacts of invasive species are among the most serious economic, social, and environmental threats of the 21st century (Wittenberg & Cock, 2001).

Phytophagous mites are prone to become IAS in agricultural systems because they are harmful to host plants, may act as vectors of plant diseases, quickly develop resistance to pesticides, are difficult to detect, are able to survive adverse conditions, reproduce parthenogenetically, and may rapidly adapt to new host plants. There are numerous historical examples of inadvertent introductions of phytophagous mites in new areas where they met favorable conditions to develop in the absence of their natural enemies, resulting in marked crop damage and negative social-economic impact (Navia et al., 2007).

During the last several years, the tarsonemid mite *Steneotarsonemus spinki* Smiley has become an important invasive phytophagous mite in the Caribbean and in continental Central America, where it has spread quickly and has caused serious damage to rice (*Oryza sativa* L.) crops. This mite is not yet known to occur in the most important rice growing countries in South and North America. In 2004, rice production in the Americas was 36,556,725 tonnes per year, about 6% of the world production. Of the total American rice production, 65% is from South America, 28% from North America, and 7% from the Caribbean and Central America. Actually, Brazil is the greatest producer in the Americas, with 13,140,900 tonnes per year, about 36%. In 2005, the area of rice harvested in South America was 6,043,541 ha and in North America it was 1,361,380 ha (FAO, 2005). Thus, the invasion of the rice mite represents a serious threat to the American rice production.

Historical information

Steneotarsonemus spinki was described from specimens collected on *Sogotoca orizicola* Muir [= *Tagosodes orizicolus* (Muir)] (Hemiptera: Delphacidae) in 1960, in Baton Rouge, LA, USA. The holotype and paratypes were deposited in the United States National Collection of Acarology (Smiley, 1967). Although the description of *S. spinki* was based on specimens collected in the USA, there is no record of the mite occurring in agricultural crops in this country. There is, however, evidence that *S. spinki* occurred in rice crops in Asia since the early 1900s. In 1930, it was reported that rice sterility was caused by a minute and agile arthropod identified in 1978 as *S. spinki*, in India (Teng, 1978, in Jagadiswari & Prakash, 2003). This mite has been considered a serious rice pest in China, Philippines, and Taiwan (Smiley et al., 1993), causing substantial yield losses in crops since 1966 (Cho et al., 1999; Xu et al., 2001). In 1985, *S. spinki* was considered a pest in all tropical regions of Asia (Reissky et al., 1985, in Almaguel et al., 2004). In 1999 it was mentioned as a pest of rice in Thailand and as a pest of rice seedlings in greenhouses in Korea (Cho et al., 1999). Recent reports from India, between 1999 and 2003, mentioned *S. spinki* on rice in Andhra Pradesh, Uttar Pradesh, Orissa, and Jharkhand (Jagadiswari & Prakash, 2003), and in 2002 it was reported to have spread to Sri Lanka (Cabrera et al., 2002b).

However, it was in the Americas that *S. spinki* assumed its largest economic importance based on severity and the extent of its damage. It was first reported in the Caribbean region, in Cuba, in 1997 (Ramos & Rodriguez, 1998). Later it was detected in Bonao in the Dominican Republic in areas where imported seeds from Asia are usually grown and it spread to Haiti (García et al., 2002; Valès et al., 2002; Ramos

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& Rodríguez, 2003). In continental Central America, *S. spinki* was first reported in Panamá in October 2003, in the provinces of Coclé and Panama (Chepo) (García, 2005), and later also in Bocas del Toro, Coclé, Los Santos (Tonosí), and Chiriquí (Chiriquí) where it has caused 40-60% yield losses in rice crops. The rice mite was detected in Costa Rica in April 2004 in the Province of Guanacaste and its presence was confirmed in March 2005. In 2005, the mite was reported in Nicaragua (Sanabria, 2005; Rodriguez, 2005) and in Colombia (Herrera, 2005), the latter representing the first observation of the mite in South America. Currently, in Colombia, it occurs in the areas of Casanare, Meta, Tolima, Hulia, and North of Santander, but in very low populations and without any significant yield losses to the rice crop in these areas (Herrera, 2005). At present, *S. spinki* has not been found in other South or North American countries.

Damage

Damage caused by *S. spinki* infestations can be classified as 'direct', when it is due to feeding, or 'indirect', when it is due to toxin injection during feeding or due to transmission of phytopathogens, especially fungi.

The cheliceral stylets of *S. spinki* perforate the epidermal cells of rice plants (Chow et al., 1980). Mite feeding seems to cause necrotic lesions on the upper surface of sheaths and on the region between the rice grain and its shell (Chow et al., 1980). Rice plants damaged by *S. spinki* can get atrophied and present developmental problems, such as deformed panicles and inflorescences, necrotic and dehydratated tissues, brown spots on the shell of grains, sterility, or a decrease in grain quality and reduction of the number of panicles (Cho et al., 1999; Kim et al., 2001, Almaguel et al., 2003).

Simultaneously with the dissemination of *S. spinki* in Asia and again in the Caribbean region, an increase in the incidence of infections with *Sarocladium oryzae* (Sawada) (Moniliaceae) in rice has been observed (Chow et al., 1980; González & Cárdenas, 2003; Ramos & Rodrígues, 2003). This phytopathogenic fungus may cause rotting of the sheath, and together with *S. spinki* infestations, cause a significant reduction in crop production. According to Almaguel et al. (2003), the presence of *S. spinki* in association with *S. oryzae* in Cuba is responsible for epidemic explosions of sterility symptoms in panicles and rotting of the sheath. The mite may carry the spores of this fungus (Lo & Hor, 1977; Chow et al., 1980). Besides *S. oryzae*, other species of phytopathogenic fungi in the genera *Pyricularia*, *Rhynchosporium*, and *Rhizoctonia*, have been reported in association with *S. spinki* in Cuba, causing spotted grains and sheaths (Almaguel et al., 2003).

In Cuba, normal development of infested rice plants has been observed until the formation of panicles. However, after a period of time the panicles remain erect instead of bending down, as would be expected due to its increased weight as the grain fills. Unfortunately, by this time it is not possible to implement control measures and farmers would experience crop losses (RI Cabrera, pers. comm.).

The mite has also been associated with *Spiroplasma citri* Saglio (Spiroplasmataceae) in sterile rice plants in Taiwan (Chow et al., 1980), and later with spherical particles, similar to those of a virus, according to a scanning electron microscopy study of specimens, in Japan (Shikata et al., 1984). In India, grains infected with *S. spinki* were usually colorless and associated with a series of pathogenic fungi and bacteria isolated from the rice grains, viz., *S. oryzae*, *Fusarium graminearum* [*Gibberella zeae (Schwein)*], *F. moniliforme* J. Sheld., *Curvularia lunata* [*Cochliobolus lunatus* R. R. Nelson & Haasis], *Alternaria padwickii* (Ganguly), and *Burkholderia glumae* (Kurita & Tabei) Urakami et al. (sin. *Pseudomonas glumae* Kurita & Tabei). However, in many cases no pathogenic agent could be isolated from these colorless grains, which confirmed the hypothesis that toxins injected by large mite populations can cause chemical reactions responsible for the discoloration of grains (Jagadiswari & Prakash, 2003).

Economic impact

Economic losses caused by *S. spinki* in rice crops in China range between 5 and 20%, on average, but in some cases they may reach up to 70% (Xu et al., 2001). Economic losses of 30% were reported in rice fields (paddies) in the southern region of the Yangtse river, in the middle of the 1970s. In more severe cases, yield losses reached values of 70 and 90% in consecutive crops grown in these same areas (Xu et al., 2001). In Taiwan, *S. spinki* was considered as one of the most important rice pests (Lo & Hor, 1977; Lo & Ho, 1979; Chow et al., 1980). In India, losses resulting from the attack of *S. spinki* ranged from 1 to 20% of the infected rice area in 23 villages investigated, and 50% in the village of Guden, all located in Godovari county (Rao et al., 2000). In Orissa, the population ranged from 7 to 600 mites per bud and grain sterility from 4 to 90% (Jagadiswari & Prakash, 2003).

In Cuban agriculture over the last few years, *S. spinki* has been considered as one of the main pests. In the first year in which *S. spinki* infestations were confirmed in rice crops in Cuba (1997/8) only 40,000 tonnes of rice were harvested from the 120,000 tonnes that were expected. A 70% reduction of yield was estimated. During subsequent years, yield losses caused by *S. spinki* infestations ranged from 30 to 60% (Ramos & Rodríguez, 2000). According to García et al. (2002), under laboratory conditions, yield losses are estimated to be between 11 and 21%. In the community of San Paul, Cuba, the presence of *S. spinki*, and its association with fungi, was responsible for 85% loss of rice productivity: from 5.6 tonnes/ha in 1997 to 0.8 tonnes/ha in 1998 (Romero et al., 2004).

In Costa Rica, during 2004, the first year of *S. spinki* infestation, yield losses of 96,000 tonnes of rice were observed in the Province of Guanacaste, which represents about 45% of the rice production of the entire country and an estimated economic loss of US$ 11million (Barquero, 2004). In areas where phytosanitary measures recommended by the 'Servicio de Sanidad Vegetal' were adopted, 3 tonnes/ha of rice were harvested in the first year, under high infestation pressure. In the second year, with an average infestation of 3 mites/plant, yields of 8 tonnes/ha with no pesticide application were obtained. Generally, more damage occurs in the second crop, however the adoption of phytosanitary measures resulted in a considerable increase of productivity. Farmers in Costa Rica adopted the same measures used in Cuba and the Dominican Republic (Sanabria, 2005).

Host plants

Rice has been considered the only host plant of *S. spinki* (Smiley et al., 1993). The survival rate of *S. spinki* on plants of 73 species – invasive plants and species that grow near rice fields, including 44 monocotyledons – was investigated in Taiwan (Ho & Lo, 1979). The mite was unable to develop on any of the host plants tested. We ourselves also tried to rear this mite on 10 species of fungi and verified that it is

strictly phytophagous. However, eggs, larvae, and nymphs of *S. spinki* were collected from an invasive plant *Oryza latifolia* Desv., commonly occurring in rice crops of Costa Rica and Panama, and the mite could complete its life cycle on this host (Sanabria, 2005).

Dissemination pathways

There are indications that *S. spinki* can be transported through the trade and transport of rice seeds, although this has not been confirmed. Many mites of various developmental stages can be found in rice crop remnants (Lo & Hor, 1977; Lo & Ho, 1979). Injuries of leaves of young plants occur as a result of the use of infected seeds, which indicates the possibility that the mite disseminates through seeds (Rao et al., 2000). In Korea, it is suspected that *S. spinki* has been introduced through the acquisition of rice grains for genetic improvement programs. The mite was identified for the first time on imported seeds used in controlled environment experiments in greenhouses. In order to avoid the occurrence of this mite under open field conditions, seeds must be disinfected before use for rice production. According to Cho et al. (1999), quarantine surveillance must be intensified in imported rice from areas where the pest is present. Mite populations are mainly found on the internal surface of stem sheaths, but when populations grow, mites can also be found on rice panicles (Chow et al., 1980) or on grains, as described by Almaguel et al. (2003). Kim et al. (2001) collected *S. spinki* from the surface of grain husks. Cho et al. (1999) reported that the milky stage in grain formation was the selected stage for feeding and reproduction of *S. spinki.* Agricultural workers and machinery are also probable dissemination agents.

This mite, like several other species of the Tarsonemidae, is transported over short distances by wind or through insects or birds that visit rice plantations (Almaguel et al., 2003). The mite may be transported by air currents over short and possibly also long distances. It is also worth emphasizing that mites may disperse from one plant to another alone, without the use of dispersal agents and regardless of wind, especially when plant density is high and plants touch one another.

In flooded rice crops, *S. spinki* may disseminate from one flowerbed to another through crop debris present on the water flows that circulate through them. Mite individuals may be collected on small pieces of vegetal tissue that get dislodged and drift with these water flows (RI Cabrera, pers. comm.).

In experiments aimed to understand *S. spinki* dispersal between neighbouring rice crops by wind, adhesive traps were installed around rice crops for various time periods. A significant difference between time periods was found, regardless of the force and direction of the wind, indicating that mite dissemination is not entirely stochastic and that there is dispersal behavior. For instance, the traps indicated that more mites disseminated between 12:00 and 15:00 hours (RI Cabrera, pers. comm.).

Biology

The rice mite reproduces both sexually and by arrenotokous parthenogenesis, implying that virgin females only produce males. The females can mate with their male descendents and subsequently produce males and females. This reproductive strategy contributes to a fast increase in populations. In Cuba, *S. spinki* completed its life cycle from egg to adult under laboratory conditions at 20, 24, and 30 °C in 11, 7, and 3 days, respectively (Ramos & Rodríguez, 2000; Almaguel et al., 2004). In contrast, Asian populations, at the same temperatures, developed slower, amounting to 20 (20 °C), 13-17 (24 °C), and 3-8 days (30 °C) (Lo & Ho, 1979; Xu et al., 2001).

In Cuba, climatic conditions favoring development of the rice mite were 25.5-27.5 °C and 83.8-89.5% r.h. They can complete development at temperatures ranging from 20 to 34 °C (Cabrera et al*.,* 2003). Periods of less rain and more sunshine are more favorable to *S. spinki* proliferation. Santos et al. (1998) report about 16 °C as minimum temperature for complete development; at lower temperatures only embryonic development was observed.

Control and management measures

Several studies have been conducted since the 1970s in order to implement an integrated management program of *S. spinki*, with the objective of reducing the use of insecticides and hence improve environmental safety. Alternative strategies, such as the use of resistant rice varieties, sampling of pests, determining economic injury level, and the careful selection and timing of control measures, reduced the number of applications of chemical products to one or two applications during the crop cycle (Cheng & Chiu, 1999).

Biological control

Biological control, an important component of integrated pest management, requires the identification and recognition of natural enemies with the objective of protecting and increasing their populations in the field. However, rice presents some characteristics that can make its implementation difficult: the crop annual cycle and the presence of debris between one crop and the next (Cheng & Chui, 1999; Ramos & Rodríguez, 2000). Due to the low efficiency of chemical pesticides to control *S. spinki* populations, biological control has been considered a sustainable alternative control option (Ramos & Rodríguez, 2003). Several mite species in the Phytoseiidae and Ascidae (Mesostigmata) have been reported as *S. spinki* predators around the world. In Asia, *Amblyseius taiwanicus* Schicha [*Neoseiulus taiwanicus* (Ehara)] and *Lasioseius parberlesei* Bhattacharyya were reported to be associated with *S. spinki* (Lo & Ho, 1979, 1980). In Cuba, *S. spinki* predators included the ascids *Aceodromus asternalis* Lindquist & Chant, *Asca pineta* De Leon, *Hypoaspis* sp., *Lasioseius* (nr. *tridentis*), *Lasioseius* sp., and *Proctolaelaps bickleyi* (Bram), and the phytoseiids *Galendromimus alveolaris* (De Leon), *Galendromus longipilus* (Nesbitt), *Galendromus* sp., *Neoseiulus paraibensis* (Moraes & McMurtry*), N. baraki* (Athias-Henriot), *N. paspalivorus* (De Leon), *Proprioseiopsis asetus* (Chant), and *Typhlodromus* sp. (Ramos & Rodríguez, 1998; Cabrera et al., 2003; Ramos et al. 2005; Ramos & Moraes, unpubl.).

Given that several predator mites are associated with *S. spinki* infestations, the next step should be to advance their use for biological control to reduce *S. spinki* populations. This requires the evaluation of their predatory efficiency and assessment of their compatibility with other control methods in rice production areas.

In addition to predatory mites, biological control agents such as the acaropathogenic fungi, *Hirsutella nodulosa* Petch and *Entomophthora* sp., may be used (Cabrera et al*.,* 2002b; Almaguel et al., 2003).

Resistant varieties

Several research projects have been initiated aimed to obtaining rice varieties resistant to *S. spinki* infestations. In Cuba, the varieties that have shown better results were RI-7, IACuba-19, Perla de Cuba, PP-2, RI-6, PP-1, RI-3, RI-13, IACuba-21, J-104, JMR IACuba-31, and Reforma (Botta et al., 2002; Romero et al., 2004; Rosa, 2004). Evaluations for resistance to both *S. spinki* and *Sarocladium oryzae* showed that Reforma and INCA LP-5 exhibited a lower incidence of mites, while the varieties Perla de Cuba and Reforma had less fungus infection (Hernández, 2005). In Costa Rica, Fedearroz 50, CFX 18, and CR 4477 are considered more tolerant to rice mite infestations and have been recommended in management programs (C Sanabria, pers. comm.).

Chemical control

Among the difficulties found in controlling *S. spinki* in Asia and Cuba are its behaviour and high reproductive potential. Its preferred location on plants makes them almost invulnerable to the action of chemical and biological products used for control. Moreover, systemic products that are used to control pests in hidden plant parts are not effective to control populations of this mite (Chow et al., 1980; Almaguel et al., 2000). In Cuba, Cabrera et al. (1998) evaluated the effects of several chemical products on *S. spinki* and reported that chemical control of this mite is very difficult. Chemical control is only recommended as an emergency action of the last resort (Ramos & Rodríguez, 1998; Cheng & Chui, 1999; Almaguel et al., 2000; Ramos & Rodríguez, 2000).

In Cuba, the pesticide Triazophos (Hostathion 40 CE) was effective in controlling the rice mite (Cabrera et al., 1999, 2002a). Bromopropilato, Dicofol, Diafentiuron, and Edifenphos were tested under laboratory conditions and were more than 95% effective in controlling adults (Cabrera et al., 2005). The chemical products recommended to be used in integrated management of the rice mite in Caribbean and other Central American countries were: Abamectin, Biomite, Dicofol, Triazophos, Endosulfan and Ethoprophos (Almaguel et al., 2005). The treatment of seeds with Benomyl 5 PM plus TMTD (Thiran) 200 ppm was also recommended because it significantly decreases the percentage of sterile and spotted grains and increases yield in areas known to be infested (García et al., 2002).

Cultural methods

A set of cultural procedures has been established to reduce populations of *S. spinki*, delay its arrival into the crop, and reduce yield losses and production costs (Ho & Lo, 1979; Cabrera et al., 1998; Ramos et al., 2001; Hernández et al., 2003; Romero et al., 2004; Hernández et al. 2005; Sanabria, 2005). The main measures are:

- elimination of crop debris and invasive plant species that can act as a source of infestation. These measures should be adopted in the production area and in neighbouring areas;
- cleaning (disinfestation of) new crop areas;
- cleaning machinery and equipment if used by different farmers or in different areas, avoiding the dissemination of the mite from one area to the other;
- allowing prepared soil to remain plant-free for at least 2 weeks before planting;
- using seeds of controlled quality and origin, disinfesting them before planting;
- preparing ahead of planting time;
- applying nitrogen fertilizers following different treatments;
- decreasing the height of the water layer;
- decreasing plant density in the crop;
- coordinate sowing time in neighboring production areas;
- avoiding to plant rice in adjacent areas during or just after harvest time and always take the wind direction into account;
- using rice varieties with higher resistance to *S. spinki* damage;
- monitoring the crop 15 days after planting, especially in areas downwind of infested areas, to obtain an early diagnostic of its presence in the crop and implement control measures.

Final considerations

The serious damage caused by the rice mite in most of the countries where it has been recorded and its fast spread in the Caribbean and continental Central America indicate that this mite represents a potential threat to other countries in America where it has not yet been recorded. The introduction and establishment of *S. spinki* in other countries in South and North America has the potential to cause a dramatic reduction in rice productivity and could result in a steep increase of agrochemical applications needed to control the mite. This would lead to an increase of the production costs and risks of environmental contamination, pesticide intoxication of farmers, and residues in foods. Rice is of primary socio-economical importance, especially in South American countries. The introduction of *S. spinki* into Colombia can be considered as the 'entry door' to South America.

The dissemination of this mite over short distances occurs through wind and water, but over long distances dissemination may occur by the transport of rice seeds. Evaluations of the macroclimatic conditions for the establishment of *S. spinki* in South America, using CLIMEX (Sutherst, 1999), showed that the ecoclimatic index ranges from medium to high in many important production areas, indicating that climatic conditions are extremely favorable for development of the mite (Navia et al., 2005). Therefore, it is essential to reinforce the use of quarantine measures to avoid or delay the introduction of this mite pest in areas of South and North American. Plant protection officers and rice producers must be aware of the symptoms caused by the mite to quickly detect the mite in the event of its introduction. The development of a contingency plan for rice mite control is of vital importance. In most countries currently invaded by *S. spinki*, procedures such as the implementation of integrated management techniques based on cultural control methods, the use of resistant rice varieties, and biological control have been recommended. The expertise developed in Caribbean and Central American countries for managing the rice mite will be very useful in dealing with the problem in newly affected countries in the region. However, the establishment and utilization of the management practices requires time, effort, and resources.

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Importation of a New World tick, *Dermacentor albipictus* (Acari: Ixodidae), with a horse from the USA into Germany

Gabriele Liebisch, Arndt Liebisch & Stephan Paufler
Laboratorium für klinische Diagnostik ZeckLab, Up'n Kampe 3, D-30938, Germany. E-mail: liebisch@zecklab.de

In February 2006 an American Bashkir Curly horse was imported via air from Montana, USA into Germany. Already on arrival at the airport of Amsterdam about 30 fully engorged ticks dropped off the horse, and during the following 4 days in the stable in Germany more than 200 engorged ticks were collected. The tick species was identified as *Dermacentor albipictus*, which is also called 'winter tick'. Hosts for the ticks are large wild and domestic mammals, such as moose, cows, and horses. On these animals the tick undergoes a one-host life cycle. Larvae become active in summer, nymphs and adults engorge during November and February on the same host. This New World tick species occurs in woodlands of North America up to 60° N in Canada. The ecological conditions in Europe may give a suitable environment for establishing a tick population for *D. albipictus*. From Germany the occurrence of two similar ticks, *Dermacentor marginatus* and *D. reticulatus*, is known. This underlines the importance for an exact species diagnosis of ticks. Ticks of the genus *Dermacentor* include important vectors of disease agents, such as viruses, rickettsiae, *Babesia*, and *Theileria*. Unfortunately, there exist no veterinary regulations by the EU (Brussels) or the OJE (Paris) for prevention of importation of ticks. The horse was treated with permethrin wipe-on. Additionally, all visible ticks were collected by hand dressing and burned. Engorged female ticks were collected for experimental use. In an outdoor experiment female ticks laid eggs under natural German winter conditions. Larvae emerged 25 weeks after engorgement of the females.

Key words: *Dermacentor albipictus*, importation, horse, Germany

Human influence on tick distribution

The influence of human activity on tick distribution, density, and tick-borne diseases in man and animals is important for human and animal health. Ticks were originally parasites of wild hosts, but since man domesticated cattle and horses for food production and transportation, almost 100 tick species, or 10% of the ca. 800 tick species in the world, became adapted to domesticated animals. Former parasites only of deer, antelopes, and wild carnivores, became parasites of domesticated cattle, camels, small ruminants, dogs, and birds. When in historical times man became more mobile and moved greater distances with domestic animals, the distribution boundaries, incidence, and disease relationship of many tick species were concurrently altered (Hoogstraal, 1973a).

Already from historical times on, striking examples of human influenced changes in tick distribution are known. The interest of man in the Asian zebu cattle (*Bos indicus*) probably accounts for the enormous geographical spread of a one-host tick species of the genus *Boophilus.* This 'cattle tick', *Boophilus microplus*, is the vector for babesiosis in cattle and became a most serious economic problem after being introduced with cattle into Australia, southern Africa, and South and Central America. Another one-host tick species of cattle, *Boophilus annulatus*, was formerly a parasite mainly of deer in the tropical and subtropical regions of America. It became adapted to domestic cattle and it was probably brought from the Mediterranean homeland by the early Spaniards and their horses to north-eastern Mexico. From there it spread into the United States with devastating results for the newly developing cattle industry (Hoogstraal, 1973a, 1977). The fight to control the tick-borne Texas fever (babesiosis) lasted years and became extremely costly for the cattle industry in tropical and subtropical countries. While the tick species was eradicated in the southern USA, it became established in the Near and Middle East, and in some regions of Africa.

Further examples of distribution of ticks appear in the genus *Hyalomma.* For centuries *Hyalomma* ticks were moved with camel caravans over Asian and African deserts and semi-deserts. When camels were entering more humid (irrigated) lands, as there are, e.g., the river valleys of Euphrat, Tigris, and Nile, some of the *Hyalomma* spp. met with cattle of European breed (*Bos taurus*) and became parasitic. During adaptation to the new host in the irrigated environment, in some the nature of their host relationship was changed from the three-host type in the deserts on camels to a two-host type on cattle in the stable. Ever since they became known for their vector capacity of theileriosis with considerable losses in cattle (Hoogstraal & Kaiser, 1959; Liebisch & Zukari, 1976).

Another example of impressive geographical spreading is the 'brown dog-tick', *Rhipicephalus sanguineus*, known for its almost worldwide distribution. In its homelands in Africa the tick is a parasite of a variety of wild and domestic mammals. However, when it was introduced into Europe, Asia, Australia, and the Americas it developed a host preference for domestic dogs. More recently, *R. sanguineus* is introduced into Central and North Europe chiefly by dogs travelling with tourists. In many of the new and cooler environments, *R. sanguineus* will not thrive under natural conditions. However, the tick became well adapted to the domestic dog, living in centrally heated dwellings. Veterinarians report thousands of tick larvae, nymphs, and adults moving on walls and carpets in living rooms during the winter season.

Harry Hoogstraal, one of the world's most outstanding tick researchers, highlighted this global dispersal by formulating a general rule for tick distribution: 'If a species occurs in two Faunal Regions or on two continents, man has been responsible' (Hoogstraal, 1973a).

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Case report

Now that man may transport animals with ticks over thousands of miles by air, the Hoogstraal rule has become even more important. In February 2006 an American Bashkir Curly horse was imported into Germany. The 7-year-old mare was born in Montana, USA, and lived free as a mustang in an Indian Reservation in Montana. The horse was transported via Minnesota to Texas and from there by air to Amsterdam. After a tour of ca. 70 days the mustang arrived at its final destination, in the woodlands of Westphalia, Germany.

American Bashkir Curly horses (Fig. 1) are of medium size, have a gentle nature, and are easy to train. Today, not only in Germany, the American Curlies are loved for Western riding. They are hardy and able to survive extreme winter conditions. One of the breed characteristics is the long curly hair coat, which provides excellent hiding places for small ticks, e.g., larvae and nymphs of some one-host tick species.

Already on arrival at the airport of Amsterdam about 30 big engorged female ticks dropped off the horse. During the following 4 days, at the stable in Westphalia, the horse owner collected more than 200 adult ticks per day.

Tick identification and biology of *Dermacentor* spp.

The tick species was identified in our laboratory as *Dermacentor albipictus.* For identification the excellent illustrated atlas of American *Dermacentor* ticks was very helpful, a publication of the tick research group at the Rocky Mountain Laboratory in Hamilton, Montana (Yunker et al., 1986). The tick species is called 'moose tick', because it is often found on moose and deer, or 'winter tick', because of its activity on the hosts during the cold season. The species has a one-host life cycle, remaining on the same host for each of the three feeding phases. In its homelands in the Americas from Canada to Mexico, large wild and domestic mammals, such as moose, deer, horse, and cattle are the preferred hosts. Most frequently, the rather big adults are seen on the host.

Non-engorged females are reddish-brown, with a 3 × 6 mm oval body and a silver-grey striped scutum. Fully engorged females (Fig. 2) are 9 × 14 mm, much bigger than the known European *Dermacentor* spp. Engorged females drop to the ground usually in the late winter. The males are 4 × 7 mm, somewhat larger than in the European species. They are also painted with silver-grey and brown stripes on the dorsal side (Fig. 3).

In Europe adult *D. albipictus* could be misidentified and mixed up with two endemic *Dermacentor* species, *D. marginatus* and *D. reticulatus*, which also feed on horses. However, there are some significant differences. Both European species feed on horses only in the adult stage. Both species occur in distinct and ecologically different landscapes (Liebisch & Rahman, 1976). *Dermacentor marginatus* is distributed on grassy lands in warm and dry areas of south-west Germany, where adults become active twice a year during spring and autumn. The favourable hosts there are sheep on pasture. *Dermacentor reticulatus* seems to be a species of the relict Fauna, which occurs in remains of natural forest in areas with higher humidity, often along river banks. Adults are found on deer, wild boar, and fox. Both species also feed on domestic dogs and horses. On horses both species prefer to feed in body regions with long hair, e.g., at the base of the mane or the tail. Often adults of both species are found attached to the skin and feeding in groups close together.

At first sight adults of the three species are alike, because of their oval body, size, and the brown and silver-white painted stripes on the dorsal scutum. From the dorsal view, the males of *D. reticulatus* are recognized by two retrograde spurs at the caudal border of the 2nd palpal segments (Fig. 4). Such spurs lack in males of *D. marginatus* and *D. albipictus.* Males and females of the two endemic species in Germany are recognised by the oval shape of their spiracular plates, bearing a blunt caudo-dorsal projection (Fig. 5A, B). The spiracular plates of males and females of *D. albipic-*

Figure 2 Fully engorged females of *Dermacentor albipictus* are much bigger than European spp.

Figure 1 American Bashkir Curly horse with long hair winter coat provides comfortable hiding places for 'overwintering' larvae and nymphs of *Dermacentor albipictus.*

Figure 3 The male of *Dermacentor albipictus* resembles European species (dorsal view).

tus are oval-round, they lack the caudo-dorsal projection, and they have chacteristically large goblet cells (Fig. 5C).

Typical for *D. albipictus* ticks is their seasonal activity. The larvae became active during late summer and autumn. They form dense clumps on the tips of grasses and twigs (Samuel & Barker, 1979). Larvae are small (0.7 × 0.7 mm) and hosts may become repeatedly infested when passing by, during August and September. The larvae will engorge and moult to nymphs in about 20 days. When engorged, nymphs resemble white or blue-grey rice grains. They moult to adults and remain on the same host during November and February. Adults engorge over a period up to 1 month.

Between the long hair coat of Curly horses the attached larvae of *D. albipictus* are almost impossible to notice and also nymphs are difficult to see. Often high numbers of larvae stay unnoticed, and nymphs are not discovered until they have become engorged – often *D. albipictus* infestation is not diagnosed until adults emerge. Larvae usually start to feed at places where they are more likely to be brushed off from the vegetation onto the host, i.e., at the ventral and frontal sides of the animals body. Secondarily, ticks may become scattered over the whole body surface as we found it in our Curly horse.

Reproduction experiment

Before treatment of the imported horse, on February 26, 2006, four fully engorged female ticks were collected. Two of them were kept indoors under room temperature conditions. They began egg laying after 4 weeks. Larvae hatched 64 days later. Two other females were placed into small vessels with plastic covers and buried outdoors in the garden about 5 cm deep, and covered with ground. They were kept and observed for survival, egg laying, and larval hatch (Table 1). Temperature was measured during winter at the soil level in the vessel: 0 to –3 °C during the first 3 weeks. This period was followed by 51 days with soil temperature between 0 and 10 °C, and a further 30 days with 10-14 °C. The observed period from dropping-off of the engorged females until start of egg laying under local outdoor conditions was 92 days. The period of egg laying lasted ca. 35 days. Egg development until the start of larval hatching was 40 days, and 9 days later all larvae had hatched. The total period from drop off after engorgement of the females until emerging of larvae accounted for 176 days or 25 weeks, under outdoor conditions.

Can *Dermacentor albipictus* thrive in Germany?

Dermacentor albipictus is widely distributed in the woodlands of North America. It occurs in as far north as 60°, and extends south to Texas and northern Mexico. Considering the bioclimatic conditions, North Europe may give a suitable environment for the tick to thrive in continental Eurasia. In 2002, a letter was published to the editor of the Veterinary Record on the importation of *D. albipictus* into Europe, when the tick came with a horse to Norway (Lillehaug et al., 2002). To our knowledge this is the only previous account.

In our outdoor tick experiment we demonstrated that *D. albipictus* lays eggs and produces larvae under natural winter conditions of northern Germany. Also the type of vegetation with woodlands and swamps resembles the homelands of the tick quite much. Suitable hosts for the one-host tick species – wild large mammals, like deer in the forests and cattle and horses on pastures – are also available. Therefore, when the tick is introduced and escapes our control, it most likely can thrive and spread in Europe or even to Eurasia.

The small larvae and nymphs attached to a host may easily escape the search and control measures, especially with the long and curly hair of a horse as we met in the present case (Fig. 1). There are other one-host ticks, e.g., in the

Table 1 Development of *Dermacentor albipictus* under natural conditions in Germany.

Tick status	Date	Day no.
dropp off	February 23	0
begin egg laying	May 26	92
end egg laying	June 30	127
begin larval hatch	August 9	167
end larval hatch	August 18	176

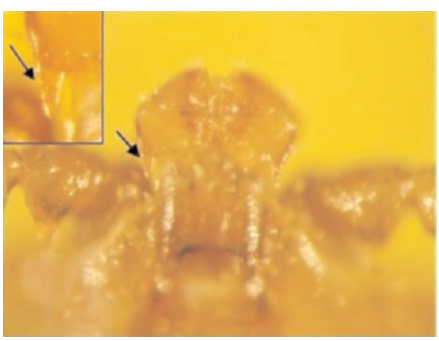

Figure 4 Second palpal segments of *Dermacentor reticulatus* with two retrograde spurs at the caudal border.

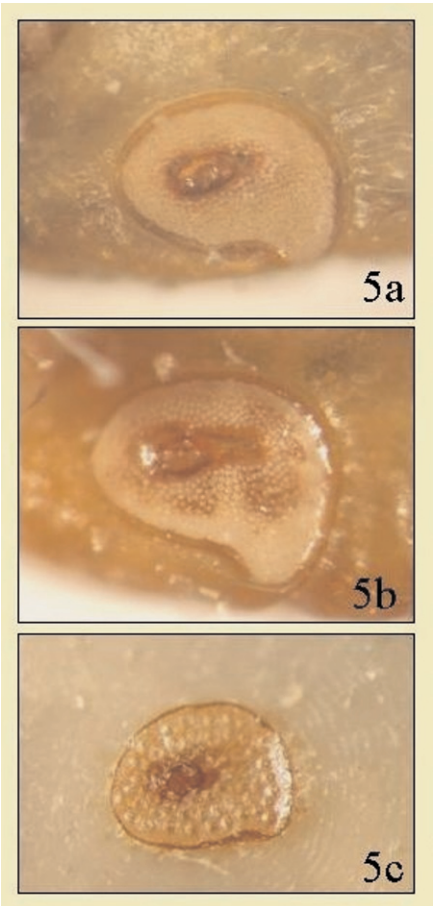

Figure 5 Frame of spiracular plate of (a) *Dermacentor marginatus* and (b) *D. reticulatus*, with distinct caudodorsal projection, and of (c) *D. albipictus*, with indistinct projection and moderately large goblet cells.

genus *Boophilus*, which are also very small and can easily be overlooked when imported. However, not all ticks transported by man or even by birds thrive in a new environment because they need other climatic conditions. We know, e.g., from our own experience, that two-host *Hyalomma* species are year by year repeatedly introduced into Germany with migrating birds. However, they cannot thrive in the new environment because of unfavourable climatic conditions.

Treatment and control of *Dermacentor* in horses

Because *D. albipictus* seems suited to thrive in Europe, treatment of the horse infested with adult *D. albipictus* became of special importance. According to German regulations, any chemical product applied to horses and (other) animals for human consumption must be considered as a medicine product and has to be formally registered, according to indication and animal species. Unfortunately, at present no acaricide is registered in Germany for use on horses, with the indication for tick control. This displays a gap in medication. Permethrin is the only substance registered for use on horses. However, the registered indication is to repel biting and nursing flies and other Diptera on the pasture. It is on the market as a 'wipe-on' formulation. We sometimes advise practitioners to use 'permethrin wipe-on' also to prevent infestation with the endemic *Ixodes ricinus* ticks in horses, because we know it is effective. With at least the same or even better efficacy the pyrethroid deltamethrin could be applied. This very effective acaricide/insecticide is available on the German medical market as a 'pour-on' formulation, for use against ectoparasites in cattle and sheep. The only acaricide formulation for spraying on the market in Germany is the organophosphate phoxim. It is registered for use in small ruminants, swine, and pets. Therefore, in the present case of tick infestation in the horse, only permethrin wipe-on was allowed for the horse. All ticks detected during hand dressing were collected and burned. Additionally, the horse was treated with wipe-on permethrine.

Disease vectoring and economic impact

World-wide, ticks in the genus *Dermacentor* are known as vectors of pathogenic viruses, rickettsiae, spirochetes, and protozoa in man and animals. In Germany, the Old World species *D. marginatus* is involved in transmission of various diseases: the Spring-Summer-Encephalitis virus in man; *Coxiella burnetii*, the causitive agent of Q-fever, in man and animals (Liebisch, 1979); and *Rickettsia slovaca*, which also causes diseases in man. *Dermacentor reticulatus* is known from many European countries as a vector of encephalitis viruses, rickettsiosis, and is feared as vector of babesiosis in dogs and horses.

From the medical and veterinary standpoint, the most important ticks of North America are 12 species of the genus *Dermacentor* (Yunker et al., 1986). They include the most important vectors of diseases, e.g., *D. andersoni*, vector of Rocky-Mountain-spotted fever. Fortunately, reports of feeding *D. albipictus* on man are rare. Hunters or other people active in woods, sitting or placing a hand on the ground, may be attacked when contacting groups of tick larvae. In New Hampshire (USA) as many as 50 larvae have been reported on a glove or pant leg after such an encounter. *Dermacentor albipictus* is a one-host tick, which reduces the chance to transmit pathogens. However, the tick is known as a vector of *Anaplasma marginale*, the causative agent of bovine anaplasmosis in cattle (Zaugg, 1990). In case of sequential infestations of overwintering beef cows, the losses in meat production may become considerable (Teel et al., 1990). The death of more than 110 cattle and 44 horses after infestation with the winter tick were reported from two farms in Sasketchewan, Canada (Cameron & Fulton, 1926). *Dermacentor albipictus* may also have a considerable economic impact on wildlife in the northern USA and Canada: it may cause serious loss of winter hair, decrease in body weight, and even considerable mortality in moose and deer (Samuel, 1989; Glines & Samuel, 1989; Teel et al., 1990). This gives proof of the tick's capacity to become a pest, possibly due to its host relation to rickettsiae. In case *D. albipictus* thrives and becomes endemic in Europe, nobody can foresee the kind of (European) pathogens which may become transmitted by this New World tick.

Sanitary and veterinary regulations

No veterinary regulations exist with the European Community (Brussels) or the 'Office International de Epizooties' (Paris) for prevention of importation of ticks. The German 'Tierseuchengesetz' takes no special notice of parasites. In section 2a of the regulation it is only stated that 'toll officials may stop the importation or exportation of live animals ... or parts of animals and other objects which could be carriers of infections'. No parasites or vectors are mentioned. The 'objects' do not include such important parasites and vectors of pathogens as ticks. Apart from mosquitoes, ticks include the most important vectors of disease agents in man and animals!

Harry Hoogstraal reported 68 different viruses from more than 80 tick species. Tick-borne viruses known or believed to cause disease in man or domestic animals numbered already 20 in 1973 (Hoogstraal, 1973b). Ticks of the genus *Dermacentor* are known to transmit such important viruses like Russian spring-summer encephalitis, European tick-borne encephalitis, and Omsk hemorrhagic fever. We follow sanitary and veterinary regulations for travelling and exporting. However, in the European Community we do not strong enough consider the danger for human and animal health by importation of ticks and tick-borne diseases agents.

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Agricultural Acarology: Biological Control

Concepts of classification of the Phytoseiidae: Relevance to biological control of mites

J. A. McMurtry
PO Box 4487, Sunriver, OR 97707, USA. E-mail: jmcmurtry@chamberscable.com

Early phytoseiid mites colonizing the plant habitat probably were generalized types with omnivorous food habits. Various life styles and 'body plans' evolved, some adapted to exploiting certain foods, others to cope with specific plant characteristics. Marked specialization for utilizing spider mites, producing copious webbing, as prey probably evolved independently in at least four of the 16 tribes across two of the three subfamilies. Specialization for other types of mite prey is less clear although some trends are evident. Species with generalist feeding habits seem to be predominant in nearly all tribes. Two genera in the tribe Euseiini evolved unique characteristics presumably for utilizing pollen as a food source. Some 'body plans' seem more closely related to the morphology of the host plant than to food source. The tribe Kampimodromini serves as an example of this feature. The classification system of Chant & McMurtry (1994, 2002-2006) is examined in relation to the main genera containing species showing promise for biological control. Challenges at the species level are discussed.

Key words: Phytoseiidae, phytoseiid classification, biological control

The family Phytoseiidae has received much attention in the last 45 years because of the potential of these mites as biological control agents. Kostiainen & Hoy (1996) listed 4,664 references on the Phytoseiidae since 1960. Moraes et al. (2004) list more than 1,200 references on taxonomy alone. The taxonomic classification of the family has had a somewhat chaotic history, often leaving workers in biological control confused on which system of supraspecific classification to apply to their work. Before 1959, less than 30 species were known (Nesbitt, 1951). Chant (1959) listed and figured about 165 species and placed most in the genus *Typhlodromus* with the subgenera *Typhlodromus* and *Amblyseius*; and eight other genera, most of which contained only a few species. Muma (1961) made history with a generic concept comprising 43 genera for ca. 180 species. Many of his genera were monotypic. For a long time thereafter, systematics of the Phytoseiidae took the direction of either the 'Chant school' [later Chant (1965) for ca. 450 spp.], or the 'Muma school' [later Muma, Denmark & Deleon (1970) for ca. 600 spp.]. Karg (1983) proposed a system comprising 32 genera. Most other taxonomic monographs, some excellent, covered only limited regions or a limited number of genera. By 1993, more than 1,650 species had been described (Chant, 1993). The recent system proposed by Chant & McMurtry, resulting from a 15-year project (Chant & McMurtry, 1994, 2003a,b, 2004a,b, 2005a,b,c, 2006a,b), proposes 84 genera in 16 tribes for some 2,300 species, as listed in the catalog by Moraes et al. (2004). Keys and diagnoses of genera and subgenera of the world were presented by Chant & McMurtry (2007).

How is this relevant to workers in biological control? To address this question, it seems important to consider the following: (1) hypothetical pathways in which the phytoseiids may have evolved in their exploitation of the plant environment, (2) associations of phytoseiids with different foods and plant characteristics (Are there any morphological adaptations? Does this relate to the classification system of Chant & McMurtry?), (3) genera and species groups of phytoseiids that show more promise for biological control of mites than others, and (4) challenges of systematics of the Phytoseiidae at the species level in relation to biological control of mites.

POSSIBLE PATHWAYS OF EVOLUTION IN THE PLANT ENVIRONMENT

I suggest that the early phytoseiids colonizing plants evolved from a 'protophytoseiid' ancestor that lived in the soil or under bark of trees (Fig. 1). They were probably morphologically conservative, with unspecialized (generalist) food habits. From these generalized types it might be assumed that morphological and behavioral novelties evolved, presumably in response to various habitat and/or food conditions. Extant examples of proposed ancestral morphological types might include *Austroseiulus australicus* in the subfamily Typhlodrominae (Chant & McMurtry, 1994), and *Chileseius camposi* and *Macrocaudus multisetatus* in the Amblyseiinae (Chant & McMurtry, 2003a; Moraes et al., 2003). These species are among the most setose in the family, with medium-length subequal setae on the dorsal shield and few leg macrosetae. The family has come to exploit the foliage habitat to a greater extent than any other family of Mesostigmata has done (McMurtry & Rodriquez, 1987). Potential foods these early phytoseiids encountered on tree, shrub, or grass habitats probably included: (a) mites, such as tydeids, eriophyoids, tarsonemids, tetranychids, tenuipalpids, (b) insects, such as scale crawlers, mealybugs, aphids (including honeydew), thrips, and (c) nematodes. All of these are now shown to be potential foods. Potential foods of plant origin would have included pollen, nectar, fungi, and leaf sap – also known to be fed upon by today's species.

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Various derived types evolved (Fig. 1), probably in multiple events, still with generalist food habits, but with morphological adaptations to various foliage types and conditions. One of these lines probably gave rise to specialized pollen feeders. A few events occurred in which specialization evolved to exploit various prey types, such as tetranychid mites that develop in patches with heavy webbing, with possible morphological adaptations for living in these prey patches. Species in various genera presently occurring in soil/humus habitats may have been derived from foliage-inhabiting groups, probably representing several independent events.

CONSIDERATION OF BIOLOGICAL CONTROL POTENTIALS ACCORDING TO LIFE STYLES, TRIBES, AND GENERA

We might divide phytoseiids into several tentative categories of 'life styles', based mainly on food habits (Types I-III; McMurtry & Croft, 1997; Croft et al., 2004). I begin with the general feeders, because these are the dominant types across the family, and I propose that the more specialized types evolved from these groups. These are considered by subfamily, tribe, and some of the better-known genera.

General feeders (Type III)

Some basic characteristics include: medium to low reproductive potential, and wide range of foods accepted, including various groups of mites, small insects, pollen, honeydew, and nectar. The reproductive potential on spider mites may be lower than on some other prey, such as eriophyoid mites. These types do not aggregate in patches of spider mites, but rather may be hindered in their movement by dense webbing. They are more apt to aggregate in protected areas of foliage rather than in patches of prey. These general feeders are often dominant in stable ecosystems, e.g., orchards receiving minimal sprays. There are many types of body plans, but no apparent morphological adaptations to prey have been documented. However, some groups show apparent adaptations to foliage texture. *Phytoseius*, *Kampimodromus*, and *Paraphytoseius* species are often found on hairy leaves, are small, laterally compressed, and have characteristic types of setae.

Typhlodrominae

The following genera are mostly characterized by conservative (ancestral) body plans and do not seem to be highly ambulatory on foliage:

Typhlodromini – Some *Typhlodromus* species, like *pyri* and *caudiglans*, are known to have a higher reproductive potential on eriophyoids than on spider mites, although various studies on these species indicate that they are effective in the control of tetranychid mites on deciduous fruits (McMurtry & Croft, 1997; Blackwood et al., 2004).

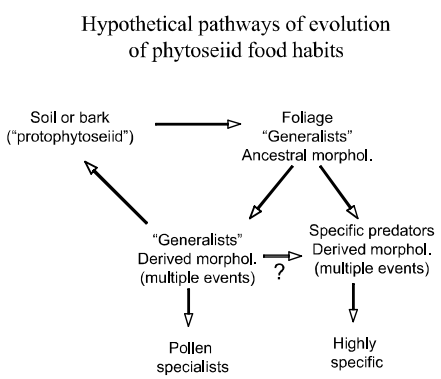

Figure 1 Theoretical pathways of evolution of food habits in the Phytoseiidae.

Paraseiulini – *Paraseiulus soleiger* was shown to have potential for control of tetranychids (McMurtry & Croft, 1997).

Metaseiulini – *Metaseiulus* species, e.g., *arboreus*, *citri*, have not been observed to respond to increases of tetranychid populations (Mahr, 1978). Eriophyoid mites were more favorable than tetranychids for *M. citri* and *M. flumenis* (Blackwood et al., 2004). Their distribution is predominately in the Western Hemisphere.

Phytoseiinae

Documented cases in which *Phytoseius* species are considered important in biological control are scarce if not absent, although there are many reports of associations with eriophyoids (Kostiainen & Hoy, 1997). They seem to be more adapted to certain leaf types. *Phytoseius* species are small and laterally compressed, and are able to move through dense leaf hairs. All are highly derived morphologically (Chant & McMurtry, 1994).

Amblyseiinae

Neoseiulini – The large genus *Neoseiulus* (>300 species) contains species of importance. Most *Neoseiulus* species generally have a conservative morphology. *Neoseiulus barkeri*, in the *barkeri* species group, and *N. cucumeris*, in the *cucumeris* species group, are well-known in biological control programs, especially for thrips (McMurtry & Croft, 1997). Species in the *desertus* group are mainly found on willow and poplar trees, and are often associated with galls of eriophyoid mites (Prischmann et al., 2005). However, we know too little of their biologies to assign them to a specialist category. The *paspalivorus* group of *Neoseiulus* is known mainly from grasses, and also coconut palms – their flat, elongate form may be an adaptation to life on grasses. *Neoseiulus paspalivorus* and *N. baraki* are associated with *Aceria guerreronis* in Sri Lanka. The morphology of these species is appropriate for accessing the area between the bracts and the surface of a coconut fruit (Moraes et al., 2004.)

Kampimodromini – Most species in this tribe have derived body plans: thick setae, small size, laterally compressed, and sometimes with large glands on the dorsal shield. *Paraphytoseius* species bear a superficial resemblance to *Phytoseius* species (convergence). At least one species, *Kampimodromus aberrans*, is documented to be an effective biological control agent (McMurtry & Croft, 1997). With many species, there seems to be a closer association with host plant features than with certain types of food (McMurtry & Croft, 1997; Kreiter et al., 2003). This trait may apply to most groups of this tribe. Some species in this tribe, particularly those in the genus *Eharius*, appear to be primarily phytophagous (Athias-Henriot, 1960; DL Mahr, pers. comm.; JA McMurtry, unpubl.). One wonders if there are some species in this tribe that do not feed at all on animal prey.

Typhlodromipsini – The biologies and biological control potentials of species in this tribe are poorly known, although *Aristaseius masseei* and *Typhlodromips sessor* are common species on apple trees. Are they valuable predators? Of the many *Scapulaseius* species, known mostly from the Asian/Pacific regions, *S. newsami* is used for control of *Panonychus citri* in China, and pollen from groundcover plants is beneficial (Ragusa Di Chiara, 1991). Also, *S. nicholsi*,

S. newsami, and *S. okinawanus* have been reported as predators of spider mites (Kostiainen & Hoy, 1996).

Amblyseiini, subtribe Amblyseiina – *Amblyseius* species (with >350 spp. the largest genus in the family) generally have highly derived morphologies, with long 'caudal' setae Z5, some setae minute, and numerous prominent leg macrosetae. The few species that have been recorded as being significant biological control agents include *A. andersoni*, reported to be important as a predator of spider mites on deciduous fruits and grape (McMurtry & Croft, 1997), and *A. swirskii*, a predator of mites on citrus and other subtropical crops in the Middle East, and recently reported to be an effective thrips predator in greenhouse crops (Bolckmans et al., 2005). *Transeius* species, e.g., *caspiansis* and *tetranychivorus*, are known from various crops in Iran and India, respectively, and deserve further study (Hajizadeh et al., 2002; Nangia & ChannaBasavanna, 1984).

Amblyseiini, subtribes Arrenoseiina and Proprioseiopsina – There is little information regarding the biological control potential of species in these subtribes, containing 48 and 125 known species, respectively.

Euseiini – *Typhlodromalus* species, e.g., *peregrinus* and *aripo*, are common in the New World and the latter was introduced to Africa for control of cassava green mite (Yaninek & Hanna, 2003). *Amblydromalus manihoti* is another sucessful introduction to Africa from Brazil for control of cassava green mite (Yaninek et al., 1998). *Amblydromalus limonicus* feeds on *Oligonychus punicae*, *Eotetranychus sexmaculatus*, and *Panonychus citri* in California (McMurtry & Croft, 1997; pers. obs.). Species in this genus have a more highly derived morphology than *Typhlodromalus*, with some long, but mostly minute setae and smooth dorsal shield (Chant & McMurtry, 2005b).

Broadly specific spider mite predators (Type II)

All of the groups included below probably evolved independently. These predators are characterized by long median dorsal setae, possibly creating a wedge effect for moving through the dense spider mite webbing (Sabelis & Bakker, 1992), generally high reproductive potential, and commonly living in highly webbed tetranychid mite colonies. Increases in their populations are usually dependent on tetranychid densities, although other prey, such as eriophyoid mites, may be important alternate food sources.

Typhlodrominae

Typhlodromini – The *rickeri* group of *Typhlodromus* (*Anthoseius*) is the only assemblage in the tribe that might be categorized in type II. *Typhlodromus rickeri* (introduced from India and established but not widespread in California) is closely associated with *Eotetranychus sexmaculatus* in California, but rust mites are also favorable for reproduction (McMurtry & Croft, 1997).

Metaseiulini – *Galendromus* species all seem to be closely associated with spider mites in webbed colonies. *Galendromus occidentalis* is well known as an important predator of *Tetranychus* spp. on various crops (McMurtry & Croft, 1997); *G. helveolus* (introduced) and *G. annectens* (native) live in webbed nests of *Oligonychus perseae* (Aponte & McMurtry, 1997) and are also associated with *E. sexmaculatus* in avocado orchards in California (pers. obs.). I would give any *Galendromus* species priority in biological control investigations.

Amblyseiinae

Neoseiulini – Some *Neoseiulus* species (probably a minority) in the *cucumeris* group, e.g., *fallacis*, and the *barkeri* group, e.g., *womersleyi*, fit this category and they rank among the most important predators of tetranychid mites in agricultural systems. If they have long median dorsal setae, they probably prefer spider mites as prey. *Neoseiulus californicus* has medium length setae and may be closer to a type III (Croft et al., 1998).

Because these more specialized predators are more dependent on their spider mite prey than those in the generalist categories, they may be displaced by species in the generalist categories when their prey becomes scarce or in stable ecosystems in which the prey species always remains at a low level (Croft & McMurtry, 1997).

Specialized predators of *Tetranychus* spp. (Type I)

Amblyseiini

Phytoseiulini – This category currently includes only the *Phytoseiulus* species. This single genus in the Phytoseiulini is highly derived and shows no obvious relationship to any other group. *Phytoseiulus* species are characterized by very high reproductive potential, long median dorsal setae (derived independently from those of other groups), and a strong affinity for *Tetranychus* species. All life functions are carried out in the heavily-webbed spider mite colonies. The plant habitat probably is less important than the prey species in determining the distribution of these predators.

Specialized pollen feeders (Type IV)

Amblyseiinae

Euseiini – This type is tentatively limited to the genera *Euseius* and *Iphiseius*. The reproductive potential of species studied is commonly highest on pollen. The highest population buildups may occur during bloom periods of the crop (e.g., avocado) or adjacent trees (windbreaks) in the absence of prey species. These mites have specialized mouthparts, short, stubby chelicerae and a wide deutosternum (Flechtmann & McMurtry, 1992), and their reproductive potential can be rated as medium. They are considered potentially valuable predators of spider mites forming light webbing, and possibly thrips (McMurtry & Croft, 1997), but only if the predator:prey ratio is high (1:2?).

Other types?

An additional type or subtype might be justified for the intriguing relationship between *Schizotetranychus celarius* and *Typhlodromus bambusae* on bamboo. Both prey and predator reportedly have a low reproductive potential in this stable habitat and there is some evidence of coevolution (Saito, 1990; McMurtry & Croft, 1997).

Are there specialized predators of eriophyoids, or tenuipalpids, or certain insects? Maybe, we just have not studied these situations closely enough to be able to answer this question. Most tribes across the family predominately contain species with general feeding habits. This feature might explain why phytoseiids have been so successful in foliage habitats. General feeders have many different 'body plans', from generalized to highly derived types. A seemingly fruitful area awaiting further study is to examine the possibility of subdividing Type III into several subtypes

SOME CHALLENGES AT THE SPECIES LEVEL

Obviously, accurate determination of species is critical in classical biological control programs. It is important to sort out cryptic species complexes. The *Euseius* species on citrus and avocado in California are an example. What was once assumed to be a single species is now known to be four, plus a fifth one which stands out as being an introduced species: (1) *E. tularensis*, widespread on citrus, rare on avocado; (2) *E. hibisci*, widespread on avocado, on citrus only near the coast; (3) *E. quetzali*, on deciduous fruits and nuts, blackberry, wild plants, rare (1 collection) on avocado; (4) *E. obispensis*, known only from avocado in San Luis Obispo County; and (5) *E. stipulates*, introduced from the Mediterranean region in 1971. In earlier years it was recovered on citrus, where it actually displaced native *Euseius* in some coastal orchards, never on avocado. Recent collections (2006-7) show that it has moved to avocado, as well as grape and raspberry (E. Grafton-Cardwell, pers. comm.) Rarely is there more than one *Euseius* species present in a given habitat (Congdon & McMurtry, 1986). These results are based on extensive sampling, in California and also in Mexico and Central America, on morphological analyses and on cross-breeding experiments, all before molecular techniques were available. Edwards et al. (1998) and Noronha et al. (2003) showed that molecular characterization of three closely related species of *Amblydromalus* and two closely related *Euseius* species, respectively, were consistent with morphological analyses, and thus confirmed the validity of these species.

The detection of biotypes may be critical to successful biological control programs. For example, a population of *Phytoseiulus longipes* from South Africa showed a low feeding rate and no promise as a predator of *Tetranychus evansi*, whereas a Brazilian population showed a strong preference for *T. evansi* in comparison with *T. urticae.* (Furtado, 2006). Also, *Amblydromalus manihoti* sent from Colombia to Africa never became established despite several attempts, whereas a strain from northeasten Brazil is established in Ghana and Benin (Yaninek et al., 1998). Drukker et al. (1997) demonstrated the possibility of selecting populations (strains of *P. persimilis* better adapted to tomato plants). Different strains may be identifiable by molecular analytical methods (Yli-Mattila et al., 2000).

Variation within populations have important implications in systematics and biological control. Setal lengths may be unreliable for diagnosing different species because there can be seasonal variation in setal lengths (Chant, 1955; Swirski & Amitai, 1965; Tixier et al., 2003). Populations of *Kampimodromus aberrans* were found to be highly structured. Molecular analyses showed that there was little genetic exchange between populations on grape with those of surrounding areas (Tixier et al., 2002). These results call into question the importance of surrounding vegetation as a reservoir for phytoseiid mites in a crop. The importance may vary with each situation.

Conclusions

The classification system of Chant & McMurtry does seem to have relevance to food habits and biological control potential of member species of different genera in various tribes, and on which groups priorities might be placed. Can we dismiss any genera as having no promise for biological control? Probably not, because we have detailed knowledge on too few species in the family. However, we can predict which genera likely contain the more specific predators, i.e., *Galendromus*, *Neoseiulus*, and *Phytoseiulus.* Although we need more work on evaluations of generalist types in different situations in order to increase the predictability of the classification system, we do have some clues to where priorities might be placed for study and utilization of these generalist groups. Genera that might be given priority in biological control programs include *Typhlodromus*, *Kampimodromus*, *Neoseiulus*, *Typhlodromips*, *Sapulaseius*, *Amblyseius*, *Transeius*, *Typhlodromalus*, and *Amblydromalus*, if species from one or more of these genera are present in a given system. Detection of cryptic species, biotypes, and population variation is essential in order to better exploit the use of phytoseiids as biological control agents. Molecular techniques are valuable tools to provide additional information for making decisions in taxonomy and biological control. As we learn more about phylogeny and classification, we should be better able to postulate paths of evolution of biological traits, especially feeding habits and habitat preferences, contributing to a more scientific basis for biological control endeavors. This paper represents only a small beginning toward relating classification and systematics of the Phytoseiidae to practical use in biological control of mites. I hope it will stimulate thinking toward future work along these lines.

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Biological control of mites in European vineyards and the impact of natural vegetation

Carlo Duso, Serge Kreiter[1], Marie-Stéphane Tixier[1], Alberto Pozzebon & Valeria Malagnini[2]

Department of Environmental Agronomy and Crop Science, University of Padova, Viale dell'Università, 16 - 35020 Legnaro (PD), Italy. E-mail: carlo.duso@unipd.it; [1]Montpellier SupAgro, UMR Centre de Biologie et de Gestion des Populations 1062 INRA/IRD/CIRAD/SupAgro, Campus International de Baillarguet, CS 30 016, 34988 Montferrier sur Lez cedex, France; [2]FEM, via E. Mach, 1 38010 San Michele all'Adige (TN), Italy

In vineyards, generalist phytoseiids are important in keeping phytophagous mites at economically acceptable levels. Among these predators, *Typhlodromus pyri* and *Kampimodromus aberrans* have proven to be most effective, because they increase in numbers in response to mite pests and alternative prey/food, they persist under conditions of prey scarcity, and they can tolerate several fungicides and insecticides. Natural colonization of commercial vineyards by phytoseiids may take several years. Therefore, strains showing field resistance to certain insecticides (e.g., organophosphates) and fungicides (e.g., mancozeb) are of practical interest. Here we report results obtained with releasing *T. pyri* and *K. aberrans* strains with different pesticide histories, with emphasis on factors affecting their persistence, i.e., alternative food availability (pollen or downy mildew), leaf morphology, and selective pesticides. Natural vegetation surrounding vineyards may impact the densities of phytoseiids in neighbouring crops. For example, phytoseiid densities on plants surrounding vineyards under IPM in Southern France (Languedoc-Roussillon) were correlated with leaf structures, and *K. aberrans* density appeared positively affected by high trichome densities and presence of domatia. Also pollen density was significantly correlated with trichome density and domatia (hair tufts). Predatory mites disperse mainly by air currents and hence their dispersion depends on wind intensity and direction. Crop colonization potential (speed, intensity, uniformity) was directly associated with phytoseiid densities and the proximity of natural vegetation. A deep, dense, and tall woody area with suitable host plants constitutes the stablest source of phytoseiids. Natural colonization of vineyards by phytoseiid mites has great potential and it may well be promoted by careful management.

Key words: Phytoseiids, *Kampimodromus aberrans*, dispersal, colonisation, woody areas, hedges, leaf morphology, pollen, fungi, IPM

Phytophagous mites, tetranychids in particular, have been considered good examples of pests induced by the use of pesticides. This hypothesis also holds for mite pests on grape: tetranychid mites have a negligible impact in undisturbed ecosystems or in organic vineyards, but in commercial vineyards they become pests due to treatments with pesticides that harm their natural enemies. The spread of integrated pest management (IPM) in European viticulture in the 1980s and 1990s was associated with a decrease in the economic importance of tetranychids. In several parts of Europe the reduction in pesticide use, the selection of compounds that are relatively safe for beneficial arthropods, and the application of threshold levels for chemical control have increased the potential of natural enemies of tetranychid mites.

Whereas various predatory insects exert a significant role in suppressing mite outbreaks in vineyards, they are attracted mainly by rather high densities of tetranychid mites. Hence, their role in pest control is significant only when relatively high tetranychid threshold levels are applied (Duso & Pasqualetto, 1993). Phytoseiidae in European vineyards are widely recognized as effective control agents, irrespective of the pest threshold level applied. A negative correlation between tetranychids and phytoseiids in the field was reported by the first researchers working on this topic (e.g., Mathys, 1958). The numerical response of phytoseiid mites to densities of tetranychid mites is often spectacular and, in contrast to insect predators, phytoseiids can persist under conditions of prey scarcity (Ivancich Gambaro, 1973; Girolami, 1981; Baillod et al., 1982).

European vineyards are dominated by generalist phytoseiids

In the last two decades faunistic surveys have been conducted on phytoseiids on grape in Europe. More than 20 species have been recorded in vineyards in Italy (Castagnoli, 1989; Nicotina, 1996; Ragusa di Chiara & Tsolakis, 2001) and France (Kreiter et al., 2000; Tixier et al., 2000b). *Typhlodromus pyri* Scheuten is the most common phytoseiid species on grapes in central Europe, *Kampimodromus aberrans* (Oudemans) is the most important in various southern regions, and *Typhlodromus exhilaratus* Ragusa plays a significant role in the Mediterranean viticulture. Other species frequently found are *Amblyseius andersoni* (Chant) and *Phytoseius finitimus* Ribaga. These species are considered to be generalists (type III, after McMurtry & Croft, 1997), since they are characterised by their close association to host plants, a wide food range, and an intra-plant distribution wider than that of their prey. They can persist when prey densities decline by surviving on alternative foods, they can regulate their population densities, and compete successfully with other predators (McMurtry, 1992; McMurtry & Croft, 1997). These features can be recognized at various levels in generalist phytoseiids encountered in European vineyards.

Evaluating *Amblyseius andersoni*, *Kampimodromus aberrans*, and *Typhlodromus pyri* in tetranychid control

Laboratory studies on food ranges have shown that *T. pyri* and *A. andersoni* develop and reproduce on tetranychids [e.g., *Panonychus ulmi* (Koch) and *Eotetranychus carpini* (Oudemans)], eriophyids [e.g., *Colomerus vitis* (Pagenstecher)], and pollen. *Amblyseius andersoni* exhibited shorter developmental times and higher oviposition rates than *T. pyri*, suggesting a higher intrinsic rate of increase (Duso & Camporese, 1991). *Kampimodromus aberrans* showed a similar food range, and demographic parameters closer to *T. pyri* than to *A. andersoni* (Schausberger, 1992; Kasap, 2005; C Duso, unpubl.).

When released in experimental vineyards in north-eastern Italy, *T. pyri* and *K. aberrans* responded better to tetranychid population increases than *A. andersoni* (Duso, 1989).

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Additional observations showed that in some situations *A. andersoni* can control *P. ulmi* in vineyards (Vila et al., 1989; Camporese & Duso, 1996), but confirmed its poor performance towards *E. carpini* (Duso & Vettorazzo 1999). *Kampimodromus aberrans* proved to be the most effective phytoseiid in controlling *E. carpini* in various European regions (Ivancich Gambaro, 1973; Duso, 1989; Villaronga et al., 1991; Kreiter et al., 1993).

Amblyseius andersoni proved to be a key predator of *P. ulmi* in fruit orchards in northern Italy, south-west France, and other European regions, and its pesticide resistance contributed to this status (Ivancich Gambaro, 1986; S Kreiter, unpubl.). Its role in vineyards is certainly less important than in orchards: populations may crash suddenly despite pesticide resistance and resurge late in the season. This phenomenon is most likely due to this species' relative humidity requirements. Moreover, *A. andersoni* is less well able to persist in conditions of prey scarcity than *T. pyri* and especially *K. aberrans*. In northern Italy *K. aberrans* seems to have adapted better than *T. pyri* to harsh environmental factors such as high temperature, interspecific competition, and unfavourable leaf morphology (Duso & Pasqualetto, 1993; Duso & Vettorazzo, 1999). The ability of *K. aberrans* to outcompete *T. pyri*, observed repeatedly, is not due to higher predation rate (Schausberger, 1997), but probably to the smaller amount of food needed to survive (C Duso, unpubl.). In the laboratory, *T. pyri* exhibited a higher competitive ability towards *K. aberrans* (Schausberger, 1998), suggesting that these contrasting results may depend on differences between geographic races.

Typhlodromus pyri and *K. aberrans* reached higher population densities on cultivars having hairy leaf undersurfaces, whereas *A. andersoni* showed the opposite (Duso, 1992). This can affect colonisation patterns in vineyards comprising cultivars with different leaf morphology, as well as interspecific competition among phytoseiids (Camporese & Duso, 1996; Duso & Vettorazzo, 1999).

Impact of *Typhlodromus exhilaratus* and *Phytoseius finitimus* on tetranychids in vineyards

Typhlodromus exhilaratus is very common in central and southern Italy, Spain, southern France, and Greece (Castagnoli, 1989; Villaronga et al., 1991; Tixier et al., 2000b; Papaioannou-Souliotis et al., 1999). Its food range includes tetranychids, eriophyids, and pollen (Ragusa, 1979, 1981), and its intrinsic rate of increase is higher on *E. carpini* (and pollen) than on *P. ulmi* (Castagnoli & Liguori, 1986a,b; Castagnoli et al., 1989). In field conditions, *T. exhilaratus* exhibits a rapid numerical response to *E. carpini* populations and this phenomenon is sometimes favoured by the presence of eriophyoids (Liguori, 1987, 1988). *Typhlodromus exhilaratus* easily adapts to conditions of low relative humidity, a fundamental factor for its persistence in vineyards of southern Europe (Liguori & Guidi, 1995). The effectiveness of *T. exhilaratus* in controlling tetranychids has not been compared with that of other phytoseiids.

Phytoseius finitimus is another very common species in Italy, Greece, and other Mediterranean countries (Castagnoli, 1989; Nicotina, 1996, Papaioannou-Souliotis et al., 1999, Kreiter et al., 2000; Ragusa & Tsolakis, 2001). Its food range has been poorly studied in relation with mites occurring on grapes, but it certainly includes tetranychids, eriophyoids, and pollen (Rasmy & El-Banhawy, 1975). This species may have potential for controlling *P. ulmi* but seems to be ineffective towards *E. carpini* (Duso & Vettorazzo, 1999). *Phytoseius finitimus* populations reach higher densities on hairy grape cultivars, which may affect interspecific competition (Duso & Vettorazzo, 1999).

Role of alternative prey for generalist phytoseiids

The importance of alternative prey for phytoseiids has been widely debated in relation to the control of tetranychids (Helle & Sabelis, 1985). Generalist feeding habits are a fundamental requirement for the persistence of predatory mites and for the success of biological control. Generalist phytoseiids can prey upon tenuipalpids, eriophyoids, tydeids, winterschmidtiids, young stages of thrips, and coccids. These preys can support survival, development, or reproduction to various measures. However, it must be stressed that some of these phytophagous species can reach pest status. Low to moderate populations of *Colomerus vitis* (Pagenstecher) have no impact on grape yield, but the case of *Calepitrimerus vitis* (Nalepa) is different because this species can cause economic damage and is more risky to manage (Duso & de Lillo, 1996). Some phytoseiid species have a higher fecundity on eriophyoids than on tetranychids (Lindquist et al., 1996). The occurrence of eriophyoids can increase the potential of generalist phytoseiids (such as *T. pyri* and *T. exhilaratus*) in responding to tetranychid population build-ups (Liguori, 1987, 1988; Engel & Onhesorge, 1994a,b).

The role of tydeids as alternative prey for phytoseiids has been emphasized for a long time since the contributions dealing with *Galendromus occidentalis* (Nesbitt) in California, USA (Flaherty & Hoy, 1971). However, laboratory studies and field observations do not support this hypothesis for the most common generalist phytoseiids in European vineyards. Tydeids are the preferred prey of some species of the genus *Paraseiulus*, in particular *P. talbii* (Athias-Henriot), but these species are hardly considered key predators of phytophagous grape mites (Camporese & Duso, 1995).

Interspecific predation among generalist phytoseiids has been widely investigated. *Typhlodromus pyri* and *K. aberrans* can prey upon immature or adult stages of con- or heterospecific phytoseiids. Since these species partly inhabit the same plants, their immature or adult stages can be regarded as potential prey for competitive phytoseiids under conditions of food scarcity (Schausberger, 1999a). Adult females of *T. pyri* and *K. aberrans* are able to discriminate between con- and heterospecific immatures and they prefer to prey upon heterospecifics when given the choice (Schausberger, 1999b). Croft et al. (1996) report data on interspecific competition between *A. andersoni* and *T. pyri*.

Factors affecting the persistence of phytoseiids when prey is scarce: windborne pollen and pathogenic fungi

It is well known that generalist phytoseiids can develop and reproduce on pollen, but the impact of pollen on phytoseiid populations in vineyards has only been studied at a small spatial and short temporal scale. Studies in Germany showed that *T. pyri* populations peaked following phases with large pollen availability on leaves (Engel & Onhesorge, 1994b). Long-term studies in north-eastern Italy confirmed a similar relationships for *T. pyri*, *K. aberrans* and, to a lesser extent, *A. andersoni* (Duso et al., 1997; Malagnini et al. unpubl.). The latter studies showed that grape leaves are excellent pollen traps, and that pollen trends typically have three major phases: in spring (May-June) the leaves host much pollen (especially arboreal pollen, Poaceae, *Vitis vinifera* L.),

used by overwintered females and the first generations of predatory mites; in early summer pollen densities decline due to drought and phytoseiid numbers also decline; in late summer, phytoseiid abundance may increase again, sustained by weed flowers (Poaceae, Plantaginaceae, Chenopodiaceae, etc.).

Grape pathogenic fungi can also play an important role as alternative foods for generalist phytoseiids. Grape downy mildew (GDM), *Plasmopara viticola* (Berk. & Curtis ex. de Bary) Berlese & De Toni, and grape powdery mildew (GPM), *Uncinula necator* (Schwein.), are the most important worldwide grape pathogens. Late-summer spread of GDM foliar symptoms has been associated with sudden population increases of *A. andersoni* and, to a lesser extent, *T. pyri* (Duso et al., 2003). This phenomenon has been observed repeatedly in northern Italy, and is partly explained by the ability to develop and reproduce when reared on GDM in the laboratory (Pozzebon & Duso, 2008). Additional effects of GDM involve interspecific competition between *A. andersoni* and *T. pyri*: GDM provide advantage to *A. andersoni* over *T. pyri*. Further studies should be addressed on the impact of GDM availability on spider mite biological control by generalists (Pozzebon, 2006).

An interesting case of interactions between predators and prey mediated by GDM concerns the phytoseiid *P. talbii* and the tydeid *Tydeus caudatus* Dugès. The latter can develop and reproduce on eriophyoids (e.g., *Col. vitis*) or on GDM (M Lorenzon, unpubl.) and is the preferred prey for *P. talbii* (Camporese & Duso, 1995). GDM positively affected tydeid populations and consequently the response by the tydeid predator, *P. talbii*. Whether GDM can improve the control of grape eriophyids by tydeids still needs to be shown (Duso et al., 2005).

The effects of GPM on generalist phytoseiids has been the subject of recent investigations. Reared on this food source, *A. andersoni* and *T. pyri* were able to develop from egg to adult, but did not reproduce, suggesting the role of supplementary food for GPM (Pozzebon et al., 2009). This would imply enhanced persistence of these species in vineyards infected by powdery mildew. Research on interactions between powdery mildew and phytoseiids is required for species adapted to the Mediterranean climate where this pathogen is more aggressive.

Several generalist phytoseiids can feed on leaf sap (McMurtry, 1992; Kreiter et al., 2002). This would partly explain the ability of *A. andersoni* and *T. pyri* to persist when prey are scarce, but the real impact of this phenomenon requires in-depth investigation.

Effect of non-prey food on phytoseiid coexistence

The effects of non-prey foods on generalist phytoseiids may be more or less pronounced depending on the mite species. In systems with multiple species present, a specific non-prey food can mediate interspecific competition. Laboratory studies showed that *T. pyri* females developed faster on pollen than on GDM mycelium and that they laid more eggs when fed on pollen than on GDM. No differences in developmental times of *A. andersoni* were seen when they were reared on pollen or on GDM, whereas differences in oviposition confirmed the higher performance on pollen than on GDM (Pozzebon et al., 2008). In the field, however, the impact of these non-prey foods on *T. pyri* and *A. andersoni* was different. On leaves, *T. pyri* population densities were positively correlated with pollen densities. In contrast, a non-significant linear relation was observed between *T. pyri* densities and GDM leaf symptom extent. *Amblyseius andersoni* population densities appeared to be positively related to the level of GDM leaf symptoms but not to pollen densities. The coexistence of these predatory mites in the same vineyard seemed to be due to a distinct preference for two different non-prey foods, irrespective of the results emerging from laboratory data. The pollen used in the laboratory (*Papaver rhoeas* L.) may have had a higher impact on *A. andersoni* demographic parameters than the pollen occurring in the vineyard (especially Poaceae and *Vitis vinifera* L.). Moreover, windborne pollen found on leaves by *A. andersoni* could be of lower quality than that provided every 3-4 days in the laboratory. Also, *A. andersoni* may be less effective than *T. pyri* in extracting nutrients from deteriorating pollen.

How can we manage alternative non-prey foods for generalist phytoseiids?

The management of alternative non-prey foods for generalist phytoseiids is crucial for the progress of biological control in vineyards. Some arboreal plants can provide large amounts of pollen. An interesting case of natural vegetation interacting with vineyards was observed on hilly areas close to the Italian Alps, where hop hornbeam (*Ostrya carpinifolia* L.) flowering was overlapping with grape sprouting. Large densities of hop hornbeam pollen were found on newly developed leaves colonized by overwintering females of *T. pyri*. Phytoseiid oviposition and the first generation were favoured by pollen abundance (Duso et al. unpubl.).

We also investigated the potential role of an experimental hedgerow in providing pollen (and phytoseiids) to a neighbouring vineyard. Among the plants included in these hedgerows, elderberry produced large amounts of pollen. Populations of the predatory mite *Euseius finlandicus* (Oudemans) increased on elderberry after flowering but the importance of this phenomenon for grapes was low in terms of pollen and predatory mites (Duso et al., 2004b).

The main component of windborne pollen in vineyards of northern Italy (and other European regions) comes from grasses (Poaceae). Nowadays, grasses are widely used as cover crops in vineyards. Field experiments showed that the amount of windborne pollen (mainly Poaceae) on the grape canopy can be increased by reducing the frequency of grass mowing. A moderate increase of phytoseiid densities was observed after this practice (Girolami et al. 2000, Malagnini et al. unpubl.).

The management of downy mildew is more complicated. GDM infections occurring in late summer are not economically important. In vineyards where *A. andersoni* is largely dominant, late-summer GDM infections could represent the most important factor for the persistence of this predator. However, it should be stressed that when *A. andersoni* and *T. pyri* coexist, the latter can be displaced by the former. Since *T. pyri* is more effective than *A. andersoni* in controlling tetranychids on grapes, infections by GDM could thus affect pest control by phytoseiids.

Interactions between pesticides and phytoseiids in vineyards

Pesticides applied in controlling grape pests and diseases can exert pronounced effects on phytoseiid survival, development, and reproduction, and may alter their response to the ecological factors mentioned above. The role of *T. pyri* in

European viticulture has almost certainly been overestimated, probably because organophosphate (OP) resistance appeared first in this species (Baillod et al., 1982; Maixner, 1990; Vidal & Kreiter, 1995). At the same time, *A. andersoni* has been considered an important bio-control agent of tetranychids in vineyards due to its high resistance to OPs and EBDC (Ethylenebisdithiocarbamate) fungicides (Duso et al., 1992; Angeli & Ioriatti, 1994). Taking the latter factor as a priority, an OP resistant strain of *A. andersoni* was released in France and Switzerland in the 1980s (Caccia et al., 1985; Vila et al., 1989). However, this approach appeared to be unsuccessful.

The role of *K. aberrans* in European vineyards has been neglected, probably because resistance developed later than for other species (Corino, 1989; Marchesini & Ivancich Gambaro, 1989). The spread of *K. aberrans* in some regions of Italy and France is most likely due to the recent appearance of strains able to survive repeated applications of EBDC (mainly mancozeb) and OPs (Vettorello & Girolami, 1992; Posenato, 1994; Auger et al., 2004b).

Little is known about pesticide resistance in *T. exhilaratus* and *Ph. finitimus*. Their spread in commercial vineyards suggests that they can tolerate common pesticides.

Should we release phytoseiids in vineyards?

The natural colonization of phytoseiids can require several years, and can be favoured when natural vegetation is contiguous to vineyards. Phytoseiid strains occurring on natural vegetation are more susceptible to pesticides than those existing in vineyards and their settlement has a lower success rate if certain pesticides are used (Tixier et al., 1998, 2000a). Therefore, the interest for strains showing field resistance to several insecticides (e.g., OPs) and fungicides (e.g., mancozeb) has increased. Various strains of *T. pyri* and *K. aberrans*, most of them OP-resistant, were released in north-Italian commercial vineyards. The spread of grape yellows in the 1990s resulted in an increase of pesticide use; consequently, susceptible strains were able to persist in organic farms only. A strain of *K. aberrans*, collected in a vineyard treated with EBDC fungicides and OPs, was released in several experimental vineyards seriously infested by *P. ulmi*. The *K. aberrans* strain proved to control *P. ulmi* effectively and persisted for 10 years, despite unfavourable climatic conditions and repeated use of the EBDC and OP pesticides (Facchin et al., unpubl.).

Negative implications of augmentative releases of phytoseiids (e.g., low genetic variability of released predators) are matter of discussion. This strategy is often preferred to natural colonization in some areas, but poor results have been obtained in others, without a clear explanation.

Surrounding natural vegetation as a source of phytoseiids for dispersal into crops

Corino (1989) from Italy, Kreiter & Sentenac (1995) from France, and many other authors have reported an increase in phytoseiid densities in vineyards managed through integrated farming. This process has been named 'colonization' and involves the presence of relatively rich and varied surrounding vegetation from which natural enemies disperse towards cultivated areas. Few studies have actually dealt with the process of colonization, but the occurrence of phytoseiid mites in surrounding vegetation is well documented as it is considered to be a potential reservoir of phytoseiids for vineyards (e.g., Boller et al., 1988; Kreiter & Sentenac, 1995; Tixier et al., 1998, 2000a; Duso et al., 2004a). In some cases, species frequently found on natural vegetation, such as *T. pyri* or *K. aberrans*, have been found to be dominant in neighbouring vineyards (Boller et al., 1988; Tixier et al., 1998).

In the Languedoc region of southern France, phytoseiids have been found in the surroundings of experimental grape fields: in woody areas, border hedges, and neighbouring grape fields, *K. aberrans* represented on average 75% of the phytoseiids present (Tixier et al., 1998, 2000a). Diversity and densities appeared to be correlated to floristic diversity and to the abundance of suitable plants for the development of *K. aberrans*. This floristic diversity is itself linked to the local physical (climatic and edaphic) conditions, to vegetational succession (equilibrium status vs. colonizing process), and to the history of land use by men, all of which affect plant communities. In soils with colonizing vegetation in abandoned and replanted lands, *T. exhilaratus* seems to be dominant, a pioneer or real crop-colonizing species (Tixier et al., 2005), whereas in vineyards at higher altitudes and on the border between Mediterranean and Oceanic climatic conditions, *T. pyri* seems to replace and perhaps displace *K. aberrans* (Barbar et al., 2005; Kreiter et al., 2006).

Both diversity and abundance are affected by plant composition, due to the close relationship between plant leaf characteristics (i.e., pilosity) and mite development, espe-

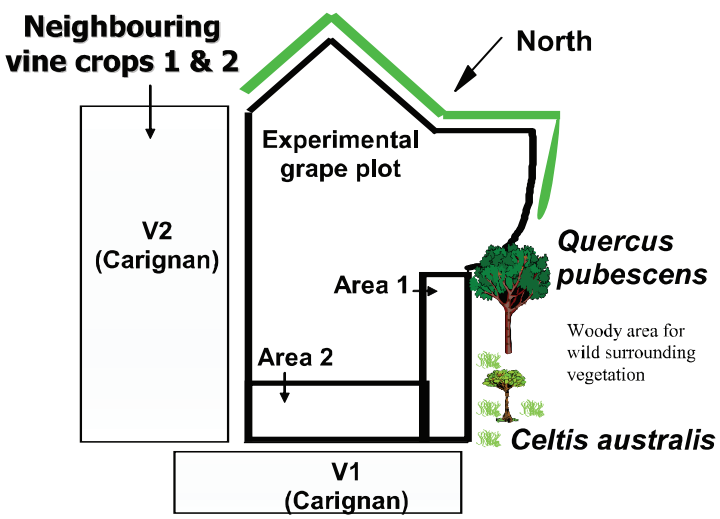

Figure 1 The experimental grape field (1) where colonisation was studied (Pouzolles, Languedoc, France).

cially for *K. aberrans* (Tixier et al., 1998, 2000a; Kreiter et al., 2002). Higher trichome and domatia densities are correlated to higher predator densities. Trichome spacing of 160-180 µm may allow *K. aberrans* to actively move in between trichomes, whereas larger predators cannot. Domatia ratings that reflect domatia complexity also seem to affect densities (Kreiter et al., 2002). Such leaf structures may allow habitat partitioning and more phytoseiid species to provide pest regulation at lower densities than single species (Croft & Slone, 1998). Plants providing substrates, liquid, and pollen and whose leaves are characterised by pilosity and domatia, positively affect phytoseiids, especially generalist species (Karban et al., 1995; Walter, 1996; McMurtry & Croft, 1997). For *K. aberrans*, these effects may also be present in one or more respects.

Finally, although several aspects of plant-phytoseiid interactions are described, not many aspects have been subjected to direct experimentation. Most of our conclusions are based on correlations or inferences from field-derived data. What is needed now are laboratory tests in simplified systems where variables can be controlled and single-factor relationships evaluated.

Phytoseiid mite dispersal or the colonization processes of plots

Phytoseiid mites disperse aerially and by ambulatory means (Sabelis & Dicke, 1985). Their dispersal is thought to be motivated by the declining conditions of their habitats, i.e., overcrowding, poor quality food, abundance of predators, and yearly plant senescence (Price, 1984). Hamilton & May (1977) developed a dispersal model and concluded that it was to the advantage of insects living in both stable and unstable environments that a portion of their offspring should disperse.

Our experiments showed that several phytoseiid species are liable to disperse in an aerial and/or ambulatory manner (Fig. 1; Tixier et al., 1998, 2000b, 2002a; Kreiter et al., 2006). This complies with previous observations on phytoseiid dispersal studied in the laboratory (Sabelis & Dicke, 1985) and in the field (Hoy et al., 1985). Furthermore, aerial dispersal contributes to a greater extent than ambulatory dispersal (Tixier et al., 1998, 2000b; Kreiter et al., 2006). Fallen leaves and animal carriers (phoresy) can also transport phytoseiids, but this dispersal mode appears to be relatively insignificant (Kreiter & Tixier, unpubl.). The number of dispersing mites and the number of those observed in cultivated fields increased together (Tixier et al., 1998, 2000b; Kreiter et al., 2006) (Fig. 2). However, this cannot be considered proof that dispersing phytoseiids effectively colonized the fields. Some species appear to disperse only by ambulatory displacement (soil or herbaceous stratum species according to Moraes et al., 1986), others only by aerial dispersal (tree inhabitants) (Moraes et al., 1986; Tixier et al., 1998, 2000b; Kreiter et al., 2006). However, *K. aberrans*, *Typhlodromus intercalaris* Livshitz & Kuznetsov, and *T. pyri* have been trapped in both aerial and soil traps (Tixier et al., 1998, 2000b; Kreiter et al., 2006). After initiating aerial dispersal, these species might use ambulatory dispersal in order to reach a new plant.

Among the eight species trapped in the Languedoc, *K. aberrans* was the most abundant. Some data (Kreiter et al., unpubl.) revealed this species to have a low dispersal rate. An environment particularly rich in *K. aberrans* would be a source population for dispersal to poorer environments and the numbers of *K. aberrans* trapped could represent only a very small proportion of the populations present in the source area. Males and females have similar dispersal properties, both in the air and on land (Tixier et al., 1998, 2000b; Kreiter et al., 2006). Most of the literature available to date presents females, especially gravid ones, as being the most dispersive, but these results were obtained in laboratories and concerned type-I species (McMurtry & Croft, 1997), which are biologically very different from types III and IV which are dominant in vineyards and orchards. Equally, a considerable number of immatures were found in the aerial traps in our experiments (Tixier et al., 1998, 2000b; Kreiter et al., 2006), which is also an uncommon result, especially for predator-types III and IV.

Soil and aerial trap catches support the view that phytoseiids disperse from woody areas and from not very far away (Fig. 3). Phytoseiid mite species were trapped between 30 and 90 m in experimental grape fields studied in the Languedoc (Fig. 3; Tixier et al., 1998, 2000b; Kreiter et al., 2006). Aerial movement might be adopted for long-distance dispersal, whereas ambulatory dispersal would be better adapted to shorter displacements. *Kampimodromus aberrans* was observed to disperse the farthest, whereas *T. pyri* and *T. intercalaris* were found mostly in traps located near the border of the woody area. This would confirm observations that *T. pyri* only disperses over short distances (Dunley & Croft, 1992; Sentenac & Valont, 1999).

Wind seems to be the main vector for aerial dispersal of phytoseiids and particularly of *K. aberrans* (Fig. 4). In our study, the N-NW winds, varying from 14 to 31 km/h in speed, facilitated dispersal of phytoseiid mites (Tixier et al., 1998, 2000a; Kreiter et al., 2006).

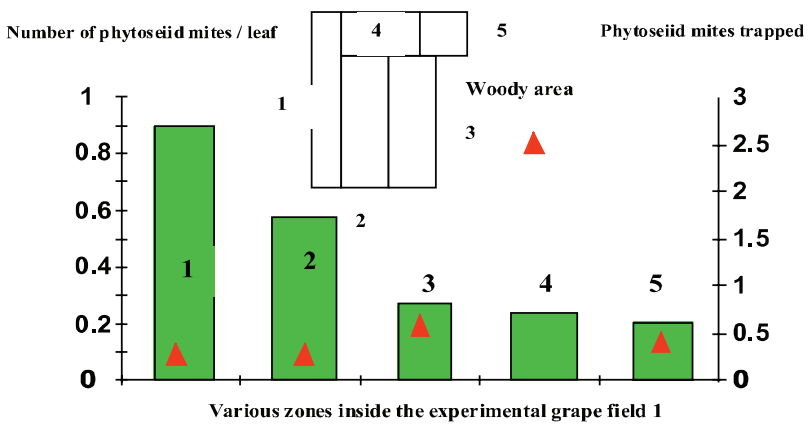

Figure 2 Comparison of mean densities of phytoseiid mites on leaves (histogram) and the mean phytoseiid mites trapped (triangles) inside experimental grape field 1 (Pouzolles, France; see Fig. 1) during 3 years.

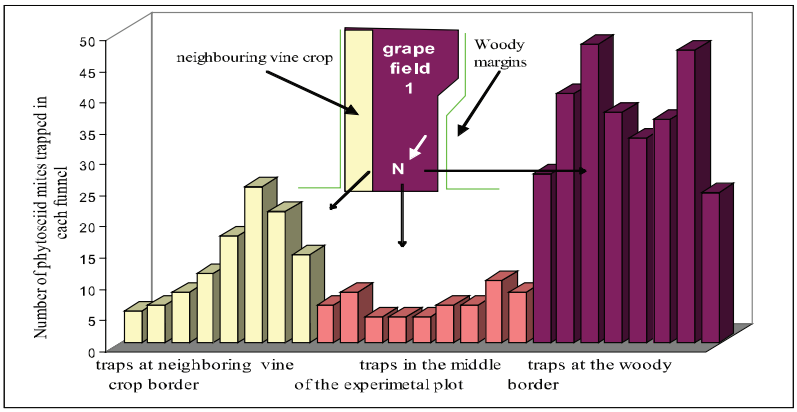

Figure 3 Number of phytoseiid mites trapped from 13 May to 01 October 1998 (>80% *Kampimodromus aberrans*) and location of trapping within the experimental grape field 1 (Pouzolles, France; see Fig. 1).

Relationship between *Kampimodromus aberrans* populations in vineyards and natural vegetation?

To answer this question, a molecular typing (RAPD) of specimens collected in vine crops and in the neighbouring vegetation was carried out. The similarities between the genetic patterns of these mites were studied for two sampling dates, May and July. For both dates there was genetic similarity between females originating from *Quercus pubescens* Willdenow and those from *Celtis australis* L. (Tixier et al., 2002a,b). In a same way, the RAPD patterns of females from vineyards surrounding the experimental plot were also similar from each others. However, differentiation in genetic patterns was observed between mites collected in the experimental plot and mites observed in the surroundings (in woody margins and in neighbouring vine crops), irrespective of geographic distance (Tixier et al., 2002a,b) and despite the great number of migrants arriving in the experimental plot and certainly originated from the woody area (Tixier et al., 1998, 2000b). Such results may be explained by the low dispersal ability (distance or/and frequency) of *K. aberrans* (Fauvel & Cotton, 1981; Perrot-Minnot, 1990; Tixier et al., 1998; Jung & Croft, 2001) or to the highly aggregated distribution of this mite (Malison et al., 1995; Tixier et al., 2000b). Genetic distances among and between populations were lower in July than in May. Such genetic homogenisation is common for multivoltine organisms (De Barro et al., 1995). However, despite this homogenisation, we still observed differences between populations even after many *K. aberrans* individuals had dispersed into the experimental vineyard.

We expected that molecular typing would allow the identification of the source of *K. aberrans*; however, no strong correlations between genetic and geographic distances were observed.

These data describing weak relationships between mites from woody margins and nearby vine plots confirmed the population density trends observed by Tixier et al. (1998, 2000b; Kreiter et al., 2006). During a 3-year study, mite densities in some sites of the experimental plot did not increase even though high numbers of phytoseiids dispersed into this

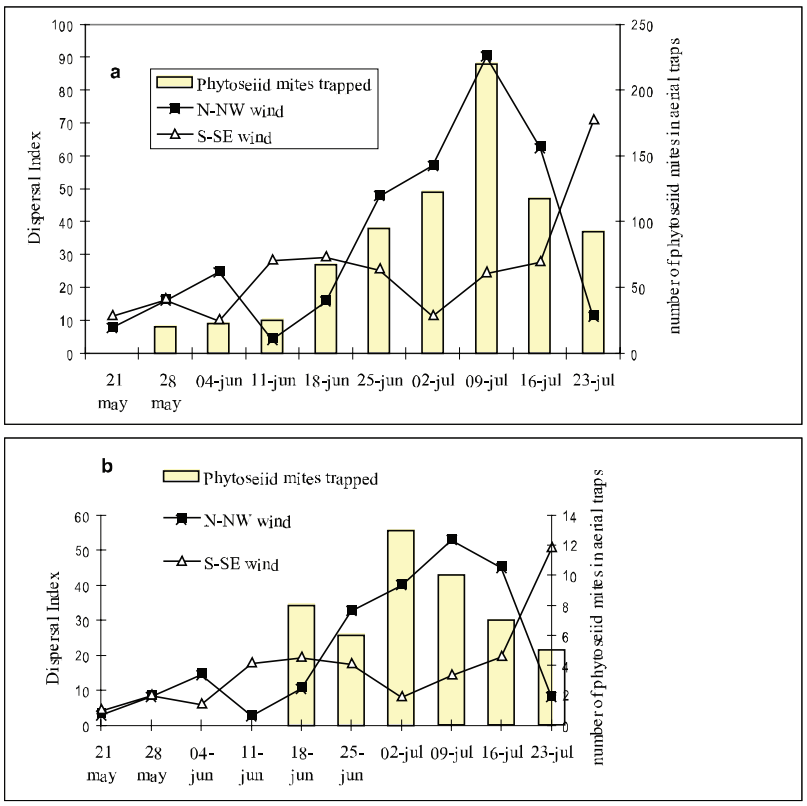

Figure 4 Correlations between total number of phytoseiid mites trapped in funnels full of water and a dispersal index for N-NW and S-SE major wind directions in experimental grape field 1 (A) and 2 (B) (Pouzolles, France; see Fig. 1). r^2 (N-NW wind) = 0.81 and 0.48, and r^2 (S-SE wind) = 0.04 and 0.02, for experimental grape fields 1 and 2, respectively. Dispersal index = number of days of wind from S-SE or N-NW × weekly mean wind velocity × richness of surrounding vegetation in phytoseiid mites / leaf.

area. Apparently, not all immigrants settled and colonised. The results of our studies suggest that little gene flow occurs between experimental and surrounding environments. Within-site selection factors may reduce the number of migrant phenotypes able to settle. Pesticide applications, for instance, could select for different phenotypes in vineyards vs. surrounding natural vegetation. The leaf characteristics of the vine variety could also select for specific phenotypes of *K. aberrans* (Kreiter et al., 2002). Populations of another natural enemy, *Diaeretiella rapae* McIntosh (Hymenoptera: Braconidae), collected on several plants located <1 km from each other, were also very different (Vaughn & Antolin, 1998). Given the close relationships between phytoseiids, especially *K. aberrans*, and their host plants (Kreiter et al., 2002), we suggest that population differentiation could be due to selection pressure arising from plant-related factors. Further experiments must be conducted in order to confirm this hypothesis.

If females from surrounding areas dispersed into the experimental vineyard, then it seems that only mites with specific phenotypes (and genotypes) succeeded in colonising it. Such conclusions are similar to those of Roderick (1992, 1996) considering the evolution of phenotypes during post-colonization. This study also suggests that selection limits colonization. The RAPD tests confirm the results obtained from earlier artificial releases of *K. aberrans* showing that released mites rarely settle well (Kreiter et al., 1993). These conclusions have implications for the biological control of mite pests and emphasize the importance to identify factors that may contribute to the settlement of *K. aberrans* and of phytoseiid mites in general.

Is settlement of migrants within plots always achieved?

The settlement of migrants may be a limiting factor for the colonisation process, confirming the results obtained when releases of *K. aberrans* were conducted (Kreiter et al., 1993). In order to explain this, the susceptibility of mites living in vineyards and in the surrounding natural vegetation towards a pesticide (quinalphos) was studied (Tixier & Kreiter, 2003). Pesticide resistance was observed for the two populations tested. The population collected on oak in the woody margin and that from the experimental vineyard had resistance coefficients of 52 and 313, respectively, and their regression slopes were significantly different from that of the reference.

Even if quinalphos has been applied regularly only 3× a year since 1998, this short period has been sufficient to allow resistance selection and development. Selection is probably promoted by multiple pesticide applications per year, the mites' pseudo-arrhenotokous sex determination system, and the existence of several generations per year. This resistance could also explain the higher competitiveness of *K. aberrans* in cultivated areas in comparison with other phytoseiids dispersing into the plot. Furthermore, it was the first time that pesticide resistance was observed in populations living in an untreated environment outside cultivated plots. Pesticide drift could sufficiently affect neighbouring populations, to select resistant genotypes present in this neighbouring natural vegetation even if the resistance level is lower than that of the populations living in the experimental vine plots. Even if the LC_{50} of populations collected on vine and oak are different, high mortality was observed for both populations treated at the recommended concentration (87 and 70% for females collected on oak and vines, respectively). This toxicity is certainly lower in field conditions and seems to show that quinalphos applications do not appear to affect migrants dispersing from oak more than mites already present in the vine plot. Hence, quinalphos spraying is probably not the main reason that explain that migrants do not settle well in vine plots. However, other pesticides, especially fungicides, are also applied and these may affect mite population dynamics (cumulative effects).

Further studies are thus needed in order to identify the other factors involved in the poor success of migrant settlement, as pesticide sprayings do not seem to be the only factor involved. Could reproduction incompatibilities between migrants and individuals already present in the plot explain the low gene flow observed? Do host plant shifts during dispersal of phytoseiids affect settlement in the vineyards? Indeed, several studies with more specific predators [e.g., *Neoseiulus fallacis* (Garman) and *Neoseiulus californicus* (McGregor)] show that host plants can influence and delay settlement (Castagnoli et al., 1999; Lester et al., 2000). Finally, what about the survival of mites during dispersal? Answers to these questions are needed in order to enhance the use of phytoseiids naturally occurring in vineyard surroundings.

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Does agroforestry affect phytoseiid mite communities in vineyards in the South of France?

Ziad Barbar, Marie-Stéphane Tixier, Brigitte Cheval & Serge Kreiter
ENSA.M–INRA, Unité d'Ecologie animale et de Zoologie agricole, Laboratoire d'Acarologie, 2 Place Pierre Viala, 34060 Montpellier cedex 1, France. E-mail: barbar@ensam.inra.fr

The abundance and diversity of phytoseiid mites were surveyed from April to September 2003-2005 in several grape crops in the South of France, with Grenache and Syrah cultivars, co-planted with rows of *Sorbus domestica* or *Pinus pinea* and in plots with monocultures of grapes. Densities of phytoseiid mites differed on the two tree species. *Pinus pinea* seemed to be a better host than *S. domestica*. *Typhlodromus exhilaratus* was the dominant species in the crops and on co-planted rows of *S. domestica* and *P. pinea*, whereas *T. phialatus* was the most abundant species in plots with monocultures of trees. Agroforestry management does not seem to affect mite diversity in vine plots. The densities of phytoseiid mites in vine crops may well be affected by the co-plantation of trees, especially in 2005. Although the densities observed during 2003 and 2004 were probably low due to very dry and hot climatic conditions, the agroforestry management seems to have had a significant impact on mite densities in 2005. Further experiments should be carried out to confirm this effect.

Key words: Phytoseiidae, *Typhlodromus exhilaratus*, *Typhlodromus phialatus*, vineyards, *Pinus pinea*, *Sorbus domestica*

This study deals with the impact of crop floristic diversification on phytoseiid mite communities in vineyards in the South of France. Many phytoseiid species are natural enemies well-known for their efficiency in controlling crop pests (McMurtry & Croft, 1997).

Plant diversity in uncultivated areas surrounding crops may increase natural enemy occurrence (density and diversity) (Altieri & Letourneau, 1982; Escudero & Ferragut, 1999; Zacarias & Moraes, 2002). Diversification of agrosystems could be achieved by the management of crop borders or of the vegetation inside the crop fields (Flaherty, 1969; Boller et al., 1988; Coli et al., 1994; Tsolakis et al., 1997; Lozzia & Rigamonti, 1998; Kreiter et al., 2000; Tixier et al., 2000a,b; Nicholls et al., 2001; Duso et al., 2004; Barbar et al., 2005).

Agroforestry management modifies microclimatic conditions, creates alternative habitats and foods, thereby affects arthropod diversity (Stamps & Linit, 1998). Although some studies have shown the importance of agroforestry system (trees and/or shrubs combined with crops) in pest management (Linit & Stamps, 1995; Altieri & Nicholls, 2002), little is known about the impact of agroforestry systems on communities of predatory mites in vineyards.

The present study aims to test the hypothesis that agroforestry grape plots have higher mite abundance and diversity than monocultural grape plots.

MATERIAL AND METHODS

The study site

The experiments took place in vine crops in Restinclières (15 km north of Montpellier, Hérault, France). Here two cultivars, Syrah and Grenache, were planted in 1997 on a reclaimed fallow (30 years old). Rows of *Sorbus domestica* L. and *Pinus pinea* L. were also planted within crops in 1997. Five to six phytosanitary treatments per year were applied (fungicides against powdery mildew and downy mildew, insecticides against *Scaphoideus titanus* Ball), with pesticides selected for their minimal side effects on phytoseiid mites, when possible. No acaricide was used during the 3-year study or before. Grape crops were surrounded by uncultivated areas mainly composed of *Pinus halepensis* Viller and *Quercus coccifera* L. Two kind of plots were included in the surveys: (1) plots including rows of *S. domestica* (grape crop I) or rows of *P. pinea* (grape crop II), (2) control plots comprising only grape, only *S. domestica*, or only *P. pinea* (Table 1).

Sampling

Sampling was done from April to September, 5× in 2003, 6× in 2004, and 3× in 2005. In grape crops I and II, 30 leaves per row of grape, *S. domestica*, and *P. pinea* (a branch of 10 cm of pine was considered the equivalent of a leaf) were collected randomly. In monocultures 60 leaves per plot were sampled. Leaves were put in plastic bags and brought back to the laboratory in a freezer. Phytoseiid mites were counted and removed from each leaf of grape and *S. domestica* using a binocular microscope at 40× magnification. For mite extraction from each twig of *P. pinea* Boller's (1984) 'dipping-checking-washing-filtering' method was used. To compare phytoseiid densities found on *P. pinea*, *S. domestica*, and vine, leaves were dried in a sterilizer at 50 °C during 2 days and then weighted. Phytoseiid abundance was recorded as numbers of phytoseiids per g dried leaves (Majer & Recher, 1988).

Mite identifications

All mites were slide-mounted in Hoyer's medium and identified with a phase-contrast and interferential contrast microscope, based on the taxonomic keys of the generic revisions of Typhlodrominae, Phytoseiinae, and Amblyseiinae (*Ambly-*

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Table 1 Characteristics of the sampled plots in the study site of Restinclières (Hérault-France).

	Experimental grape crop		Monocultural		
	I	II	grape crop	*P. pinea*	*S. domestica*
Surface (m²)	4,600	3,900	1,000	3,400	2,500
Plantation density (m)	2.5 × 1	2.5 × 1	2.5 × 1	3 × 2	4 × 3
Syrah cultivar (rows)	11	10	9	-	-
Grenache cultivar (rows)	9	12	11	-	-
Rows of *Sorbus domestica*	4	-	-	-	5
Rows of *Pinus pinea*	-	5	-	12	-

Table 2 Results of variance analysis and mean comparison test ($\alpha = 0.05$) on phytoseiid mite densities in sampled grape crops in Restinclières (Hérault - France).

Comparison	Kruskal-Wallis test	Newman-Keuls mean mite density	
Sampling date	P<0.001	2003	0.10b
		2004	0.06c
		2005	0.22a
Grape cultivars	P<0.001	Grenache	0.07b
		Syrah	0.14a
Grape crops	P = 0.05	Experimental grape crop I	0.11a
		Experimental grape crop II	0.10a
		Monocultural grape crop	0.10a

Means sharing a letter (within a 'comparison') are not significantly different.

seiini, *Neoseiulini*, *Kampimodromini*) (Chant & McMurtry, 1994, 2003a,b, 2004), and the catalogue of Moraes et al. (2004) for all other genera of Amblyseiinae.

Data analysis

Variance analysis (Kruskal-Wallis test), followed by a Newman-Keuls mean comparison test ($\alpha = 0.05$) (Statistica® version 7.1, 2005) were carried out to compare (1) mite density on vine, in grape crops I and II (agroforestry), and in the monocultural grape crops, (2) mite density on *S. domestica* and *P. pinea* rows in grape crops I and II, and in plots with tree monocultures, (3) mite density on the two grape cultivars, and (4) mite density on *S. domestica*, *P. pinea*, and grape (no. mites/g leaf dry weight).

RESULTS

Phytoseiid mite abundance in vine crops with and without agroforestry management

During 3 years, phytoseiid mites were found in all grape crops studied. Highest densities were observed in 2005 (mean no./leaf = 0.22) and the lowest in 2004 (mean no./leaf = 0.10) (Table 2). Mite densities in agroforestry-managed grape crops and those in monoculture groups did not differ significantly when the 3 years were grouped ($H_{2,18185} = 6.05$, P>0.05; Table 2). However, within each year, there were differences. In 2003, mite abundance did not differ between grape crop I and the grape monoculture, but a significant difference ($H_{2,6568} = 141.97$, P<0.001) was found between these two plots and grape crop II, with the lowest densities. In 2004, mite densities were highest in grape crop II ($H_{2,8313} = 47.78$, P<0.0001), but densities remained altogether low during this year. In 2005, densities were different in the three grape crops, with the higher densities in the agroforestry-managed grape crops I and II ($H_{2,3300} = 15.28$, P = 0.0005).

In all grape crops (agroforestry-managed or monoculture), mite densities were higher on Syrah than on Grenache ($H_{1,18185} = 98.78$, P<0.001; Table 2). The two cultivars have very different leaf architecture and this observation confirms the association between leaf characteristics (pilosity and domatia) and development (i.e., survival, fecundity) of various phytoseiid species (Duso & Vettorazzo, 1999; Kreiter et al., 2002).

Phytoseiid mite abundance on *Sorbus domestica* and *Pinus pinea*

Phytoseiid density on *S. domestica* (co-planted or not) was very low during the 3 years. On *P. pinea* a significant difference in phytoseiid density was observed between the two modalities (co-planted or not), when grouping the 3 year samples ($H_{1,2891} = 86.87$, P<0.001) or not (2003: $H_{1,1250} = 15.87$, P = 0.001; 2004: $H_{1,1200} = 112.06$, P<0.001; 2005: $H_{1,525} = 12.73$, P<0.0001).

Phytoseiid mite densities on *Pinus pinea, Sorbus domestica* and vine plants

Mite density on *P. pinea* (co-planted or not) during 3 years was significantly higher than on *S. domestica* ($H_{5,22088} = 523.85$, P<0.001). The highest densities were found in the *P. pinea* monoculture, then on *P. pinea* co-planted in grape crop II. Mite densities in the *P. pinea* monoculture were not significantly different from densities found on grape cv. Syrah.

Phytoseiid mite diversity

In all grape crops (agroforestry or not), *Typhlodromus* (*T.*) *exhilaratus* Ragusa prevailed (>98%) (Fig. 1). The other species [*Typhlodromus* (*T.*) *phialatus* Athias-Henriot, *Paraseiulus triporus* (Chant & Yoshida-Shaul), *Typhlodromus* (*T.*) *pyri* Scheuten] were only observed in vine plots in 2003.

Five phytoseiid species were found on co-planted *P. pinea*: *T. exhilaratus* (87%), *T. phialatus* (12%), and sporadically *Typhlodromus* (*Anthoseius*) *recki* (Wainstein), *Kampimodromus aberrans* (Oudemans) and *Neoseiulus bicaudus* (Wainstein). In the *P. pinea* monoculture, the main species was *T. phialatus* (97%).

Typhlodromus exhilaratus was the only species found on co-planted trees of *S. domestica*, whereas *T. phialatus* was the main species in the monoculture of *S. domestica* (67%).

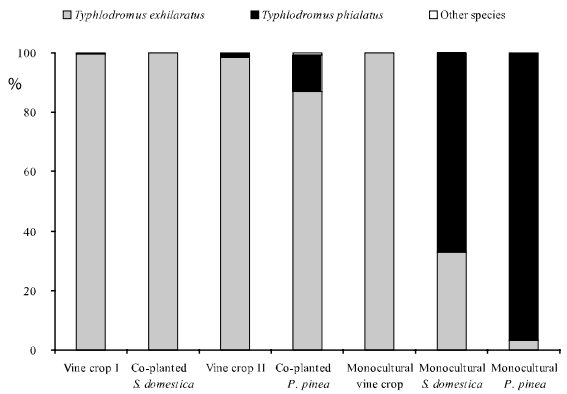

Figure 1 Distribution of Phytoseiid mite species (%) in the sampled plots in the study site of Restinclières (Hérault- France).

DISCUSSION

In the various grape crops, phytoseiid densities were different from year to year and within a same year. This variability in time could be due to different weather conditions between years and to the use of insecticides (i.e., June, 2003) potentially toxic for phytoseiid mites. Some conclusions can be drawn on the influence of agroforestry management on mite abundance and diversity in vine crops.

Sorbus domestica and *P. pinea* co-planted in vine crops or not could provide shelter to phytoseiid mites. However, different phytoseiid mite species were observed on co-planted or monocultures of trees. *Typhlodromus phialatus* was the prevailing species in monoculture tree plot (either *P. pinea* or *S. domestica*), whereas *T. exhilaratus* prevailed in co-planted trees of either species. *Typhlodromus phialatus* was also the prevailing species in uncultivated areas surrounding the experimental plots, whereas *T. exhilaratus* was scarcely found (Barbar et al., 2005). Even if previous studies on the same site showed dispersal of *T. phialatus* and *T. exhilaratus* in the plot (Tixier et al., 2006), it seems that only *T. exhilaratus* could develop both on grape and co-planted trees. Why? Interspecific predation between *T. exhilaratus* and *T. phialatus* could affect the settlement of *T. phialatus* in grape crops. Meszaros et al. (2007) showed in lab experiments that adult females of *T. exhilaratus* attacked larvae and protonymphs of *T. phialatus* and had a greater fecundity than *T. phialatus*. However, these lab data do not explain the dominance of *T. phialatus* in plots with monocultures of trees or in uncultivated areas surrounding grape crops. Thus, other factors seem to be involved. Some authors have assessed toxicities of pesticides on *T. exhilaratus* and *T. phialatus* populations (Castagnoli & Liguori, 1987; Grande & Ingrassia, 1988; Rodrigues et al., 2002). Barbar *et al*. (2007) studied fungicide and insecticide side effects on different populations on *T. phialatus* and *T. exhilaratus* from the same experimental site. They showed a better survival after insecticide (chlorpyriphos-ethyl) of *T. exhilaratus* than of *T. phialatus* (100% mortality at a lower concentration than the recommended rate), suggesting that pesticide application could act as a selective filter allowing the better settlement of *T. exhilaratus* than of *T. phialatus*.

Higher mite densities and a higher frequency of occurrence in time were observed on *P. pinea* than on *S. domestica*, both for *T. phialatus* and *T. exhilaratus* (on monocultural and co-planted trees, respectively). It thus seems that *P. pinea* is a more suitable host plant than *S. domestica* for both phytoseiid species. Phytophagous mites (Tetranychidae, Tenuipalpidae, Eriophyidae) have been observed on the two host plants under study (Barbar, unpubl.). Possibly, different densities of prey species (especially high densities of Tenuipalpidae on *P. pinea*) have a different impact on phytoseiid mite development on *S. domestica* and *P. pinea* (Kreiter et al., 1993; McMurtry & Croft, 1997; Duso & Vettorazzo, 1999). However, more specific studies are needed to test these hypotheses and to draw conclusions on the factors affecting phytoseiid densities on *S. domestica* and *P. pinea*.

In the present study, phytoseiid mites were observed on co-planted *S. domestica* and *P. pinea* and these trees could thus constitute a reservoir for these predators. However, one may wonder if they could constitute a better phytoseiid mite source than rows of grape vine. The densities of phytoseiid mites observed on co-planted *P. pinea* were similar to those observed on cv. Grenache, whereas densities observed on co-planted *S. domestica* were much lower. All factors being equal, a row of *P. pinea* would act as a row of Grenache, providing lower mite densities than a row of cv. Syrah. However, volumes of canopies (numbers of leaves, height of trees, plantation densities) of grape and tree rows are different. To assess a real comparison of mite abundance on vine and tree rows, the determination of the number of leaves per tree and per vinestock would be required.

In the present study, predatory mite abundance in vine crops planted with the two trees was sometimes higher than in the plot with a monoculture of grape. However, these data cannot be generalized, especially in 2003 and 2004. The results obtained in 2003 and 2004 do not allow definitive conclusions because of the particularly dry climatic conditions in 2003 affected densities of phytoseiid mites with a subsequent effect in 2004. In 2005, the populations increased again and during this year a positive effect of agroforestry on mite densities on grape was observed, irrespective of the tree planted. We can thus hypothesize that trees would act as reservoirs for these predators. To determine mite migration between vine and co-planted trees, a molecular typing was carried out. Although more adapted molecular markers have to be applied, the prelimilary results seem to show a dispersal between the populations collected on grape vines and the co-planted trees (Barbar, 2007).

In conclusion, our starting hypothesis was that agroforestry management affects mite density and diversity. This first study concerning impact of agroforestry on grape crop phytoseiid communities showed that: (1) mite density seems to be affected but only in 2005, when mite densities were higher and when impact of external factors was low (low humidity associated with temperatures as high as in 2003), and (2) agroforestry management does not affect phytoseiid mite diversity in grape plots. However, these trends have to be confirmed by further experiments.

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Manipulating plant-arthropod conversations to improve conservation biological control of mites

David G. James, Sandra Castle Del Conte, Tanya S. Price, Tessa R. Grasswitz, Lawrence C. Wright, Victor Reyna, Oscar Garcia & Joe Perez

Department of Entomology, Washington State University, Irrigated Agriculture Research and Extension Center, 24106 North Bunn Road, Prosser, WA 99350, USA. E-mail: david_james@wsu.edu

The potential of using synthetic herbivore-induced plant volatiles (HIPV) (e.g., methyl salicylate, MeSA) as a cultural tool to enhance conservation biological control of mites is being researched in commercially grown hops in Washington State, USA. Compared to unbaited blocks, hop yards baited with controlled release sachet (CRS) dispensers of synthetic MeSA recruited larger (3-5×) populations of spider mite predators: *Stethorus* spp. (Coleoptera: Coccinellidae), *Orius tristicolor* (Hemiptera: Anthocoridae), *Geocoris pallens* (Hemiptera: Geocoridae), *Deraeocoris brevis* (Hemiptera: Miridae), Chrysopidae, Hemerobiidae, Nabidae, Thripidae. The enhanced community of mite predators controlled spider mite populations in MeSA-baited hops without miticide intervention. All unbaited blocks required at least one miticide for spider mite control. Direct application of natural MeSA (oil of wintergreen) to hop plants contained in canola oil or rosemary/peppermint oil pesticide formulations, resulted in greater attraction of two mite predators (*O. tristicolor* and *Stethorus* spp.) to treated than to untreated plants. MeSA-mediated stimulation of the plants to produce predator-attracting volatiles is suggested as the likely mechanism. The use of synthetic or natural versions of HIPV/plant-signaling compounds like MeSA as 'Herbivore-Induced Plant Protection Odors' (HIPPOs), has the potential to provide a novel yet practical strategy for improving the efficacy and reliability of conservation biological control of mites in a variety of agricultural ecosystems.

Key words: HIPV, HIPPO, methyl salicilate, synthetic lure, hop, multitrophic interaction, integrated mite management

The use of conservation biological control (CBC) as a component of integrated mite management in agriculture is a strategy that is increasing in importance and popularity. Concurrent with the increasing use of CBC in agriculture has been a realization that 'generalist' natural enemies (i.e., those that have a broad prey preference) can often play a major role in mite suppression (James, 2001). Thus, CBC as a strategy that enhances guilds or communities of both specialist and generalist natural enemies is now viewed as a mite management strategy, very likely to improve crop protection. Another factor that has encouraged and enhanced the use of CBC in many crop systems is the availability and use of pesticides that are narrow-spectrum and safe to many beneficial insects and mites (James, 2002, 2003d, 2004).

CBC research in many crop systems is focused on improving reliability by strengthening the natural enemy community both in terms of population density and species diversity (Cardinale et al., 2003). Inevitably there are two aspects of this problem that need to be addressed: (1) attraction of beneficial arthropods to the crop during early cropping phases, and (2) maintenance of these populations throughout the life of the crop. Kean et al. (2003) identified 'spatial attraction' of natural enemies as the best way of enhancing CBC. Their results suggested an almost linear relationship between natural enemy attraction and prey equilibrium. The use of semiochemical attractants (e.g., host/prey-derived chemicals) to increase recruitment and retention of beneficial arthropods in crop ecosystems, is an area of opportunity for enhancement of CBC.

Herbivore-Induced Plant Volatiles (HIPV) offer the best potential for developing effective and practical semiochemical-based strategies for manipulating natural enemy populations. Plants attacked by herbivores emit specific chemical signals. These are the 'words' of a complex language used to 'warn' other plants of impending attack and to recruit predatory/parasitic arthropods for 'bodyguard' services. Such plant 'bodyguards' respond to plants in distress, and benefit from the food/host resources available (Sabelis et al., 1999). A voluminous body of literature now exists on this phenomenon, first demonstrated in a series of elegant laboratory investigations based on a bean plant-spider mite-predatory mite system (Sabelis & van de Baan, 1983; Sabelis et al., 1984; Sabelis & Dicke, 1985). The qualitative and quantitative characteristics of HIPV can vary according to the herbivore involved, the plant species, and even genotype (Turlings et al., 1993, Takabayashi et al., 1994). HIPV may function as direct attractants and/or as plant signals. Airborne or topically applied methyl jasmonate (MeJA) can act as a plant signal by causing the emission of volatiles in some plants mimicking those produced in response to herbivore damage (Hunter, 2002). There is some evidence that methyl salicylate and hexenyl acetate may also function as plant signals (Shulaev et al., 1997; Ozawa et al., 2000; Engelberth et al., 2004). The use of HIPV as signalers or elicitors of 'correct' and complete blends of natural enemy attracting emissions, is an attractive possibility for manipulating predator populations for mite management.

Compared with the abundance of laboratory studies on HIPV, there is a dearth of field-based studies (Hunter, 2002). The first demonstration of the impact of HIPV in the field came from research on biocontrol of psyllids in pear orchards in The Netherlands (Drukker et al., 1995), which showed that densities of predatory bugs (Anthocoridae) increased with the density of caged psyllids. Shimoda et al. (1997) recorded more predatory thrips on sticky cards near spider mite-infested bean plants than on traps near uninfested plants. Bernasconi et al. (2001) trapped more natural enemies near plants damaged and treated with caterpillar regurgitant, than near undamaged, untreated plants.

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The first direct evidence for the potential of synthetic HIPV as field attractants for beneficial insects came from this research group (James, 2003a,b,c) which demonstrated attraction of a number of insect species and families to methyl salicylate (MeSA) and (Z)-3-hexenyl acetate (HA) in Washington hop yards. Mite predators attracted to MeSA included *Chrysopa nigricornis* Burmeister (Chrysopidae), *Geocoris pallens* Stål (Geocoridae), and *Stethorus punctum picipes* (Casey) (Coccinellidae). Three predators were attracted to HA, a predatory mirid, *Deraeocoris brevis* (Uhler), an anthocorid, *Orius tristicolor* (White), and *S. punctum picipes*. Subsequent synthetic HIPV/trapping studies revealed that at least 13 species or families of beneficial insects responded to one or more synthetic HIPV (James, 2005). Thirteen HIPV attracted one or more species/family of beneficial insect.

Evidence for recruitment and retention of beneficial insects in grapes and hops using controlled-release (CR) dispensers of MeSA, was presented by James & Price (2004). In a replicated experiment conducted in a juice grape vineyard, sticky cards in blocks baited with MeSA captured significantly greater numbers of five species of mite predators (*C. nigricornis*, *Hemerobius* sp., *D. brevis*, *S. punctum picipes*, *O. tristicolor*) than unbaited blocks. Monitoring conducted in a MeSA-baited hop yard indicated development and maintenance of a beneficial arthropod population that was nearly four times greater than that in an unbaited reference yard. The large population of mite predators in the MeSA-baited hop yard was associated with a dramatic reduction in spider mite numbers, and sub-economic populations were maintained for the rest of the season. The evidence presented in James & Price (2004) is highly suggestive that the use of controlled-release MeSA in a crop could increase recruitment and residency of populations of mite predators. Here, we report additional data from field experiments on the use of synthetic HIPV to enhance CBC of spider mites.

MATERIALS AND METHODS

Recruitment of mite predators to hop yards

Controlled-release dispensers containing synthetic MeSA (5 g, 98%, Chem-Tica International, Costa Rica) were deployed in four hop yards in south-central Washington State during May-September 2004. A control yard with similar characteristics (size, variety, etc) was also established, 1-2 km from each MeSA site. Dispensers were stapled to supporting posts (ca. 0.5 m above ground) in the yards. Dispenser deployment density for the hop yards was 180 (A), 447 (B), 516 (C), and 556/hectare (D). Mite predator and spider mite populations were monitored weekly at each site (including controls) by examining leaf samples, conducting canopy shake sampling and by retrieving/replacing four yellow sticky cards stapled to poles; see James & Price (2004) for full sampling methodology. Insecticide and miticide applications were kept to a minimum at all sites and where possible, chemicals known to have minimal effect on mite predators were used. Sticky cards were positioned randomly within each hop yard or vineyard and separated by at least 10 m. After collection, they were examined in the laboratory and all beneficial insects identified and counted. Trap data were analyzed using either the Mann-Whitney Rank-Sum Test, or the Kruskal-Wallis ANOVA on ranks.

Attraction of mite predators to hop plants sprayed with pesticide/MeSA formulations

In order to test the hypothesis that MeSA may recruit mite predators to crops via plant signaling we conducted experiments using spray formulations containing small amounts of natural MeSA (oil of wintergreen), applied to hop plants during spring prior to mite colonization. During May-June 2005, groups of four hop plants (cv. Columbus, Chinook, and Mt Hood) (three replicates of each variety in a randomized block design) were sprayed weekly with a Canola oil/2% MeSA formulation, or left unsprayed. Abundance of winged mite predators (e.g., lacewings, minute pirate bugs, etc.) was monitored using yellow sticky cards examined/replaced weekly. During May-July 2006, individual hop plants (cv. Mt Hood, Chinook) (three replicates of each variety in a randomized block design) were sprayed weekly with Canola oil/2% MesA, Canola oil only, Ecotrol® – a commercial pesticide product from EcoSMART Crop Protection, TN, USA, containing rosemary/peppermint oils and MeSA (oil of wintergreen) –, or left unsprayed. The abundance of mite predators was monitored as described above. Trap data were analyzed using either the Mann-Whitney Rank-Sum Test, or the Kruskal-Wallis ANOVA on ranks.

RESULTS

Recruitment of mite predators to hop yards

All of the unbaited control yards were treated with at least one miticide (for twospotted spider mite, *Tetranychus urticae* Koch) and/or insecticide [for hop aphid, *Phorodon humuli* (Schrank)]. The control yard for site B was treated with abamectin and imidacloprid, pesticides known to be harmful to some mite predators (James, 2001; James & Vogele, 2001), thus this pair was not used in this analysis.

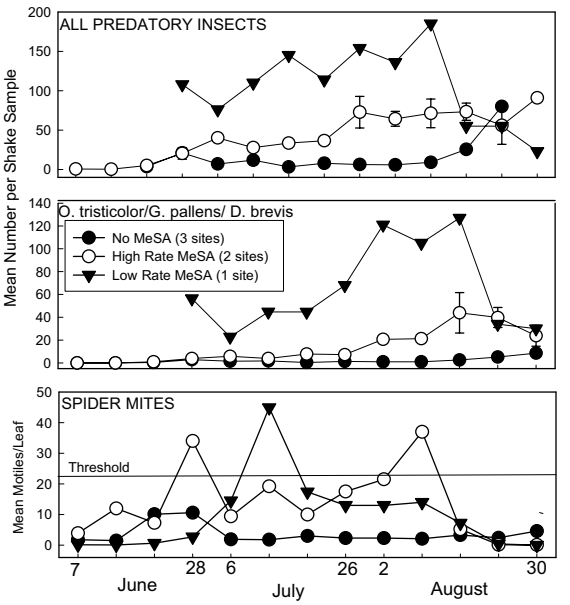

Figure 1 Mean (± SE) abundance and phenology of mite predators and spider mites in hop yards baited with low (180 dispensers/ha) and high (516 or 556 dispensers/ha) deployment rates of MeSA or left unbaited.

The remaining control yards were treated with bifenazate and/or pymetrozine, both of which are considered safe to most mite predators in hops (James, 2002). MeSA sites A and D were not treated with any insecticide/miticide, whereas site C received one application of the aphicide, pymetrozine and another of *Bacillus thuringiensis*.

The MeSA-baited hop yards developed larger populations (3-5×) of predatory insects than corresponding unbaited yards (Figs 1 and 2). Mite predator species that were significantly more abundant in MeSA-baited yards than unbaited yards included *O. tristicolor*, *G. pallens*, *D. brevis*, and *Stethorus* spp. (Fig. 2). Other mite predator species and families recorded and quantified but pooled here as 'predatory insects' included lacewings (Chrysopidae, Hemerobiidae), lady beetles (Coccinellidae), predatory thrips (Thripidae), and damsel bugs (Nabidae). Numbers of the hemipteran mite predators, *O. tristicolor*, *G. pallens*, and *D. brevis* were combined and were six times more abundant in the high deployment rate MeSA yards than in the corresponding control yards (Fig. 2). The difference was even greater between the low deployment rate yard and corresponding control yard (21.5×). Similarly, mite-eating lady beetles (*Stethorus* spp.) were 23.5× more abundant in the low-rate MeSA yard than in the control yard (sticky card data) (Fig. 2). Predatory insect abundance was greater and earlier in establishment in the low-rate yard than in the high-rate yards (Fig. 1). Spider mite populations in the MeSA-baited yards briefly exceeded the recommended miticide spray threshold (Fig. 1). In the low-rate MeSA yard spider mites exceeded 40 motiles/leaf for a week in early July but stayed below the threshold for the rest of the season. Similarly, populations in the high-rate yards briefly climbed above 30 motiles/leaf in late June and again in mid-August. However, hop cone yield and quality were not affected in the MeSA yards and were comparable to those obtained in the non-MeSA yards. Large populations of predatory insects remained in the MeSA-baited yards during August despite the low numbers of spider mites. Other arthropod prey (e.g., thrips) was also present and may have helped sustain the generalist mite predator community.

Attraction of mite predators to hop plants sprayed with pesticide/HIPV formulations

The anthocorid hemipteran, *O. tristicolor*, was the only mite predator present in sufficient numbers in the 2005 experiment to evaluate. The abundance of this predator was approximately 4-8× greater on sticky cards attached to Canola oil/MeSA-treated Mt Hood and Chinook hop plants than on those attached to untreated plants of the same varieties. In contrast, there was no difference in abundance of *O. tristicolor* on cards attached to Columbus hop plants treated with Canola oil/MeSA or left untreated (Fig. 3).

In 2006, 2-3× as many *O. tristicolor* were captured on sticky cards attached to Canola oil/MeSA, Canola oil only, and Ecotrol®-treated plants (both varieties), than on untreated plants. The mite-eating lady beetle (*Stethorus* spp.) was 2-4× more abundant on sticky cards attached to Ecotrol®-treated plants (both varieties) (Fig. 4).

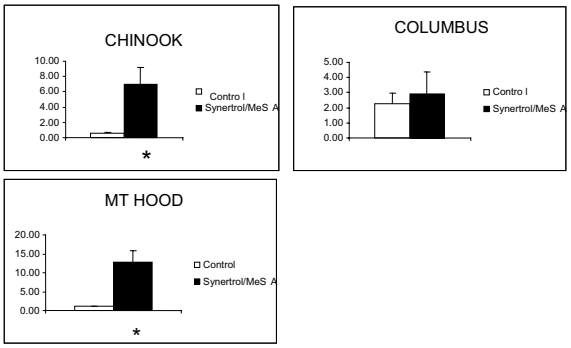

Figure 3 Attraction of *Orius tristicolor* to hop plants (three cultivars: Chinook, Mt Hood, Columbus) sprayed weekly with a canola oil (Synertrol®)/2% MeSA formulation or left untreated (Mean + SE number/sticky card/week). Asterisks indicate significant difference from control (P<0.05).

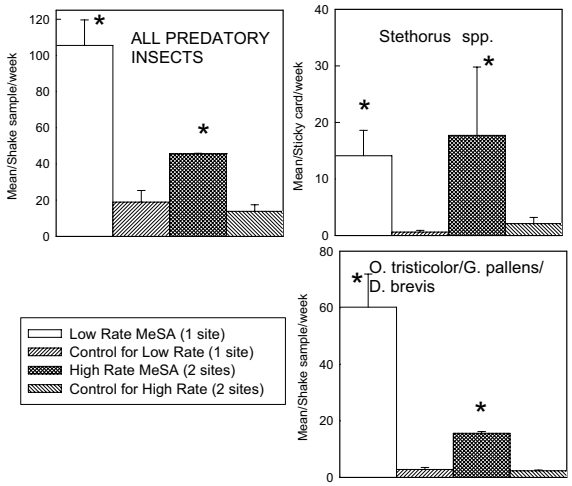

Figure 2 Mean (+ SE) abundance of mite predators (all taxa combined), *Stethorus* spp., *O. tristicolor*, *G. pallens*, and *D. brevis* in MeSA-baited and unbaited hop yards during May-September 2004. Columns marked with asterisks are significantly greater than the corresponding control column (P<0.05).

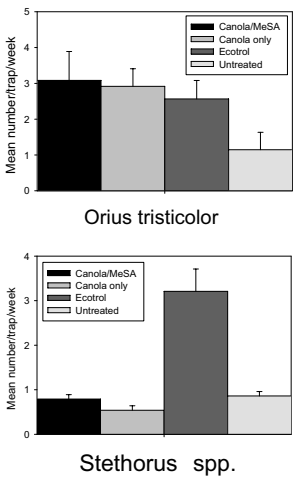

Figure 4 Attraction of *Orius tristicolor* and *Stethorus* spp. to hop plants (Mt Hood and Chinook, data combined) sprayed weekly with a canola oil (Synertrol®/2% MeSA) formulation, Ecotrol®, or left untreated. All treatments were significantly different from untreated (P<0.05) for *O. tristicolor*. Ecotrol was significantly different from untreated for *Stethorus* spp. (P<0.05).

DISCUSSION

The potential of CR dispensers of MeSA for enhancing the recruitment and arrestment of mite predators in crops (James & Price, 2004), is further supported by the field data presented here. In addition, the attraction of some mite predators like *O. tristicolor* and *Stethorus* spp. to hop plants sprayed with pesticide-type products containing small amounts of MeSA, raises the possibility that direct application of synthetic or natural HIPV to plants may be an alternative, effective, and practical strategy for attracting mite predators and improving mite management in crops

Hop yards baited with CR dispensers of MeSA at deployment rates of 180-642/ha recruited and harbored larger populations of predatory insects than nearby and comparable yards without dispensers, as indicated by canopy shake sample, sticky card, and leaf sample data. The data presented here are only a portion of the total data sets collected for each yard, but are typical of the results obtained. Mite predator species that appeared to respond most strongly to MeSA included *Stethorus* spp., *O. tristicolor*, *G. pallens*, and *D. brevis*. During August up to 200 *Stethorus* spp. per sticky card/week were recorded from MeSA-baited hop yards, and 100-150 predatory bugs (*O. tristicolor*, *D. brevis*, *G. pallens*, Nabidae) could be shaken from *each* hop plant sampled. The largest populations of predators occurred in the hop yard baited with the lowest number of MeSA dispensers (180/ha), suggesting that the higher deployment rates used (447-642) may have been sub-optimal. It is possible that the atmospheric concentration of MeSA in these yards, particularly during spring and early summer, was too high for optimal predator attraction. Predator populations in these yards during July, although higher than in non-MeSA yards, were not as high as in the low deployment rate yard. None of the MeSA-baited hop yards were treated with a miticide and despite short-lived increases in mite populations above the spray threshold, acceptable commercial outcomes in terms of hop cone yield and quality were achieved. The best result was achieved in the low deployment rate yard where mite numbers remained below the spray threshold for all of the cone maturation period (late July-September). The slightly larger populations of spider mites permitted to develop in the MeSA-baited yards (compared to the miticide-treated unbaited yards) may have aided predator recruitment during the summer by direct (numerical aggregation) or indirect means (natural production of HIPV). However, early-season spider mite populations in baited and unbaited yards were similar, but the size of predator populations was already differing by late June. Most of the mite predators attracted to MeSA-baited hops were generalist feeders. This community of predators will develop and maintain populations even if mites are not abundant as long as alternative prey is available. Another possible advantage of not attracting specialist predators is avoiding selection *against* responding to synthetic MeSA if nutritional rewards are inadequate.

The experiments involving direct application of MeSA to hop plants via incorporation within a pesticide-type spray treatment, indicated that the effect of MeSA may be more than just direct attraction of mite predators. The minute pirate bug (*O. tristicolor*) and mite-eating ladybeetle (*Stethorus* spp.) are unlikely to have responded to the actual sprays which dissipated within minutes of application. It is more likely that these mite predators responded to HIPV produced by the hop plants in response to exposure to MeSA. Evidence of a signaling function for MeSA, helping plants to recruit 'bodyguards' does exist. Shimoda et al. (2002) provided evidence that gaseous MeSA elicits the production of volatiles from bean leaves that are attractive to a predatory thrips species. James & Grasswitz (2005), in a vineyard study, showed attraction of two parasitic wasp species to plants exposed to MeSA. These wasps are not attracted directly to MeSA and the authors suggested they responded to HIPV produced by grape plants in response to MeSA exposure. Interestingly, in our direct application study, significant attraction of *O. tristicolor* and *Stethorus* spp. occurred with two hop cultivars (Chinook, Mt Hood), but not with the third (Columbus). Different plant cultivars have been shown to differ quantitatively and qualitatively in HIPV production and MeSA-mediated induction of HIPV may not have occurred in this hop variety.

A significant response by *O. tristicolor* was recorded for the canola oil only-treated plants as well as for the canola oil/MeSA-treated plants. The canola oil used was a commercial preparation (Synertrol®, Organic Crop Protectants, Sydney, Australia) that may have contained plant essential oils, terpenoids, and/or other components. Further studies are required to determine whether one or more of these components has a plant-signal function. Ecotrol® is an insecticide/miticide marketed by EcoSMART Crop Protection in the USA for use in agricultural crops against a broad range of pests. Its listed active ingredients are rosemary oil and peppermint oil. So-called 'inert' ingredients include an 'essential oils blend' containing an unknown amount of MeSA (oil of wintergreen). The attraction of *O. tristicolor* and *Stethorus* spp. to Ecotrol®-treated plants may have occurred as the result of MeSA or other essential oil-mediated plant-signaling. Although Ecotrol® is not marketed as a product that attracts natural enemies of pests or aids in biological control, anecdotal reports from growers and pest scouts often remark on a 'resurgence' of beneficial insects after application. Unwittingly perhaps, EcoSmart Crop Protection may be providing a 'pesticide' to growers that has a plant-signaling function which improves plant defences by recruiting predators.

The direct application results while raising the possibility of plants stimulated to produce HIPV in the presence of synthetic MeSA, does not provide the evidence needed to confirm this. Measuring and analyzing the volatiles produced by hops and other plants exposed or not exposed to synthetic MeSA should provide definitive evidence for the existence or not of this mechanism and such studies are planned.

The possible use of synthetic HIPV like MeSA either as direct or indirect enhancers of natural enemy population levels in crops (Herbivore-Induced Plant Protection Odors: HIPPO), is an exciting prospect. Recent studies (James, 2003a,b, 2005; James & Price, 2004) as well as the present work have provided evidence for the potential use of synthetic HIPV as aids to enhancing conservation biological control in crop ecosystems. However, many questions surrounding the use of these materials in integrated pest management remain to be answered. For example, what are the ecological consequences of providing synthetic HIPV to predators in the absence (or relative absence) of their prey? Will this 'misinformation' result ultimately in non-response by natural enemies to HIPV? As noted above, most if not all of the predatory insects attracted to synthetic MeSA in Washington State hop yards are generalist-feeding species (James, 2003a, b, 2005; James & Price, 2004) and the misinformation issue may not be as important with these species

as it might be to predators with a narrower host range, like *Stethorus* spp. Defining and understanding the mechanism(s) of attraction and recruitment of predatory and parasitic insects by synthetic HIPV, will be of paramount importance in the effective use of these materials in crop pest management. The data in this study suggest that using synthetic HIPV to signal plants to produce their own HIPV blends is a possibility, but more extensive laboratory and field experimentation is required before this can be confirmed. Optimal deployment (release rates, dispenser density) of synthetic HIPV for natural enemy recruitment and retention, will require a good understanding of the precise mechanisms mediating attraction of predators and parasitoids. Comprehensive studies are planned and will be reported in due course.

Acknowledgements

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Status of coconut mite *Aceria guerreronis* and biological control research in Sri Lanka

L.C.P. Fernando & N.S. Aratchige
Coconut Research Institute, Lunuwila, Sri Lanka. E-mail: head_cpd@cri.lk

The coconut mite, *Aceria guerreronis* (Acari: Eriophyidae), invaded Sri Lanka in 1997, and subsequently spread in many areas infesting 17% of the total coconut extent by 2005. It is more prevalent in the dry zone than in the intermediate and wet zones. Within each year, populations fluctuated with high peaks in June-August and relatively low levels in November-February. *Neoseiulus baraki* and *N. paspalivorus* (Phytoseiidae) are the most common predatory mites found beneath the bracts of infested coconuts; *N. baraki* is found in higher proportions in drier areas, *N. paspalivorus* in wet and intermediate areas, occasionally in cooler pockets of the dry areas. Populations of *N. baraki* constantly increased in the period 2000-2005 and their fluctuating numbers followed a pattern similar to that of coconut mite. This strong interrelationship suggests that *N. baraki* is a prospective candidate for control of coconut mites. Its use involves mass breeding and field release techniques. It turned out that *N. baraki* could be mass-reared in laboratory arenas on *Tyrophagus putrescentiae* (Acari: Acaridae). Mass releases of lab-reared *N. baraki* increased its numbers and reduced coconut mite numbers in the field, up to a period of 4-6 weeks after release. Also the use of the entomopathogenic fungus *Hirsutella thompsonii* was explored. The most effective isolate persisted for up to 16 weeks on the nuts. Over 90% of the nuts had low numbers of live coconut mites up to 4 weeks after treatment. The trials indicate that either biological control agent lacks the ability to suppress the coconut mite for a longer period. Hence, frequent applications of these natural enemies and/or interventions by use of low toxic chemicals may be required for sustainable control. Directions in biological control research of coconut mite are discussed.

Key words: *Aceria guerreronis*, biological control, coconut, distribution, population fluctuations, Sri Lanka

Coconut (*Cocos nucifera* L.) supports livelihood of millions of small and marginal farmers of the tropical world. It is the major source of dietary energy of many Asians and supplies raw material for a number of industries, such as coir manufacture, copra processing, oil milling, and confectionaries. The contribution of Asia and the Pacific region to the world coconut production is enormous, sharing 84.6% of total production and 87% in total world area with coconut (Coconut Statistics, 2004). The four major coconut growing countries – Indonesia, Philippines, India and Sri Lanka – alone contribute 77% to the world production and Sri Lanka is the fourth largest producer in the world. An area of 0.44 million ha (6.4% of the country) is under coconut, which is mainly grown in the coastal belt and extending interior to the north-west. Production in 2005 was 2576 million nuts and nearly 80% is consumed locally (Sri Lanka Coconut Statistics, 2005). Invasion of coconut mite, *Aceria guerreronis* Keifer (Eriophyidae), into the Indian sub-continent during 1997-1998 created a major setback for the coconut industries of India and Sri Lanka (Fernando, 1998; Sathiamma et al., 1998) and the beginning of a significant threat to coconut in Asia and Pacific regions.

Distribution of coconut mite in Sri Lanka

Distribution pattern

In Sri Lanka, coconut mite was first reported in late 1997 from the Kalpitiya peninsula of the Puttalum District (North-western Province). Subsequently, it spread to the other parts of the district infesting nearly 6800 ha of coconut lands causing an outbreak by the end of 1998 (Fernando et al., 2002). During the following years it invaded the other coconut growing areas and at the end of 2005, 17% of the total coconut extent was infested. The incidence of the pest in different areas varied between 2 and 100% (Fig. 1). Strikingly, in Sri Lanka, coconut mite is more prevalent and spread at a higher rate in the dry-zone (<900 mm rainfall), than in the intermediate (900-1800 mm) and wet zones (>1800 mm). Currently, out of the total infested coconut lands 66% are in the dry-zone, whereas only 24 and 10% are in the intermedi-

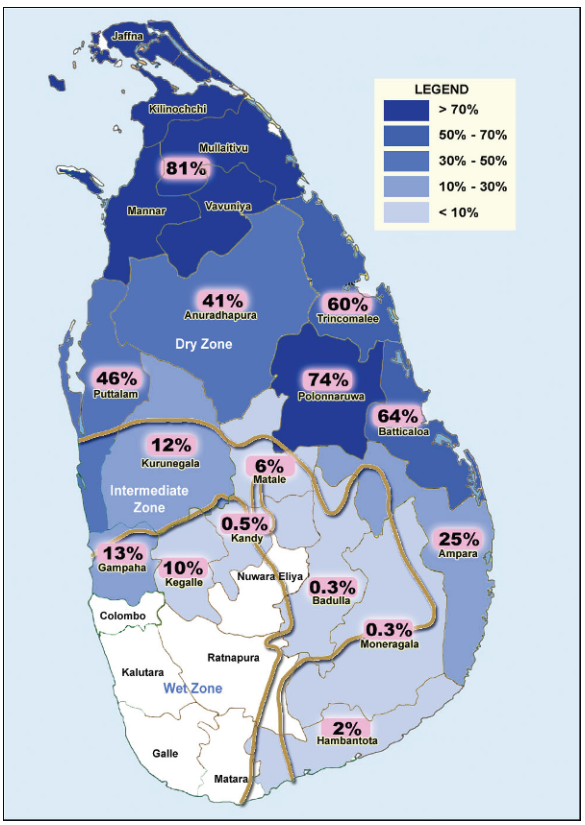

Figure 1 Percentage extent of coconut infested by coconut mite in different districts of Sri Lanka in 2005.

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ate and wet zones, respectively. Of the total coconut extent in each climatic zone, 42 and 10% of coconut lands are affected in the dry and intermediate zones, respectively, but only 2.5% in the wet zone, thus demonstrating the fast spread in the dry zone.

Annual and seasonal population fluctuations

Since knowledge on the abundance and fluctuation of coconut mite numbers annually and seasonally is crucial in planning control measures, a long-term study was initiated in two coconut-growing areas where the outbreak occurred initially, viz. Kalpitiya and Madurankuliya (Puttalum District). Mite censuses were done in February, June, August, and November, corresponding to the dry period after heavy rains, wet but less intense rainfall, dry, and wet with intense rainfall periods, respectively. The population dynamics of the coconut mite in the two sites during 2000-2005 is shown in Figure 2. Mean mite numbers varied from season to season and from area to area, and were lower at Madurankuliya than at Kalpitiya. In Kalpitiya, coconut mite populations increased from 2000 to 2003, but dropped in 2004, followed by a rapid increase in 2005. In Madurankuliya, populations increased during the first 3 years, declined during the next 2 years, and increased in the last year. Mean mite numbers peaked in June–August (Fig. 2).

Biological control research

The research programme on developing suitable management methods for coconut mite has been focused mainly on identifying (1) low toxic chemicals for short-term management and reducing the spread of the pest, and (2) biological methods for sustainable, long-term management. NeemAzal (azadirachtin 1%) (Wickramananda et al., 2003) and a mixture of neem oil and garlic (Fernando et al., 2002) were effective in reducing the pest population to about 60%. A mixture of 30% 'used engine-oil' completely controlled the coconut mites on treated nuts (Chandrasiri & Fernando, 2004). However, these chemicals require repeated applications at frequent intervals to keep the pest suppressed for a longer period. Due to the nature of the coconut palm (tall tree), the pest habitat (nuts), and the Sri Lankan coconut grower (small holder), the use of chemicals is impractical, uneconomical, and environmentally unsafe. Hence, research on developing biological methods was given the highest priority. In this direction predatory mites and the entomopathogenic fungus *Hirsutella thompsonii* Fisher were considered prospective candidates.

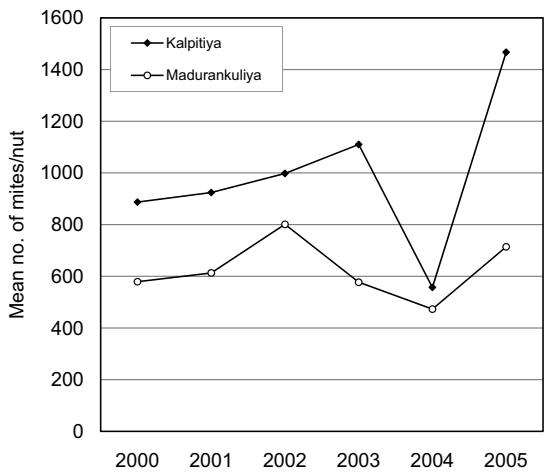

Figure 2 Mean number of coconut mites per nut in Kalpitiya and Madurankuliya during 2000-2005.

Predatory mites

Many predatory mites associated with coconut mite have been reported (Moraes & Zacarius, 2002; Singh & Rethinam, 2004). But the sheltered habitat where coconut mite colonies are usually found, i.e., the small gap between bracts and nut surface, lowers the accessibility for many of these predators. In Sri Lanka, *Neoseiulus baraki* (Athias-Henriot), *N. paspalivorus* (De Leon), A*mblyseius largoensis* (Muma) (Moraes et al., 2004), *Bdella* sp., and tarsenomid species are associated with coconut mite. The first three have been observed feeding on coconut mites. Commonly, *N. baraki* and *N. paspalivorus* are found in colonies of coconut mite beneath the bracts, whereas *A. largoensis*, a larger mite than the other two, is mostly seen outside the perianth of infested nuts.

Although *N. baraki* and *N. paspalivorus* are morphologically very similar, they have distinct geographical distributions. A survey carried out in 2005-2006 during both dry and wet periods in various agro-climatic regions where coconut mites are a pest, indicated that *N. baraki* is the most abundant species. It is found in all infested areas, but its ratio to *N. paspalivorus* varies. It occurs at a higher proportion in the dry zone, whereas *N. paspalivorus* is mainly confined to the wet zone, where its ratio to *N. baraki* is much higher. In the intermediate zone both species occur almost in equal proportions. Interestingly, *N. paspalivorus* was also found in the dry zone, in isolated pockets closer to water bodies such as lakes and irrigation channels (Table 1). In the areas where both species were recorded they were found in the same plantation and occasionally on the same nut.

Neoseiulus baraki *as a prospective candidate*

Neoseiulus baraki has several morphological and behavioural adaptations that make it a potential candidate for biological control of coconut mites. They do not prefer too much light and their bodies are flat (Moraes & Zacarias, 2002), with short distal setae (Moraes et al., 2004) which is an ideal morphological feature of a predator whose prey is in refuge inside a narrow habitat, such as under the perianth. Spatial and temporal distribution patterns showed that on infested palms the mean numbers of *N. baraki* followed a similar pattern to that of coconut mites in association with the maturity of nuts, but they reached a peak 1 month later than the coconut mites, suggesting a typical predator-prey interaction (Fernando et al., 2003). The strong influence of *N. baraki* on populations of coconut mites over time suggested that it might be a candidate for biological control of coconut mite.

According to the categorization of Gerson et al. (2003), *N. baraki* is more like a type III generalist predator species. Under laboratory conditions it developed well on pollen, but did not reproduce successfully on this food (Fernando et al., 2004). Presence of *N. baraki* in Algeria (Athias-Henriot, 1966), Thailand (Ehara & Bhandhufalck, 1977), Taiwan (Tseng, 1983), and China (Wu, 1986) where coconut mite is not reported confirms its generalist nature. However, in Sri Lanka, other than from coconut, *N. baraki* has been collected only from fruits of *Borassus flabellifer* and once on *Thangaseis calipia* (Moraes et al., 2004). Although generalist predators are not usually considered in biological control programmes, certain characteristics could be considered in using them as biological control agents, such as their habitat preferences. Although type III phytoseiids do not seem to

Table 1 Distribution of *Neoseiulus baraki* and *N. paspalivorus* in different areas of Sri Lanka in 2004-2005.

Climatic zone	District	Area	Incidence (%)	
			N. baraki	*N. paspalivorus*
Dry zone	Puttalum	Kalpitiya	100	0
		Madurankuliya	100	0
	Anuradhapura	Rajangane	100	0
	Polonnaruwa	Polonnaruwa	100	0
		Maduru-oya	100	0
	Ampara	Kohobana	100	0
		Nintavur	100	0
		Inginiyagala	81.6	18.4
	Hambantota	Tissamaharama	6.6	93.4
	Matale	Dambulla	82.2	17.4
	Puttalum	Madampe	48.6	51.4
Intermediate zone	Kurunegala	Ganewatte	98.0	2.0
		Narammala	85.8	14.2
		Makandura	53.0	47.0
Wet zone	Gampaha	Mirigama	10.6	89.4
		Weliweriya	6.4	93.6

specialize on eriophyoids they may occur at specific sites in a manner similar to specialized phytoseiids (e.g., *Phytoseiulus-Tetranychus* association) (Gerson et al., 2003). It seems that the occurrence of a large number of *N. baraki* on mite-infested coconut is strongly influenced by the anatomy of the perianth (bracts) of the nut they inhabit. Higher relative humidity and lower temperature beneath the perianth than in the surrounding environment, and protection from hyper-predators that cannot creep under the perianth may have influenced the presence of *N. baraki* beneath the bracts of coconuts. Occupation of both *A. guerreronis* and *N. baraki* in the same habitat indicated the potential use of *N. baraki* as a control agent of coconut mites.

A study in Sri Lanka revealed that the abundance of *N. baraki* varied from area to area but generally populations increased over time with two troughs in 2001 and 2004 (Fig. 3). Also the number of nuts with *N. baraki* increased over time. This result suggests that the predator has not come to a plateau and is still increasing in the field. Within each year significant seasonal variation was observed in predator densities with a peak population in June and August, which are generally months with less or no rainfall. Interestingly, mean predator numbers followed a similar trend to that of coconut mite, peaking a little later than the prey mite (Fig. 3) further indicating the strong relationship between the two.

Neoseiulus baraki has been reported on coconut palm from Puerto Rico (Howard et al., 1990) and Brazil (GJ de Moraes, pers. comm.), but its potential as a biological control agent of coconut mite has not been investigated. However, for the above reasons it was speculated that *N. baraki* can be utilized in an augmentative control programme for the management of coconut mite in Sri Lanka. This required developing technologies in mass rearing and field releases of *N. baraki*.

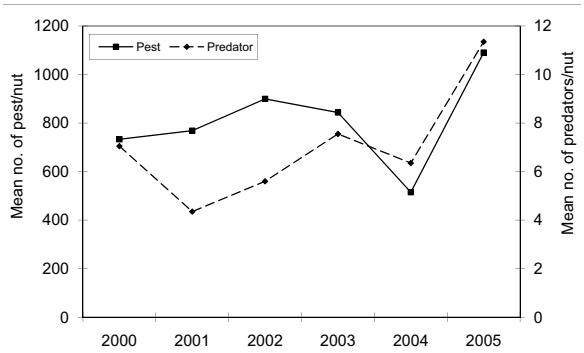

Figure 3 Population fluctuation of coconut mite (pest) and *Neoseiulus baraki* (predator) during 2000-2005.

Mass rearing and field releases of Neoseiulus baraki

Rearing of coconut mite, the natural host of *N. baraki* in Sri Lanka, in the laboratory is difficult and therefore alternative hosts were sought. The predator appeared to develop and oviposit satisfactorily on the flour mite, *Tyrophagus putrescentiae.* On *T. putrescentiae*, *N. baraki* laid on average 26.4 eggs, compared to 31.1 on coconut mite (Fernando et al., 2004). *Tyrophagus putrescentiae* developed well on a mixture of rice bran and wheat bran and could be reared on the same arena used for rearing *N. baraki*, i.e., a Scriven & McMurtry (1965) type arena, surrounded by a water barrier. On the arena the *N. baraki* population increased by a factor 24 in 3 weeks (Fernando et al., 2004). In mass production it could be reared in polypropylene sachets on *T. putrescentiae* (ADNT Kumara, pers. comm.) and on plastic trays of 43 × 32 cm, lined with black waterproof paper with the edge lined with insect glue.

The first experimental release of *N. baraki* was conducted in a coconut-mite-infested estate in North-western Province. On five palms each, 10,000 laboratory-bred *N. baraki* were released. In 8-12 weeks after release, the mean number of *N. baraki* increased significantly, and the mean numbers of coconut mites dropped in the treated block compared to the control block, until about 10 weeks after release – this reduction was statistically significant only during 4-6 weeks after release.

Hirsutella thompsonii as a prospective candidate

The entomopathogenic fungus *H. thompsonii* attacks several tetranychid and eriophyoid mites of many crop plants (Baker & Neunzig, 1968; McCoy & Selhime, 1974) including the coconut mite (Hall et al., 1980; Cabrera, 1982; Beevi et al., 1999). Hence, it is considered a potential control agent. A number of attempts have been made to control coconut mites with partial success (Espinosa-Becerril & Carrillo-Sanchez, 1986; Suarez, et al., 1989; Cabrera, 2002; Rabindra & Kumar, 2003). The variable effect of *H. thompsonii* on the coconut mite may be due to differences in isolates used and in prevailing climatic conditions. A survey in four districts of coconut-mite-,infested areas in Sri Lanka revealed that *H.*

thompsonii is found naturally on infested nuts but at a low incidence of <8%. In order to enhance levels of the fungus in the field to reduce coconut mite populations and their damage, studies were started on identifying effective local isolates of the fungus and the effect of field application.

Determination of effective isolates and field studies

Four Sri Lankan isolates with promising characteristics were tested, chosen by CABI-Bioscience in the UK. Isolate IMI 391722 had the highest ability to suppress the coconut mite population and persisted longest on the nuts. For up to 4 weeks the proportion of nuts with a high number of coconut mites stayed significantly lower on palms treated with IMI 391722 than on the untreated palms. With this isolate >90% of the nuts had dead coconut mites due to mycosis up to 10 weeks after the treatment. There was no indication of dissemination of the fungus from treated to untreated nut bunches of the same palm, nor of harmful effects on *N. baraki*. The effect of *H. thompsonii* isolates on coconut mite appeared localized and short term (Fernando et al., 2007). A study to investigate the effect of 2- and 3-monthly applications of IMI 391722 isolate indicated that damage levels could be reduced and kept at lower levels on treated than on untreated palms, although the reduction in coconut mite numbers by this treatment was not statistically significant.

Future directions in biological control of coconut mite

Our studies on *N. baraki* and *H. thompsonii* indicated that the effect of these two control agents on coconut mites could be enhanced by augmentative releases or inoculative applications. However, it seemed that they lack the ability to suppress coconut mite populations over a period longer than ca. 4 weeks after a single release or application. Therefore, how these agents could be utilized in a sustainable manner needs scrutiny.

Repeated releases or applications may be required to keep coconut mites at low levels. In the case of *H. thompsonii,* 2-3 monthly application showed no consistent reduction in coconut mite numbers over time, but damage on nuts at harvest was reduced. Importantly, this indicates that coconut mite numbers may not be directly related to the damage levels and interpretation of experimental results by numbers of coconut mites alone may not be reliable. Therefore, assessment of damage levels at harvest is essential to reliably assess the effect of any control agent on coconut mite. A coconut takes 10-12 months after fertilization to mature for harvest. Coconut mites colonize the nuts throughout this period, but peak populations are found on 5-6-month-old nuts (Fernando et al., 2003). Since the damage observed at harvest is the result of a large number of coconut mites feeding on the nuts for a long period of time, the numbers found at one point of time may not be directly related to damage levels at harvest. It is particularly disadvantageous in the case of coconut mite because sampling is destructive and population levels on a nut cannot be traced more than once. Further studies are needed of the relationship between coconut mite numbers and damage levels at harvest.

Repeated releases or applications require bulk production of *N. baraki* and *H. thompsonii*. Mass production techniques for *N. baraki* have been developed. A low-cost method of mass culturing of *H. thompsonii* and development of a possible biopesticide are progressing. Production of both these agents requires labor and involves a large cost, particularly the production of a biopesticide. Cost effectiveness of augmentative progammes requires attention and should be determined.

It appears that *N. baraki* or *H. thompsonii* ideally be integrated with other methods such as chemical control. In this respect initial reduction of the coconut mite population by low-toxic chemicals and subsequent application of biological control agents to maintain the pest at low levels may provide better results in the sustainable management of coconut mites. Selection of chemicals that do not affect *N. baraki* and *H. thompsonii* is important. Neem-based chemicals have only a slight effect on *N. baraki* (Fernando et al., 2002).

Although *N. baraki* is observed in higher numbers in prey patches, relatively few eggs are found in there, suggesting that the predator comes in to feed and/or seek shelter, but that it lays eggs elsewhere. Also, being an apparent generalist predator, it could have alternative food sources within the palm or on other plants associated with coconut (e.g., intercrops, cover crops, etc.). Conservation of the alternative food sources may be an important strategy to increase the natural population levels of *N. baraki* in the field. Hence, conservation biological control could be regarded as an important component in an integrated package of the control of coconut mite. So far, in Sri Lanka *N. baraki* was reported only from two host plants in addition to coconut (Moraes et al., 2004). It has rarely been seen on leaves and other parts of the coconut. An extensive survey of understory plants is suggested to identify other host plants and hosts of *N. baraki* to utilize them to conserve *N. baraki* in infested fields.

The differential distribution of *N. baraki* and *N. paspalivorus* in different agro-climatic regions in Sri Lanka is an important observation in the biological control of coconut mites. Several hypotheses may explain this. The presence of *N. paspalivorus* in relatively high proportions in wet and intermediate zones and in pockets close to water bodies in the dry zone suggests that it prefers cooler (micro-climatic) conditions. Alternatively, there may be differences in the vegetation (that involve alternative food for survival of the predators) in different agro-climatic regions. Or differences in characters of the nut/palm itself pertain to the climate or soil of the particular agro-climatic zone. These hypotheses remain to be tested and utilization of the two predatory mites in different areas could be considered.

Minute size and the absence of hind legs restricts the ambulatory movement of eriophyoid mites and this, in one way, is an ecological advantage to them as it restricts contact with acaropathogenic fungi (Sabelis & Bruin, 1996). On the other hand restricted ambulatory movement limits escape from predators which are usually bigger. One way of escaping predation is by spatial isolation in refuges (Sabelis, 1996). Most eriophyoid mites are refuge seekers/inducers and can live in places where they are less exposed to predators. Coconut mite is no exception to this. They live under the perianth where natural enemies have less access to them. This is an important feature that has to be considered especially in classical biological control, which is another option for control of coconut mite. Ideally, the agent should be flat enough to creep underneath the narrow space between the surface of the nut and the perianth of coconuts to reach prey colonies. The size of this space may vary depending on cultivar/variety, season, and location where the palm is grown. In other words, natural enemies that were selected from one location may not be the ideal candidate for controlling the coconut mite in a palm in another location. Therefore, size is crucial in selecting natural enemies for the control of coconut mite.

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Development of an economic rearing and transport system for an arid-adapted strain of the predatory mite, *Neoseiulus californicus*, for spider mite control

Eric Palevsky et alia
[For a complete list of authors and addresses see end of article]

Neoseiulus californicus (McGregor) is a relatively effective phytoseiid predator against spider mites reported from the Americas (North and South), Africa, the Mediterranean basin, and Japan. This study was a collaborative effort to identify and mass-rear a strain of *N. californicus* that is more efficacious under arid conditions than the standard commercial strain from California (USA). *N. californicus* strains were collected in arid areas worldwide. Discriminant analysis performed with 30 morphological variables allowed for their separation. The performance of these strains under arid conditions and on selected host plants was evaluated in laboratory and semi-field trials. Development and survival of eggs and juveniles differed among strains and humidities. Female performance (survival, oviposition) was influenced by strain but not by humidity. The Californian reference strain was superior to all others, when considering both adaptation to dry conditions and power of population increase at low humidities. Whole potted plant trials (cucumber, strawberry, and pepper) confirmed this result. Field trials were conducted to compare this new product to the standard commercial strain of *Phytoseiulus persimilis* Athias-Henriot. To identify possible influence of symbiotic bacteria on strains of *N. californicus* general primers for the 16S rDNA gene were used in a PCR. Among other bacteria, a *Spiroplasma*, closely related to the one found in various arthropods, was detected in two strains, and it was further characterized. No influence on the host's developmental time, fecundity, and sex ratio could be detected. To provide this product at an affordable cost for European growers an economic rearing and delivery system for *N. californicus* was developed and its marketing potential in Europe and neighboring countries was estimated.

Key words: Phytoseiidae, intra-specific variation, mass-rearing, symbionts

Spider mites of the genus *Tetranychus* are important pests of many food and ornamental crops. The predatory mite *Phytoseiulus persimilis* Athias-Henriot provides excellent control of two-spotted spider mite (TSSM), *T. urticae* Koch, at moderate temperatures and high humidities, but at low humidity levels and high temperatures control is not up to par (Stenseth 1979). *Neoseiulus californicus* (McGregor), native to the Mediterranean basin, South America, and California (USA) (deMoraes et al. 2004), is relatively effective against spider mites at lower humidities (Bakker et al. 1993, Rott & Ponsonby 2000, de Courcy Williams et al. 2004) and was therefore chosen as a potential predator to supplement *P. persimilis*.

Our aim was to identify an arid-adapted strain and develop an economic rearing and transport system that would allow growers to take advantage of this valuable natural enemy. As *N. californicus* is found in many different geographic and climatic regions, we expected to find substantial intraspecific variation among strains allowing for the selection of an arid-adapted strain (Castagnoli & Simoni 2003). This study was a multi-institutional, multi-national, collaborative project that was performed as five distinct workpackages, each with a specific goal. In this paper we present an overview of the results of this project as well as reference to the manuscripts resulting from each workpackage.

OVERVIEW

Workpackage 1

The objectives were to collect and rear strains of *N. californicus* which would be sent to other research teams involved in the project and to search for diagnostic tools that would allow for the differentiation of the populations of *N. californicus* studied. Through collaborations and direct collections, 10 strains of *N. californicus* were obtained and reared in the laboratory at SupAgro (Montpellier, France). The strains originated from arid regions of the Mediterranean Basin, Chile, Brazil, California, and Japan (Table 1). Among them, the population reared at Koppert NL was considered as the reference strain. To develop a diagnostic tool that could allow the differentiation among the various *N. californicus* populations, morphological and morphometrical characters were studied. Thirty female mites of each strain were mounted on slides and measurements were conducted with image analysis software coupled to a Leica microscope with a high resolution digital camera. In total, 42 morphological characters were analyzed by ANOVA and Multifactorial and Discriminant analysis. Morphological typing revealed 30 characters that allowed for efficient discrimination among strains with a low rate of error. To identify molecular markers for the distinction between strains three mitochondrial (two fragments of mt-COI and a fragment of mt-12S DNA) and two nuclear (18S r-DNA and ITS r-DNA) genes were used. The sequences obtained for the five genes were aligned and a distance matrix was constructed using the Jukes & Kantor model. Genetic variation observed among the strains with

Table 1 *Neoseiulus californicus* strain origin.

	Country, site of collection	Host Plant	Year
1	France, Hérault, Mauguio	Eggplant	2004
2	Chile, La Cruz	bean	2000
3	Italy, Tuscany, Firenze	strawberry	2004
4	Spain, Valencia, Bolbaite	strawberry	2000
5	Italy, Sicily, Palermo, Paternico	strawberry	2004
6	Greece, Thessaloniki	bean	2005
7	Japan, Ibaraki	bean	2005
8	Brazil, Sao Paulo, Piracicaba	bean	2005
9	Tunisia, Tozeur	*Convolvulus arvensis*	2005
10	USA, California -> Koppert NL	Mass rearing	2004

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the selected DNA markers was too low to be used as diagnostic tools. This result could be explained by exchange and dispersal of *N. californicus* and/or a low rate of genetic change in the strains after their dispersal from the original site of collection.

The differences between the levels of variation estimated by morphological and molecular markers could be due to a high morphological plasticity linked to environmental adaptation. In the future, it would be interesting to perform the morphological measurements in order to see if the morphological differences emphasized in the present study will be conserved (for the details of this workpackage see Guichou et al., this volume).

Workpackage 2

The objectives were to identify the most dry-adapted strain of *N. californicus* using life history experiments and to investigate the efficacy of the most dry-adapted strain under semi-field and field conditions. Initially, each involved research group received, established, and then maintained two to three strains of *N. californicus* collected in WP1. Egg hatch and development, juvenile survival and development, and adult survival and fecundity were assessed for all strains at 3-4 humidity levels. Altogether, the life history tests suggested that the Californian reference strain provided to the University of Natural Resources and Applied Life Sciences (BOKU) in Vienna and the Sicilian *N. californicus* strain are superior to the other strains when considering both adaptation to dry ambient conditions and the power of population increase at low humidities (Walzer et al., this volume). These strains were therefore selected to proceed to the next experimental level, at which their efficacy was compared with that of *P. persimilis* on single potted cucumber, pepper, and strawberry. Synthesis of the life history tests and the experiments on single potted plants suggest that under dry ambient conditions the BOKU *N. californicus* strain is the best performing strain of the eight strains tested. The superiority to other *N. californicus* strains is due to the combination of a well-developed tolerance to low humidities and a high reproductive potential. Whether or not the BOKU *N. californicus* strain is superior in spider mite suppression to the currently available commercial strain of *P. persimilis* under dry ambient conditions depends on the host plant, the availability of alternative food, and the level of spider mite infestation.

The results of our strawberry trials conducted in raised beds in greenhouses and in the open field suggest that better spider mite control would probably be achieved with sequential releases of both predators. Similarly, Rhodes et al. (2006) obtained significant reductions of *T. urticae* in strawberry with combined releases of these predators. In one out of the two seasons of testing, better results were obtained with the release of *N. californicus* only. In our cucumber trials in Austria conducted in the spring, *N. californicus* gave similar results to *P. persimilis*. El Laithy & Sawsan (2005) found similar results; up to twice as many spider mites were found on cucumbers in which *P. persimilis* was released as compared to *N. californicus*. In trials on pepper plants in The Netherlands in summer and in Israel in autumn and spring, all conducted as part of this study, *N. californicus* was a more efficacious predator than *P. persimilis* (Weintraub et al. 2006). Castagnoli et al. (2004) and Simoni et al. (2005) compared the efficacy of *N. californicus* on a number of solanaceous plants (eggplant, pepper, tomato) in greenhouses. They, too, found that *N. californicus* successfully controlled spider mites on eggplants and sweet peppers and reduced leaf damage.

This workpackage is summarized in three related papers dealing with the intraspecific variation in humidity susceptibility in the different strains of *N. californicus* in artificial cages (Walzer et al. 2007), the efficacy of two selected *N. californicus* strains in spider mite control in strawberries and sweet pepper under controlled arid conditions on whole plants (Palevsky et al., 2008) and in a field study on peppers grown in plastic tunnels under the extreme arid conditions of the Arava Valley desert of Israel (Weintraub & Palevsky, 2008).

Workpackage 3

The objectives were: to identify bacteria from *N. californicus* strains that exhibit differences in performance as determined by WP2, screen all *N. californicus* strains for the presence of specific bacteria, and determine the fitness effects of various bacteria in selected strains in which such effects are suspected.

The bacteria associated with six geographically different populations of *N. californicus* were characterized using molecular fingerprinting techniques, and a number of bacteria have been discovered. Among these, *Spiroplasma* was detected in two populations (from Sicily and Chile). *Spiroplasma*-specific primers for the 16S rDNA coding gene were used for screening various mites for the presence of that bacterium. When individual mites from *Spiroplasma*-infected and uninfected lines were screened, 75 and 60% infection rates were found in the Sicily and Chile lines respectively. A combination of general bacteria primers and *Spiroplasma*-specific primers was used for generating a 905-basepair (bp) sequence of the 16S rDNA *Spiroplasma* gene out of the Sicily and Chile *N. californicus* lines. That gene sequence is most closely related (99% similarity) to the 16S rDNA gene of the *Spiroplasma* found in the pea aphid *Acyrthosiphon pisum* (Harris), and to the male-killing agent of the butterfly *Danaus chrysippus* (L.). A 385-bp fragment of the second gene, *dnaA*, also exhibited highest sequence similarity to the genus *Spiroplasma*. The sequences of the two genes were virtually identical in the two predatory mite strains. Fitness experiments showed no significant difference in both the fecundity of and sex ratio produced by *Spiroplasma*-infected and uninfected mothers. Similarly, the infection status of the mothers did not significantly influence offspring developmental times.

Several bacterial symbionts have been reported to be associated with predatory mites from the family Phytoseiidae, including *Rickettsia*, *Wolbachia*, *Cardinium*, *Enterobacter*, and an unnamed member of the Bacteroidetes (Weeks & Stouthamer 2004, Hoy & Jeyaprakash 2005), but our findings are the first published record of *Spiroplasma* in plant-inhabiting mites (Enigl & Schausberger, 2007; Zchori-Fein et al., 2007). The genus *Spiroplasma* belongs to the class Mollicutes, which it shares with other host-associated members, such as the vertebrate-pathogenic mycoplasmas and the insect-vectored plant-pathogenic phytoplasmas. Species within the genus are known to exhibit a diverse array of relationships with their hosts, with respect to factors such as transmission modes, replication sites, and number of hosts required for successful completion of the life cycle. Although the first three *Spiroplasma* species described were plant pathogens, most

of the other species known to date have been found in insects, where the symbiotic association ranges from pathogenic to mutualistic. Within the Acari, *Spiroplasma* has so far been identified in three tick genera (Burgdorfer et al. 1973, Tully et al. 1982, 1995) and a parasitic dermanyssoid mite (Reeves et al. 2006), and generally its influence on the tick and mite host has not been determined.

The low levels of *Spiroplasma* vertical transmission as well as the relatively high rates of horizontal transmission recorded during this study were in contrast to transmission modes previously reported for symbiotic bacteria. The unstable infection made it impossible to establish infected and uninfected lines and to compare them according to the original plan. As a result, the phenotype of *Spiroplasma* in *N. californicus* is yet unknown and currently there is no indication that *Spiroplasma*-infected and uninfected lines differ with respect to their ability to withstand harsh environmental conditions. Nonetheless in another arthropod-symbiont system, the corn stunt spiroplasma and its vector, the corn leafhopper *Dalbulus maidis* (DeLong et Wolcott), it was shown that *Spiroplasma*-infected *D. maidis* have survival rates higher than non-infected individuals under conditions of starvation and cold temperatures (Ebbert & Nault 2001).

Currently, insects are thought to be the major reservoir of *Spiroplasma* in nature. However, large-scale screenings and in-depth studies are required in order to determine the prevalence and phenotype of that symbiont in various mite groups. Such efforts may result in a considerable expansion of both known host range and effects of *Spiroplasma* in mites, and may provide insights to the use of predatory mites for pest control.

Workpackage 4

The objectives were to develop a commercial rearing and transport system. The information and technology of this workpackage is owned by Koppert The Netherlands, therefore a detailed summary cannot be provided, but we present briefly the main results. Pollen from seven plant species (*Quercus ilex*, *Q. ithaburensis, Pistacia atlantica, P*. *lentiscus, P. palaestina, P. vera*, and *Zea mays*) were found suitable as an alternative food source for *N. californicus.* Two small-scale mass rearing systems were developed; one on artificial arenas and the other on soybean plants, with pollen as food source for the production of sufficient predatory mites for field trials (Argov et al. 2006). The two mite species that were found to be potentially useful as factitious hosts for mass rearing were identified and evaluated. At Koppert NL, an economically feasible mass rearing system of the BOKU strain of *N. californicus* on the most promising mite species (Bolckmans et al. 2005) was developed and no negative effects of mass-rearing have been observed. A transport/storage period of 10 days at 8°C has no impact on the quality of the product. The BOKU strain of *N. californicus* does not enter diapause under short-day conditions. A simplified standard quality control procedure was developed using pollen as food and polyacrylamide gel as water source (Argov et al. 2006).

The effects of using astigmatid mites as a diet for mass rearing *N. californicus* were summarized in two publications (Castagnoli et al., 2006; Simoni et al., 2006). An additional contribution is now in preparation concerning quality control and maintenance of predation efficacy after long term mass–rearing on alternative prey (Castagnoli & Simoni, in preparation).

Workpackage 5

The objectives were to determine the marketing potential in Europe and identify the economic benefits of *N. californicus* to the growers of selected crops. A literature survey was conducted in order to list the regions and crops susceptible to spider mites. A field study was conducted in Spain, Greece, and Israel, focusing on pepper and strawberry. In addition, interviews with growers in Italy, Spain, and The Netherlands were conducted. The literature survey supports the earlier assumptions that the current use of *N. californicus* for spider mite control around Europe is still minimal. Currently, according to the literature survey, the IPM market share is estimated at 6% of all protected crop areas. The main reason for the small market share is the large gap between costs of biocontrol agents (integrated in an IPM program) vs. acaricides (implemented in a chemical pest management [CPM] program). Based on these findings, as well as on the results of the field study, two main questions related to economics were raised: (1) What effect would this research have on the growth of IPM? and (2) What are the expectations of additional income for growers?

Our research focused on three crops known to be damaged by TSSM (rose, pepper, and strawberry) and in which IPM is successfully used in four countries (Spain, Greece, The Netherlands, and Israel). The pepper crop in Spain and roses in the Netherlands are notable due to their large market share in Europe. According to the field study, TSSM is estimated to cause damage to 3-5% of the yield (quantity and quality). On the basis of this research and estimates by experts, 30% of this damage can be prevented, increasing income by 1-1.5%. This is considerable in financial terms, and its significance increases when taking into account the importance growers ascribe to biological treatment of TSSM within the IPM package. More than 30% of the CPM growers who participated in the field study claimed that an improvement of the efficacy of biological control against TSSM would cause them to switch to IPM. A decrease of 40% in *N. californicus* production costs is expected as a result of this research, and an additional 40% decrease is expected due to other improvements resulting from this R&D effort. Overall, a 50% decrease from the 2005 price of *N. californicus* is expected, with a significant impact on the expense to the growers. The range of the reduction in expenses is between 100 euros/ha (Spanish strawberry growers) and 600 euros/ha (Dutch rose growers). For most growers included in this study, reductions are estimated at 150-200 euros/ha. This also significantly reduces the gap between CPM and IPM expenses. The area under IPM is expected to expand from the current 5,700 ha to 16,600 ha. This figure indicates long-term potential, with a slow implementation process which will be affected by other factors. The additional profit to the growers using IPM is estimated at 16.6 million euro per year. In the long run, this is an expression of the total economical value of the research.

An additional product of this workpackage is the development of methodology for quantitative-economic estimation of IPM scientific research. The results strengthen the justification and need of expansion of the research to other countries and crops, such as clementine, ornamentals, especially chrysanthemum in the Netherlands, and deciduous orchards in eastern Asia.

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Eric Palevsky[1], Phyllis G. Weintraub[2], Einat Zchori-Fein[1], Shira Gal[1], Neta Mozes-Daube[1], Yael Argov[3], Marisa Castagnoli[4], Marialivia Liguori[4], Roberto Nannelli[4], Sauro Simoni[4], Franca Tarchi[4], Silvia Guidi[4], Donatella Goggioli[4], Giuseppino Sabbatini Peverieri[4], Peter Schausberger[5], Andreas Walzer[5], Monika Enigl[5], Serge Kreiter[6], Marie-Stéphane Tixier[6], Sabine Guichou[6], Karel Bolckmans[7], Yvonne van Houten[7], Elmer van Baal[7], Jose Eduardo Belda[8], Shimon Steinberg[9], Nurit Shapira[10], Dimitrios Charantonis[11], George Tsalis[11], Raija Holmqvist[12], Sune Gullans[12], Michael Gross[13], Patrick Peternel[13], Jan van der Blom[14], Juan Carlos Perez Mesa[14], Gad Rosenthal[15] & Ofra Goldfarb[15] (order of authorship according to research institute and industry affiliation)

[1]Dept. of Entomology, Agricultural Research Organization (ARO), Ministry of Agriculture, PO Box 1021, Ramat Yishay

30095, Israel. E-mail: palevsky@volcani.agri.gov.il; [2]Dept. of Entomology, ARO, Ministry of Agriculture, Gilat Research Center, D.N. Negev, 85280 Israel; [3]Israel Cohen Institute for Biological Control (ICIBC), Plant Production and Marketing Board, Citrus Division, Derech Ha'macabim 46, Rishon Le Zion 75359, PO Box 54, Beit Dagan 50250, Israel; [4]CRA-Istituto Sperimentale per la Zoologia Agraria (ISZA), via di Lanciola 12/A, Firenze 50125, Italy; [5]Institute of Plant Protection, Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources & Applied Life Sciences (BOKU), Peter Jordanstrasse 82, A-1190 Vienna, Austria; [6]Montpellier SupAgro, 2 Place Pierre Viala, 34 060 Montpellier cedex 01, France; [7]Koppert Beheer, Veilingweg 17, 2650 AD Berkel en Rodenrijs, The Netherlands; [8]Koppert España, c/Cobre 22, Pol. Ind. Ciudad del Transporte, 04745 La Mojonera (Almeria), Spain; [9]Bio-Bee Biological Systems, Kibbutz Sdeh Eliyahu, Beit Shean Valley, 10810, Israel; [10]ARV Development Company, Sapir Center, M.P. Arava, 86825, Israel; [11]Charantonis Biological Crop Protection, 35, Safrapoleos Str., 585 00 Skydra, Greece; [12]Oy Schetelig AB, Tiilitie 14, FIN-01720 Vantaa, Finland; [13]Biohelp, Kapleigasse 16, A-1110 Vienna, Austria; [14]Coexphal, Ctra. de Ronda, 11-1º E, Apto. 330, 04004 Almeria, Spain; and [15]Kivun, 116 Menachem Begin Rd., Beit Kalka, PO Box 7201, Tel Aviv, Israel

Host Range, distribution, and morphometrics of predatory mites associated with phytophagous mites of fruit crops in Himachal Pradesh, India

Meena Thakur, C.L. Dinabandhoo & Usha Chauhan

Department of Entomology and Apiculture, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan-173 230 (Himachal Pradesh), India. E-mail: uchauhan97@rediffmail.com

A survey on host range and distribution of mite species associated with fruit crops was carried out during 1999-2001 at different altitudes and districts of Himachal Pradesh. Eight species of predaceous mites (mostly Phytoseiidae) were found in association with 11 species of phytophagous mites (mostly Tetranychidae). *Panonychus ulmi* was the major phytophagous species on apple at Shimla district, other pests included *Tetranychus urticae*, *Bryobia rubrioculus*, *Brevipalpus californicus*, and *Aculus malus*, whereas *T. urticae* was dominant on apple and other pome and stone fruits at Kullu district. At Shimla district the predaceous mite *Amblyseius finlandicus* was abundant and associated with *P. ulmi*, *B. californicus*, *A. malus*, and *Tetranychus* species on apple, pecan nut, and walnut. At Solan district *Amblyseius longispinosus* was prevalent. Its host range was restricted to *Tetranychus* species on fig, papaya, apple, and beans. *Phytoseius crinitus* was abundant on guava and citrus, infested with *Brevipalpus phoenicis* at Una district. *Phytoseius intermedius* was the dominant predatory mite associated with litchi leaves having well developed erinia due to *Aceria litchi*. *Typhlodromus homalii* was found in mixed population of *P. intermedius* on litchi leaves infested with *A. litchi* from Kangra district. *Agistemus* sp. was collected from apple leaves infested with *T. urticae* at Shimla, from cherry and walnut with *T. urticae* at Kullu, and from guava and citrus infested with *B. phoenicis* at Una. At Mandi district *Tydeus* sp. was present along with A. *finlandicus* (5 mites per 25 leaves) in mango orchards infested with *T. urticae*. *Biscirus* sp. was collected on grapes and citrus leaves. Morphometrics and diagnostic features of different species are presented in the paper.

Key words: Faunistics, orchard, species diversity, fruit trees

Mites are gaining importance economically as they cause many types of injury resulting in leaf and fruit fall, malformations, and deformities to, fruit and vegetable crops and other cultivated plants (Jeppson et al., 1975). In India, phytophagous mites were reported from the North-East in 1868 (Peal, 1868). Till the end of the 19th century only three species were known from the country. At present about 2350 species have been reported from India (Gupta & Gupta, 1999). In Himachal Pradesh, the red spider mites (*Tetranychus* and *Panonychus*) were reported to pose a serious threat to the apple crop, backbone of the state economy. At present 62% of the apple orchards are reported to be infested with mites (Ghosh, 1998). The increase in the pest status may be attributed to the lack of biological, ecological, and taxonomic knowledge, and to inappropriate management practices, such as the misuse of pesticides followed by the development of insecticide resistance (Evans et al., 1993) and the destruction of natural enemies. The importance of predaceous mites in the control of phytophagous mites in fruit crops, particularly in apple ecosystems, and their role in integrated pest management (IPM) programs are well documented (Croft, 1975; Croft & McGroarty, 1977; Croft & Slone, 1997).

Prior to the initiation of an IPM program, it is a prerequisite to determine the species of mites present in an orchard. The survey report on the various aspects of predaceous mites will be useful from a pest management point of view in Himachal Pradesh. Mites were recognized as serious pests in the state since 1992, when there was severe outbreak of European red mite, *Panonychus ulmi* (Kumar & Bhalla, 1993). The pest was previously under check due to the presence of predatory mites but excessive use of chemicals led to destruction of predators and severe outbreak of the pest. Predaceous mites belonging to the Phytoseiidae and Stigmaeidae were reported previously as predators of *Tetranychus cinnabarinus* Boisduval, *Brevipalpus phoenicis*, and *Oligonychus* sp. (Dhooria, 1998). The present contribution increases the knowledge on association of various predaceous and phytophagous mites in Himachal Pradesh, their seasonal dynamics, hosts, and the altitude at which they occur.

MATERIALS AND METHODS

Survey

A random survey during 1999-2001 was conducted in various districts of Himachal Pradesh, which is situated between 30°2'-33°N and 75°47'-79°E, and at altitudes varying from 350-7,000 m above mean sea level (amsl). The elevation of the areas surveyed for the infestation of mites varied from 430-2,769 m amsl. Commercial, abandoned orchards and isolated fruit trees (e.g., apple, walnut, pear, peach, plum, cherry, almond, pecan nut, apricot, mango, litchi, guava, fig) were surveyed for mite fauna.

Sampling

Leaf samples consisted of 20-25 leaves picked randomly from the periphery of a tree. Leaves were placed in polythene bags and transported to the laboratory. Phytophagous and predatory mites were counted on each leaf. Predatory mites could easily be distinguished from phytophagous mites as they are generally coloured and fast moving.

Preservation

Preservation of mites was done in 70% ethyl alcohol and glycerine (10:1 v/v) or AGA solution based on the Tuttle formula, as described by Jeppson et al. (1975). The preserved specimens for mounting were cleared in 35% lactic acid at 35-40 °C in an oven for 1 week.

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Table 1 Morphometrics and diagnostic features of predaceous mites.

Species	Morphometrics (mm)	No. dorsal setae (pairs)	Identifying features; setal length in mm
Amblyseius finlandicus Oudemans	0.549 × 0.272	16	Z_5 setae longer (0.075-0.080); other setae on periphery 0.057, s_4 0.030, median setae 0.016
Amblyseius longispinosus Evans	0.530 × 0.290	17	Z_5 setae 0.078-0.080, s_4 0.080-0.085; other setae small
Phytoseius crinitus Swirski & Shechter	0.434 × 0.192	15	8 pairs of setae long, thick, and serrated; Z_5 0.080, Z_4 0.085; 5 pairs of setae simple and short (0.060-0.008)
Phytoseius intermedius Evans & Macfarlane	0.456 × 0.168	15	9 pairs of setae long, broad, thick, and serrated, Z_4 0.075-0.080, Z_5 0.050-0.055; 6 pairs of setae short and simple.
Typhlodromus homalii Gupta	0.489 × 0.224	18	Z_5 setae 0.040-0.048; other setae small and of equal length (0.018-0.039)
Agistemus sp.	0.434 × 0.224	11	Setae (0.036-0.078) stout, barbed, and set on small tubercles, dorsal body showing polygonal reticulations
Tydeus sp.	0.431 × 0.286	11	Setae 0.039-0.052; pear shaped body, 4 segmented palpi
Biscirus sp.	0.721 × 0.304	10	Setae 0.039-0.081; elongate rostrum, palpi 5 segmented terminating into 2 setae

Morphological studies

Adult males and females were processed for microscopic studies as their identification was based on size and pattern, number and type of setae. The cleared specimens were mounted in Hoyer's medium (Baker & Wharton, 1952). The mounted slides were catalogued and later used for recording microscopic morphological features recognized as taxonomic characters (Gupta, 1970).

RESULTS AND DISCUSSION

Identifying features and morphometrics of predatory mites

The body size (including mouth parts) and important identifying features of the eight predaceous mite species encountered are presented in Table 1. The idiosoma of *A. finlandicus* was 0.364 mm long and 0.272 mm wide, which was comparable to 0.308 × 0.200 mm recorded for the species by Gupta (1985). The dorsal shield (idiosoma) of *Amblyseius longispinosus* was 0.371 × 0.290 mm, which was bigger than 0.325 × 0.180 mm recorded by Gupta (1985). The idiosoma of *P. crinitus* measured 0.302 × 0.192 mm, compared to 0.280 × 0.168 mm by Gupta (1985). The dorsal shield of *T. homalii* measured 0.318 × 0.224 mm, which corresponds to 0.325 × 0.188 mm recorded by Gupta (1970). The principal characteristic feature, which is in conformity with Gupta's (1970) description of the species, is that the peritreme extends beyond and then curves inwards.

The dorsal shield of *Agistemus* sp. measured 0.302 × 0.224 mm (full body size 0.435 × 0.224 mm), the dorsum had polygonal reticulations, 11 pairs idiosomal setae, which were stout, barbed, and set on small tubercles. These characters indicate a close resemblance to *Agistemus sumatrensis* and *A. pieteri* described by Ehara & Oomen-Kalsbeek (1983) from Indonesia on tea plants. However, our species was much longer than either of the two species and its identity could not be confirmed by any of the authorities to whom the specimens were sent. The morphological characters of the genus *Tydeus* (Table 1) are in conformity with the description of Jeppson et al. (1975) of *T. californicus* and *T. caudatus* on citrus from Southern California, USA. The morphological characters of the genus *Biscirus* (Table 1) are conform the description of Krantz (1978) for the genus.

Population density and distribution of mite species

Eleven species of phytophagous mites were encountered in association with eight species of predaceous mites. Most phytophagous and predaceous mites were in the families Tetranychidae and Phytoseiidae, respectively. A visit to various locations in Shimla district (2,206 m amsl) during September 2001 revealed that *P. ulmi* was the major phytophagous species on apple: 25-50 and 100-200 *P. ulmi* per leaf were collected from commercial and abandoned apple orchards, respectively (Table 2). An epidemic outbreak of *P. ulmi* (465 mites/apple leaf) has been reported by Kumar & Bhalla (1993) in the wet temperate high hills of Shimla and Kullu (1,219 m amsl) during June-August, when mean temperature ranged from 23-27 °C. In Nova Scotia, Canada, *P. ulmi* generally was abundant in orchards with high insecticide scores, resulting in absence of natural enemies (Hardman et al., 1991).

In our survey, other pests included *T. urticae*, *B. rubrioculus*, *B. californicus*, and *A. malus*. *Brevipalpus californicus* and *B. rubrioculus* were present in lower numbers than *T. urticae* (Table 2). Nymphs and adults of *B. californicus* were difficult to see because they lie flat against the leaf surface and were slow to move, although their full red colour stands out against the green background. Apple rust mite, *Aculus malus*, was present in very high density in commercial and abandoned apple orchards. Heavy infestation of this mite caused lengthwise curling of leaves which later turned rusty brown.

A survey in the same area in November 2000 indicated the absence of adults of *P. ulmi* and *T. urticae*, but the presence of *B. californicus* nymphs, *A. malus*, and overwintering eggs of *P. ulmi* in groups or scattered on roughened bark area at the base of buds, spurs, and wounds. They could serve as food for predatory mites. The presence of *A. malus* may be considered beneficial, because they are an important alternative food source for predaceous mites. They are known to sustain populations of *Amblyseius fallacis* (Croft, 1975) and *Typhlodromus pomi* (Kinsley & Swift, 1972) when their preferred food is scarce. Suski (1972) conducted an extensive study of tarsonemids on apple trees in Poland and concluded that they served as an alternative food for phytoseiids. Tydeids were depicted by Readshaw (1975) as possible prey for stigmaeids.

The predaceous mites *A. finlandicus* and *Agistemus* sp. were encountered on leaves in neglected and abandoned pome and stone fruit orchards in November 2000 and September 2001. *Amblyseius finlandicus* was the dominant species (Table 3). On okra, feeding on *Polyphagotarsonemus latus* under tropical conditions of Punjab, *A. finlandicus* was reported to peak in July (Ghai & Bhullar, 2003). It is known as an important predator of *P. ulmi* and *A. malus* on cherry and

Table 2 Phytophagous mites associated with fruit foliage in Himachal Pradesh, India.

Family/Species	Elevation (m amsl)	Period of survey	No. specimens collected/leaf	Host plant
TETRANYCHIDAE				
Tetranychus urticae Koch	1,219	July 1999, Aug 2000, 2001	25-50	pome and stone fruits
	1,463	July 1999, 2000, May 2000, Nov 2000, Aug, Sept 2001	50-100	papaya, apple
	2,206	Sept, Nov 2001	25-50	apple
	754	Sept, Dec 1999, Aug 2000, Feb, May 2001	15-30	mulberry, mango
Tetranychus ludeni Zacher	1,463	July 1999, 2000, May 2000, Aug, Sept 2001	80-100	fig
Panonychus ulmi (Koch)	2,206	Nov 2000, Sept 2001	25-50 in commercial orchards, 100-200 in neglected orchards	apple, pecan nut, walnut
	2,769	Sept, Nov 2000	30-50	apple
Panonychus citri (McGregor)	1,463	July 1999, Sept 2000, 2001	25-50	citrus
Eutetranychus orientalis (Klein)	1,463	July 1999, May 2000, Aug, Sept 2001	5-10	citrus
Bryobia rubrioculus (Scheuten)	2,206	Sept 1999, Nov 2000, Sept 2001	10-15	apple
	754	Sept 1999, Aug 2000, May, Sept 2001	1-2	peach
TENUIPALPIDAE				
Brevipalpus californicus (Banks)	2,206	Nov 2000, Sept 2001	5-25	apple
Brevipalpus phoenicis (Geijskes)	750	Oct 2000, May 2001	5-8	guava, citrus
Tenuipalpus granati Sayed	1,463	July 1999, May 2000, Aug, Sept 2001	5-10	pomegranate
ERIOPHYIDAE				
Aceria litchi Keifer	754	Sept, Dec 1999, Aug 2000, Feb, May, Sept, Nov 2001	150-200	litchi
	1,597	Dec 1999, May 2000, Aug 2001	<150	litchi
Aculus malus Zaher & Abou Awad	2,206	Nov 2000, Sept 2001	100-500	apple

Table 3 Predatory mites associated with fruit foliage in Himachal Pradesh, India.

Family/Species	Elevation (m amsl)	Period of occurrence	No. specimens collected/25 leaves
PHYTOSEIIDAE			
A. finlandicus	2,206	Nov 2000, Sept 2001	23
	754	May 2000, Sept, Nov 2001	5
A. longispinosus	1,463	July 1999, May 2000, Aug, Sept 2001	16
P. crinitus	750	Oct 2000, May 2001	10
	1,597	Dec 1999, May 2000, Aug 2001	8
P. intermedius	754	Sept, Dec 1999, Aug 2000, Feb, May, Sept, Nov 2001	38
	1,597	Dec 1999, May 2000, Aug 2001	5
T. homalii	1,597	Dec 1999, Aug 2000, 2001	14
STIGMAEIDAE			
Agistemus sp.	2,206	Nov 2000, Sept 2001	5
	1,219	July 1999, Aug 2000, 2001	15
	750	Oct 2000, May 2001	18
BDELLIDAE			
Biscirus sp.	1,463	Sept 2001	5
TYDEIDAE			
Tydeus sp.	754	Sept, Dec 1999, May, Nov 2001	20

plum (Livshits & Mitrofanoz, 1981; Belmans, 1994), and it has been described as an important predator of *P. citri*, causing severe damage to citrus in Yugoslavia (Tomasevic & Mijuskevic, 1974). In the present study, despite the presence of *P. citri*, we did not find *A. finlandicus* on citrus trees. The association of *A. finlandicus* with several phytophagous species on various host plants indicates a lack of specificity in its food habits, as stated by Gupta (1985).

The survey conducted in Kinnaur district (2,769 m amsl) during September and November 2000 revealed the presence of *T. urticae*, *P. ulmi*, and *B. rubrioculus*. No predator was encountered, except one predaceous beetle. Pome and stone fruit orchards in Kullu district (1,219 m amsl) revealed the presence of *T. urtice* in a random survey during July 1999, August 2000, and August 2001 (Table 2). The only predaceous mite present here was *Agistemus* sp. (Table 3). In these orchards *A. finlandicus* was not present, perhaps due to the difference in altitude or the absence of *P. ulmi*, its preferred host.

At 750 m amsl (Una district), the flat mite *B. phoenicis* was found feeding along the midrib on the undersurface of guava and citrus leaves during October 2000 and May 2001. Individuals were difficult to see because of their size, but their light yellow colour stands out against the green leaf surface. In tropical Punjab severe damage to mandarin orchards due to *B. phoenicis* was reported during January to February (Sandhu et al., 1979). The predators *Agistemus* sp.

and *P. crinitus* were present at this elevation, *Agistemus* sp. was seen on guava leaves even in absence of adults or nymphs of *B. phoenicis*. Microscopic examination of such leaves showed the presence of eggs, reminiscent of its fondness of eggs as observed by Oomen (1982). *Agistemus* spp. have been described as effective predators of *B. phoenicis* in Indonesia (Ehara & Oomen-Kalsbeek, 1983) and on vines in Hungary (Szendrey & Voigt, 2000).

At 1,597 m amsl (Kangra district) *P. crinitus* and *P. intermedius* were present on litchi leaves infested with *A. litchi* (Table 3). At 1,463 m amsl the phytophagous mites *T. ludeni* and *T. urticae* were found on a variety of host plants, viz., fig, apple, bean, papaya, and guava, in random surveys conducted during July 1999, July and May 2000, and August and September 2001. High numbers of *T. urticae* and *T. ludeni* were observed feeding inside webs during these months (Table 2). The only predator collected on these trees was *A. longispinosus* (Table 3). The adults of this mite appeared reddish when fully fed, due to the colour of the prey fluids. Its association with *Tetranychus* spp. is known, viz., *T. ludeni* (Peter & David, 1988), *T. urticae*, *T. kanzawai* (Lo et al., 1984), *T. cinnabarinus* (Wen & Lee, 1981).

Surveys conducted throughout Mandi district (754 m amsl) during September and December 1999, August 2000, and February, May, September, and November 2001, indicated the occurrence of *T. urticae* (mango), *B. rubrioculus* (peach), and *A. litchi* (litchi) (Table 2). The feeding activity of *A. litchi* caused twisting of leaves and the appearance of first greenish, then chocolate brown erinia on the lower surface of leaves. On litchi leaves *P. intermedius* and some *Tydeus* sp. were found in association with *A. litchi* (Table 3). The predaceous mites encountered on mango infested with *T. urticae*, growing near the litchi orchards, were *A. finlandicus* and *Tydeus* sp. (Table 3).

Conclusion

Different predaceous species were dominant at different altitudes (states). In wet temperate high hills at 2,206 m amsl – the apple growing belt of Shimla and the backbone of the state economy in terms of revenue –, *A. finlandicus* was present in high numbers. The use of *A. finlandicus* as IPM tool will reduce the cost of chemicals, the cost of crop production, and environmental hazard. In sub-temperate, subhumid middle-high hills at 1,463 m amsl (Solan), *A. longispinosus* was associated with a variety of hosts infested with *T. urticae* and *T. ludeni*, causing severe damage to fruit and vegetable crops. It can be exploited for the management of tetranychids at this altitude. In subtropical and low hills at 754 m amsl (Mandi), *P. intermedius* was present in high numbers on litchi leaves infested with *A. litchi*, and *Tydeus* sp. was present in high numbers in mango orchards infested with *T. urticae.* The preservation, multiplication, and release of these predaceous mites can reduce the use of chemicals in litchi and mango orchards at this altitude. This survey of mites associated with fruit foliage serves as a first step towards developing an IPM program that will account for the existence of natural enemies in the orchard environment.

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Winter survival and reproduction of *Amblyseius longispinosus* (Acari: Phytoseiidae), a potential predator of spider mites on roses in Himachal Pradesh, India

Usha Chauhan, Ram Kumar & Meena Thakur

Department of Entomology and Apiculture, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan-173 230 (Himachal Pradesh), India. E-mail: uchauhan97@rediffmail.com

Amblyseius longispinosus is an effective predator for the control of two-spotted spider mite, *Tetranychus urticae*, on roses. It feeds on all stages of the spider mite but it prefers nymphs and adults to eggs. The predatory mites were kept under laboratory conditions on excised, mite-infested rose leaves. Under winter conditions (T-min 0.5-5.9 °C, T-max 18.4-22.7 °C, 20-91% relative humidity), the predatory mite completed egg-to-adult development in 8.8 days. Egg, larva, protonymph, and deutonymph stages took 3.8, 1.3, 1.7, and 2.0 days, respectively, and adults survived for 19.4 days. Within 2 days after emergence of the adults, mating took place, each copulation lasting only 7-8 min. Mean fecundity was 11.2 eggs per female and all eggs hatched successfully. Thus, *A. longispinosus*, can survive and reproduce under winter conditions prevailing mid-hill in Himachal Pradesh. It may therefore be a candidate for control of two-spotted spider mites in areas with a temperate climate in India.

Key words: *Amblyseius longispinosus*, biology, winter months, oviposition, *Tetranychus urticae*

Phytophagous mites are known as serious pests of many agricultural, horticultural, and ornamental crops in India (Jhansi Rani & Jagan Mohan, 1997; Mallik et al., 1998; ChannaBasavanna, 1999). In the recent past, polyhouse cultivation of crops like rose, carnation, and several vegetables is gaining momentum in India. Rose (*Rosa* sp.) is currently the most important commercial crop. It is grown in the open field as well as under protected conditions in polyhouses. Rose plants are attacked by several pests of which the two-spotted spider mite, *Tetranychus urticae* Koch, is the most important. Pest management by biological agents, such as phytoseiid predators, has received considerable attention recently, because the *T. urticae* developed resistance to most of the available acaricides (McMurtry & Croft, 1997; Shaila, 1999).

The phytoseiid *Amblyseius longispinosus* (Evans) is widely distributed in the tropics and has been shown to be an effective predator of tetranychid mites on crops like rose, cotton, bamboo, cucumber, and strawberry, both in the field (Hegde & Patil, 1994; Mallik et al., 1998; Zhang et al., 1998, 1999; Colkesen & Sekeroglu, 2000; Kongchuensin et al., 2001; Abhilash & Sudharma, 2002) and under polyhouse conditions in tropical India (Mallik, 1974; Hegde et al., 1995; Mallik et al., 1998). However, there is no report about its effectiveness in the temperate climates that prevail in hilly regions in the Western Himalayas. This was the reason to undertake these laboratory studies in the winter months under mid-hill conditions in Himachal Pradesh.

MATERIALS AND METHODS

The culture of *A. longispinosus* was procured from Dr. B. Mallik's laboratory at Gandhi Krishi Vignan Kendra University (Bangalore, India). The culture was maintained under controlled conditions in the laboratory in an incubator (type Biochemical Oxygen Demand incubator) at 27±2 °C and 60-70% r.h. During December 2005 and January 2006, when there were quite large fluctuations in day and night temperature and relative humidity (Table 1), the culture was moved to a room with outdoor temperature and humidity, and here survival, development, life span, and fecundity were assessed.

From the stock culture, 15 pairs of newly emerged mites, either in mating or pre-mating positions, were collected and kept separately on an upside down rose leaf infested with two-spotted spider mites, on moist cotton in a Petri dish (diameter 13 cm). The predators were observed daily for fecundity, development, and survival. To ensure regular food supply, a sufficient number of spider-mite eggs and motile stages were introduced to the leaves using a camel hair brush. From the eggs laid by the predators, 25 eggs were collected and transferred to separate small rose leaflets for assessment of life-history parameters. To provide prey, spider mites in various stages of development were introduced to these leaflets every 3rd day. Shriveled leaves were replaced by fresh ones when needed. Dead spider mites were removed from the leaves on a daily basis. For recording the developmental stages of the predatory mites, observa-

Table 1 Meteorological data during the course of study.

Month	Week	Temperature (°C)		Relative humidity (%)	
		Maximum	Minimum	Maximum	Minimum
Dec 2005	1	23.0	1.8	69	43
	2	22.3	0.5	74	45
	3	21.5	1.5	77	48
	4	23.3	1.8	91	56
Jan 2006	1	19.1	2.5	87	49
	2	22.7	2.6	75	20
	3	18.4	5.9	89	52
	4	19.9	1.7	74	32

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tions were taken twice a day at 10:00 and 17:00 hours. Whenever mites moulted, the mid between two successive observations was taken as the time of moulting.

RESULTS AND DISCUSSION

From the 15 pairs of predatory mites kept for continuous observation, two females died (4 and 6 days after emergence), so that 13 pairs could be observed for mating behaviour and fecundity. Total developmental period of this predator ranged from 4-6.8 days under tropical conditions (Ibrahim & Palacio, 1994; Thongtab et al., 2001), but it took 8.8 days in the present studies under temperate climate conditions in winter.

Mating behaviour and mating period

Newly emerged males approached the female and climbed on her from the front, rear, or flanks. Unreceptive females moved away from the males. Sometimes unreceptive females got surrounded by two or three males. In this situation she lowered her idiosoma to the substrate and remained motionless until the males moved away. Generally the males became sexually mature earlier than the females. Males stimulate the females to mate by touching her dorsum with the first pair of legs and pedipalps. When the female became receptive, she raised the posterior end of her body and the male turned back and crawled underneath the female followed by actual mating in venter-to-venter position. The pre-mating phase took several hours, whereas the mating act itself lasted only 7-8 min. In mating position, females do not feed but they may move while dragging the male underneath.

Fecundity and oviposition behaviour

Egg laying started 2-3 days after last moulting. The eggs of *A. longispinosus* are oval, translucent, and much larger than those of *T. urticae*. Eggs were mostly laid near the midrib of the leaf or at the curved part of the leaf, provided there were sufficient eggs and nymphs of the prey mite. Sometimes eggs were laid on the silken threads woven by the tetranychid mite. Females laid never more than two eggs per day, most females laid one egg per 1, 2, or 3 days. The average (±SD) number of eggs laid by each female was 11.2±4.92 (n = 15) eggs (range: 0-17) over their life span of 19.4±12.94 days (range: 4-37). The most pronounced difference with other reports concerned the total number of eggs laid during the full adult life span: we found 11.2 eggs only, whereas Mallik et al. (1998) recorded 40-50 eggs/female when fed on *T. urticae*. Thongtab et al. (2001) found 19.5 eggs/female, when fed on *Eotetranychus cendanai*, whereas a total of 25.2 eggs/female were reported by Abhilesh & Sadhrma (2002) when reared on *Tetranychus ludeni*. This difference may be due to difference in season and climate, and due to differences in prey species and host plant.

Juvenile development time

Mean (± SD) incubation period was 3.8±0.71 days (range: 3.0-4.5) and all eggs hatched. The newly hatched larva had a whitish and translucent appearance and three pairs of legs. It remained near the egg shell for a few hours and fed on a few eggs or quiescent stages of two-spotted spider mite, until moulting to the next instar. The mean larval period was 1.3±0.26 days (range: 1.0-2.0. After a few hours, the larva moulted into a protonymph, which was larger and yellowish, and with four pairs of legs. They move actively in search for food, while exploring the substrate with their forelegs and pedipalps. The protonymphal stage lasted for 1.7±0.32 days (range: 1.0-2.5). The deutonymphs were larger and darker yellowish than the protonymphs. This stage lasted for 2.0±0.45 days (range: 1.5-2.5).

Adults

After a few hours quiescence, the deutonymph casted its skin and developed into an adult. The male's body was smaller than the female's, and their colour remained yellow throughout life. However, the sexually mature female changed from dark yellow to reddish, with a transparent globular structure at the centre of the idiosoma, representing the terminal oocyte. Most of the mature predatory mites were found hidden underneath the spider mites' webs. Similarly, Zhang et al. (2000) reported more females of *A. longispinosus* on bamboo leaves with webnests of *Schizotetranychus nanjingensis* than on leaves without webnests.

Moulting behaviour

Moulting behaviour of the predatory mites was quite different from that of the two-spotted spider mite. The quiescent stages of the predator were spent largely in an active, mobile state, yet became motionless for only a few hours before shedding the old integument, which took about 7-10 min.

Feeding behavior

Adult predatory mites and young larvae were mostly found feeding on eggs and quiescent stages of *T. urticae* (in agreement with Mallik et al., 1998), but protonymphs and deutonymphs mostly fed on the motile stages of this prey. Sometimes a single prey female was attacked by two or three predator nymphs. Young nymphs climbed over the adult prey and inserted their mouthparts in the anterior region of the prey to suck body fluids.

Conclusion

Our studies showed that the predatory mite *A. longispinosus* can survive and reproduce under the temperate climate conditions prevailing in the hilly regions of the Western Himalayas, albeit at a lower rate than in tropical climates. Zhang et al. (1996) also reported that this mite can survive for many days when stored at 9±1 °C, and Lee & Ahn (2000) found up to 50% survival at 15-21 °C. This further indicates that the predator may be used for control of two-spotted spider mite in various crops in Himachal Pradesh, in open fields during summer, and in polyhouses throughout the year.

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Effect of the entomopathogenic fungus *Beauveria bassiana* on three acarine pests

Abd-Allah M. Afifi, Aiman M. Mabrouk & Amal A. Asran[1]

Agric. Zoology Dept., Fac. Agriculture, Cairo University, Giza, Egypt. E-mail: amafifi51@gmail.com; and [1]Plant Pathology Institute, Agric. Research Center, Giza, Egypt

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) as control agent of European red mite, *Panonychus ulmi* Koch, citrus rust mite *Phyllocoptruta oleivora* (Ashmed), and two-spotted spider mite *Tetranychus urticae* Koch was investigated. Fungal spore suspensions at 2×10^6 and 2×10^8 spores/ml were applied to *P. ulmi* and *Ph. oleivora*. Mite mortality increased with an increase in spore concentration and exposure time; 14 days after treatment with 2×10^6 and 2×10^8 spores/ml, average mortality was 62.5 and 83.3% for *P. ulmi*, and 82.6 and 91.7% for *Ph. oleivora*, respectively. Spore suspension (2×10^8) was applied to citrus fruits and leaves to control citrus rust mite under field conditions. Seven days after spraying fruits and leaves, respectively, reduction of the mite population was 74.9 and 72.5%, and after 14 days 83.8 and 85.8%. Spore suspension (2×10^6) was also applied to cotton plants (cultivars Giza 70, 45, and 83) in the field, to control *T. urticae*. Spider mites on Giza 45 were more susceptible to fungus treatment than on the other two cultivars; 14 days after treatment, the mite population was reduced by 67.9, 78.4, and 66.5% on Giza 70, 45, and 83, respectively.

Key words: Entomopathogenic fungi, biological control, *Beauveria bassiana*, *Tetranychus urticae*, *Phyllocoptruta oleivora*, *Panonychus ulmi*

The two-spotted spider mite *Tetranychus urticae* Koch, the European red mite *Panonychus ulmi* Koch, and the citrus rust mite *Phyllocoptruta oleivora* (Ashmed) are considered important pests, because of the damage they inflict on many agricultural and horticultural crops. There is an increasing interest in pathogens of phytophagous mites, which may be attributed to their success in applications. In several cases pathogens were proven to be good alternatives for chemical insecticides (Chandler et al., 2000). Entomopathogenic fungi play an important role in regulation of natural mite populations, and are sometimes able to decimate populations of phytophagous mites (van der Geest et al., 2000).

The fungus *Beauveria bassiana* (Balsamo) has been used to control *T. urticae* (Tamai et al., 1999; Hassan, 2003; Abdel-Moneim, 2004; Afifi et al., 2004) and citrus rust mite (Alves et al., 2005). This pathogen has high stability in mixtures with some chemical insecticides (Sewify, 1989). Several other species of pathogenic fungi were tested against mite pests, such as *Hirsutella thompsonii* Fisher against *Ph. oleivora* (McCoy, 1975; Latge et al., 1988) and against *T. urticae* (Hanna & Heikal, 1995), *Neozygites floridana* Weiser & Muma against *T. urticae* (Brandenburg & Kennedy, 1982; Mietkiewski et al., 1993), and *Verticillium lecanii* (Zimmerman) against *Eutetranychus orientalis* (Klein) (Sewify & Mabrouk, 1991).

The objective of this work was to study the effect of the entomopathogenic fungus *B. bassiana* on *P. ulmi*, *Ph. oleivora*, and *T. urticae* under laboratory and field conditions.

MATERIALS AND METHODS

Conidia of *B. bassiana* were brought from the USDA-ARS Collection of Entomopathogenic Fungal Cultures, Plant Protection Research Unit, Cornell University, Ithaca, NY, USA. Conidia were cultured on autoclaved Potato dextrose agar (PDA) medium. Inoculated PDA medium was incubated for 2 weeks at 25 °C. Fungal spores were harvested by rinsing with sterilized distilled water, then filtered through sterilized cheese cloth to reduce mycelium clumping. Spores were counted using a haemocytometer. To study the effect of fungus application on *P. ulmi*, two concentrations were prepared, 2×10^6 and 2×10^8 spores/ml. Sterilized potato leaf discs (2.5 cm diameter) were put on moist cotton wool pads placed in glass Petri dishes, where a few drops of water were added daily to the cotton wool to maintain a suitable moisture level. Ten leaf discs, each containing 10 individuals of homogenous adult females were used. The same two fungal concentrations were tested on *Ph. oleivora.* Experiments were conducted as mentioned before, except that citrus leaf discs were used. Five replicates were obtained for each fungal suspension concentration. Control treatments involved sprays of distilled water only. All experiments were conducted in the laboratory at 21±2 °C and 90% r.h. Mortality (%) was determined at 3, 7, and 14 days after treatment.

In the field, the fungal suspension was applied to citrus trees (2×10^8 spores/ml) to control the citrus rust mite, and on cotton plants (2×10^6 spores/ml) to control the two-spotted spider mite. Three cotton cultivars were treated: Giza 70, Giza 45, and Giza 83. The control treatment involved sprays with water only. The numbers of mites were determined on citrus leaves and fruits and on cotton leaves, before spraying and 1 and 2 weeks after treatment. Mite population reduction (%) was calculated according to Henderson & Tilton's equation (1955).

RESULTS AND DISCUSSION

The higher fungus concentration caused higher mortality and *Ph. oleivora* was more sensitive than *P. ulmi* (Table 1). Mortality increased with the time since treatment. Fourteen days after treatment with 2×10^6 and 2×10^8 spores/ml, average mortality was 62.5 and 83.3% for *P. ulmi*, and 82.6 and 91.7% for *Ph. oleivora*, respectively.

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Table1 Effect of two concentrations (no. spores/ml) of *Beauveria bassiana* in controlling *Panonychus ulmi* and *Phyllocoptruta oleivora* at 21±2 °C and 90% r.h.

Spore concentration	*P. ulmi* mortality (%) after			*Ph. oleivora* mortality (%) after		
	3 days	7 days	14 days	3 days	7 days	14 days
2×10^6	5.0	15.0	62.5	32.4	69.4	82.6
2×10^8	41.4	70.0	83.3	40.0	87.2	91.7
Control	1.9	5.7	13.4	0.0	4.3	7.5

Table 2 Efficacy of *Beauveria bassiana* (2×10^8 spores/ml) in controlling *Phyllocoptruta oleivora* on citrus trees under field conditions.

Citrus	Treatment	Initial mite no./leaf	No. mites/leaf after		Reduction (%) after	
			7 days	14 days	7 days	14 days
Fruit	Fungus	41.1	19.04	8.9	74.9	83.8
	Water	41.4	76.4	55.3		
Leaf	Fungus	11.5	6.8	2.8	72.5	85.8
	Water	11.9	25.6	20.4		

Table 3 Efficacy of *Beauveria bassiana* (2×10^6 spores/ml) in controlling *Tetranychus urticae* on three cotton cultivars under field conditions.

Cotton cultivars	Treatment	Initial mite no./leaf	No. mites/leaf after		Reduction (%) after	
			7 days	14 days	7 days	14 days
Giza 70	Fungus	17.7	12.6	4.7	38.01	67.9
	Water	17.9	20.1	14.8		
Giza 45	Fungus	23	10.7	5.5	57.6	78.4
	Water	22	26.4	24.4		
Giza 83	Fungus	17.9	12.1	5.0	44.7	66.5
	Water	18	22.0	15.0		

The suspension of 2×10^6 spores/ml gave best results against *T. urticae* 7 days after application (Afifi et al., 2004), but in the present study this concentration caused low mortality in *P. ulmi* (15%).

Seven days after spraying with 2×10^8 *B. bassiana* spores/ml in the field, the reduction of the *Ph. oleivora* population was 74.9 and 72.5% on citrus fruits and leaves, respectively, and after 14 days reduction was 83.8 and 85.8% (Table 2). The reduction of *T. urticae* on cotton plants in the field after spraying with 2×10^6 *B. bassiana* spores/ml increased with an increase in the time of exposure since the sprays. Spider mites on cultivar Giza 45 appeared to be more susceptible to fungus treatment than mites on the other two cultivars; 14 days after treatment, the mite population was reduced by 67.9, 78.4, and 66.5% on Giza 70, 45, and 83, respectively (Table 3).

Beauveria bassiana is evidently a promising biocontrol agent against *T. urticae* as well as other pest insects, and this particular entomopathogenic fungus is easily cultured on artificial media. Hence, it can be propagated in large quantities. Furthermore, it has a long half-life compared to other pathogenic fungi, and has a relatively wide range of pest arthropods as hosts, which increase its importance as a biocontrol candidate (Hassan, 2003). This type of pathogens has high stability in mixtures with some groups of chemical insecticides (Sewify, 1989). Ali et al. (2005) used the biopesticide Bio-Fly (based on *B. bassiana*) in an integrated pest program to control *Ph. oleivora* and *Brevipalpus phoenicis* (Geijskes) on citrus trees in Egypt. Alves et al. (2005) studied the pathogenicity of *B. bassiana* to *Ph. oleivora*, one of the major pests of citrus crops. Fungus conidia were found to adhere all over the mite's body surface, especially around the anus, where vegetative mycelium was found entering the mite body. They noticed the formation of small crystals that were produced inside the mite's body during colonization of the body cavity by the fungus.

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Hirsutella thompsonii as a mycoacaricide for *Aceria guerreronis* on coconut in India: research, development, and other aspects

P. Sreerama Kumar
Project Directorate of Biological Control, HA Farm Post, PO Box 2491, Hebbal, Bellary Road, Bangalore 560 024, India.
E-mail: psreeramakumar@yahoo.co.in

Since 1998, the coconut mite, *Aceria guerreronis*, has been a chronic constraint to coconut farming in India, the third largest producer of coconuts in the world. From the beginning, biological control has been perceived as the most sustainable method for its management, despite the availability of several alternatives such as chemical and botanical pesticides, and nutrition-based cultural methods. Intensive research resulted in the identification of the mite-specific fungal pathogen, *Hirsutella thompsonii*, as the most important natural regulator of the coconut mite, and this subsequently led to the development of Mycohit, a powder formulation of the fungus. The pathogen has been evaluated as a short-term as well as a long-term biocontrol agent since 2000. Spraying of the product on young bunches resulted in high fungus-associated mortality of the mite in many locations. Two liquid variants of the formulation, viz. Mycohit-LG20 and -OS, were also evaluated across four south Indian states. The fungus was found to be capable of bringing down the mite population up to 90%, resulting in considerable reduction in pre-harvest nut damage. In several trials, the fungal treatment was superior to azadirachtin, dicofol, triazophos, and/or wettable sulphur.

Key words: *Aceria guerreronis*, coconut, eriophyid mite, *Hirsutella thompsonii*, mycoacaricide

Several years have elapsed since the coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), appeared on a vast scale in India, the third largest producer of coconuts (*Cocos nucifera* L.), with an estimated share of 18% in global production. India is the only country in the top three producers to be affected by the nut-invading mite. It almost jeopardized the entire coconut-based farming and industry. In effect, *A. guerreronis* continues to cost millions of rupees through losses in coconut production and increased plant protection and plant nutrition costs. In spite of extensive research, most strategies suggested for managing the pest still remain unsustainable and anecdotal.

Recommendations started with chemical pesticides in 1998, which eventually resulted in the declaration of a 'chemical holiday' in 2001 because of associated problems. Later control methods were based on botanical pesticides, mainly azadirachtin, and nutrition-based cultural methods, as well as combinations of several methods. From the beginning, however, biological control has been perceived by the stakeholders, especially scientists, growers, and administrators, as the most sustainable method for managing *A. guerreronis*. In two international workshops held in 2000 in Sri Lanka (Fernando et al., 2002) and in 2003 in India (Singh & Rethinam, 2003), biological control was given more emphasis than any other control strategy. In fact, *Hirsutella thompsonii* Fisher (Mitosporic fungi: Hyphomycetes), a mite-specific fungus, is the only natural enemy that has been evaluated on a large-scale as a biological control agent for the coconut mite so far.

Basic research

The striking association of *H. thompsonii* with the coconut mite, and the ability of the fungus to establish natural regulation of the pest across all the infested areas in south India, suggested that it would pay to augment the fungus to suppress the pest (Sreerama Kumar & Singh, 2000; Sreerama Kumar et al., 2001, 2002; Sreerama Kumar, 2002). Over 5,000 mite-infested, fallen as well as harvested, tender coconuts of varying age were collected during 1999-2000 for isolation of fungal pathogens. As many as 35 fungal species were repeatedly obtained from cadavers of the mites suspected to be dead from infection. Nevertheless, only *H. thompsonii*, and a few new pathogens, including species of *Acremonium*, *Lecanicillium*, and *Sporothrix*, could cause infection in the mite when 60 isolates including all isolated genera were screened under in vitro conditions. The basic research from characterization to mass production of *H. thompsonii* resulted in product development by the Project Directorate of Biological Control (PDBC) (Sreerama Kumar & Singh, 2000; Sreerama Kumar, 2002).

Development of formulations

The development of a powder formulation named Mycohit (Fig. 1), the first Indian mycoacaricide based exclusively on *H. thompsonii* [isolate MF(Ag)5; IMI 385470], has been summarized in earlier publications (Sreerama Kumar & Singh, 2000, 2002; Sreerama Kumar, 2002). Subsequently, during 2002,

Figure 1 Mycohit, a powder formulation of *Hirsutella thompsonii.*

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Table 1 Field evaluation of Mycohit at Adichunchanagiri, Mandya district, Karnataka (mean values ± SE; n = 12).

Treatment	Live mites (no. mm^{-2})				Pre-harvest damage grade	
	Pre-treatment		Post-treatment			
	4th bunch	5th bunch	Tagged bunch 1	Tagged bunch 2	Tagged bunch 1	Tagged bunch 2
Mycohit	5.7±1.55	8.8±2.48	1.2±0.42	0.3±0.26	3.2±0.37	2.8±0.35
Mycohit-T (T-04)	6.1±2.05	3.4±1.51	1.9±0.94	0.4±0.17	3.8±0.35	3.6±0.35
Cosavet-DF	3.4±0.88	2.7±0.87	1.5±0.85	1.4±0.45	4.4±0.17	4.2±0.18
Neemazal	3.6±0.90	3.2±1.05	1.0±0.28	1.5±0.55	4.1±0.33	4.3±0.20
Control	6.2±1.61	3.3±1.21	4.6±1.26	4.0±1.47	3.9±0.37	3.6±0.33
P value*	0.80	0.17	0.033	0.0074	0.11	0.0042

Pre-treatment counts were done on samples obtained before application of treatments and post-treatment counts on day 45 after the first application. Final damage grading was done on 5 August 2002 [Mycohit, Mycohit-T (T-04), and Control] and 12 August 2002 [Cosavet-DF and Neemazal].

*Data were analysed by one-way ANOVA (GraphPad Prism for Windows; www.graphpad.com). Pre- and post-treatment data were square-root transformed first [√(x+0.5)].

four new variants, viz. Mycohit-LG10, -LG20, -OS (all liquids), and -GP (granular), were developed for field evaluation in various states wherever *A. guerreronis* has been a problem. Shelf-life studies on the formulations indicated that the viability of the fungus decreased drastically over time, especially when the formulations were stored under ambient conditions. Under laboratory conditions, all four new formulations killed >80% of the mites, with Mycohit-LG20 (90.3%) performing the best. Under field conditions, the new formulations killed >75% of mites, again with Mycohit-LG20 (89.8%) performing the best.

Field evaluation

Extensive field evaluation of Mycohit was carried out initially by PDBC in Bangalore Urban and Rural districts in Karnataka. Sufficient mycoacaricide was produced for distribution to scientists at various research centres for carrying out field trials and to farmers for use and collection of data.

Protocol 1

The first-round protocol was based on the mortality of the coconut mite underneath the perianth (Fig. 2) (Sreerama Kumar & Singh, 2000; Sreerama Kumar, 2002). Three applications of Mycohit with 15-day intervals were found to be better than single and double sprays in the initial round of extensive field experiments. Fungus-associated mortality in young bunches for up to 70 days from the beginning of the experiment was taken into consideration in most of the trials.

Protocol 2

An improved and simplified protocol for the evaluation of Mycohit was developed in September 2001 for use in further multilocation trials. The importance of the pre-harvest nut-damage-grade as a parameter to judge efficacy was taken into account in this new procedure. Briefly, the assessment consisted of analyzing pre-treatment live-mite population counts on one nut each from the fourth and fifth bunches just before applying the treatments. The second and third bunches were tagged for assessing post-treatment populations on day 45 after first application, and for damage grading during the pre-harvest stage. The grading system on a scale of 1-5 was as follows: 1, no damage; 2, 1-10% damage; 3, 11-25% damage; 4, 26-50% damage; and 5, >50% damage, with reduction in size and great distortion.

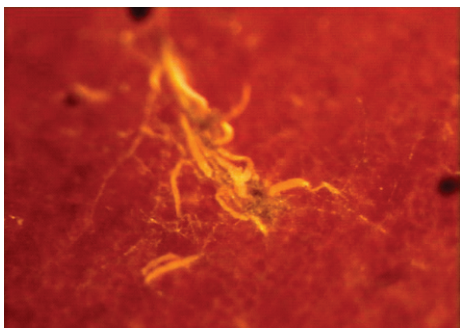

Figure 2 Mortality of the coconut mite brought about by *Hirsutella thompsonii* inside the perianth.

The revised protocol was used for the first time in a field experiment laid out at Adichunchanagiri Mahasamastanam Mutt near Bellur (Mandya district, Karnataka) for comparing the efficacy of Mycohit (1%) and Mycohit-T (T-04; 1%) – produced by the Hindustan Antibiotics Limited, a public-sector undertaking located in Pune, dealing largely with pharmaceuticals – with azadirachtin (0.005%) [Neemazal T/S 1% EC, EID Parry (India) Limited] and wettable sulphur (0.4%) (Cosavet-DF, Sulphur Mills). Including the control (treated only with water) there were five treatments, each with 12 palms (n = 12 replicates). First, second, and third sprays were given on 27/28 November, 13, and 28 December 2001, respectively.

Pre-treatment counts of live mites (no. mm^{-2} nut surface) just below the perianth were 3.4-6.2 in the fourth bunch and 3.2-8.8 in the fifth bunch (Table 1). In terms of live-mite density on tagged bunches 1 and 2 on day 45, both Mycohit versions were found to reduce the mite population: 73.1 and 92.2% reduction by Mycohit, and 59.2 and 90.2% by Mycohit-T, in comparison with the control populations on comparable bunches. Mycohit could reduce the post-treatment mite population by 89.3% over the pre-treatment population (Mycohit-T: -76.0%).

An interim grading for damage on the nuts from the tagged bunches was done on 4 April 2002 and both Mycohit versions were found to have performed better than the chemicals up to then. The final data on nut damage from the field experiment were collected in August 2002. The Mycohit treatment was significantly different from the control treatment in reducing the nut damage in tagged bunch 2 (Table 1).

Interestingly, since there was no control over the fungus spread in the field, the samples from the control palms showed pathogen-associated death, which might have eventually affected the results to some extent.

Multilocation field trials

Immediately after the release of Mycohit in May 2000, several field trials were performed through collaboration with local research centres in the southern states. Later a decision

Table 2 A brief summary of results from the multilocation field trials on *Hirsutella thompsonii* formulations.

	Formulation		
	Mycohit	Mycohit-LG20	Mycohit-OS
Maximum reduction (%) in post-treatment mite population over control	90.7	83.9	84.4
	Chemical		
	Triazophos	Wettable sulphur	Dicofol
Reduction (%) caused by chemical over control in the same trial	22.8	59.6	69.0
Place of trial*	Annehalli	Vellanikkara	TG Colony

Reduction (%) is based on the mean of two tagged bunches.

*Annehalli: Kolar district, Karnataka; Vellanikkara: Thrissur district, Kerala; TG Colony: Chitradurga district, Karnataka.

was made at the Ninth Biocontrol Workers' Group Meeting (October 2000, Bangalore) to evaluate Mycohit under the All-India Coordinated Research Project on Biological Control of Crop Pests and Weeds. However, all these earlier multilocation trials were mortality-based and these data are not presented here.

The pathogen has been evaluated both as a short-term and long-term control agent from 2000 in several states.

Short-term trials

During 2002-2004, Mycohit and its two liquid variants, Mycohit-LG20 and -OS, were evaluated based on the new protocol by eight research institutes through field trials in 10 districts across four south-Indian states, viz. Andhra Pradesh, Karnataka, Kerala, and Tamil Nadu. In comparison with control, more than 50% reduction in post-treatment mite population was observed with Mycohit, -LG20 and/or -OS in nine trials across seven districts in Karnataka and Kerala.

The fungus was found to be capable of bringing down the post-treatment population of the mite by as much as 90% (Table 2). In several trials, fungal treatment was superior to azadirachtin, dicofol, triazophos, and/or wettable sulphur. *Hirsutella thompsonii* formulations yielded a significant reduction in pre-harvest nut damage in one trial each in Kolar, East Godavari, and Thrissur districts, and in two trials in Thiruvananthapuram district.

Long-term trials

On 6 August 2002 it was decided by the group of scientists working on the coconut mite to evaluate the potential of *H. thompsonii* as a long-term regulating agent through multilocation field trials. The protocol for the experiment consisted of spraying a block of 25 palms in the middle of a coconut plantation and monitoring the spread of the fungus in eight directions through quarterly assessments and annual grading for 2 years. The trials were laid out in Karnataka, Kerala, and Tamil Nadu. The fungus was able to spread from tree to tree as indicated in the quarterly and annual assessments (data not shown).

Government support

Over the years both state and central governments have shown interest in *H. thompsonii* as a mycoacaricide for the coconut mite. The Planning Commission of the Government of India expressed keen interest in the fungal biocontrol of the pest. The Coconut Development Board, Ministry of Agriculture, Government of India, and the Indian Council of Agricultural Research under the World Bank-funded National Agricultural Technology Project sanctioned specific projects. A proposal made to the Directorate of Plant Protection, Quarantine and Storage, Government of India, for inclusion of *H. thompsonii* in the Schedule to the Insecticides Act of 1968, resulted in the addition of *Hirsutella* in the list by the Central Insecticides Board, thus enabling the registration of products based on *H. thompsonii* for field use.

Commercial interest

Hindustan Antibiotics Limited was one of the first companies to show interest in the commercialization of Mycohit. Though the experimental versions of Mycohit produced by that company performed well in the field (Sreerama Kumar, 2002), no commercial product was brought out, for reasons other than product performance and market demand. However, in 2003 a decision was taken to sell *H. thompsonii* culture to individuals, institutes, and industry for research or commercialization. Many commercial producers of biological control agents have purchased the fungus culture from PDBC for a nominal amount of Rs 2,000 (approx. USD 42) per tube provided with an agar slant. Apart from industry in India, firms based in Europe and Sri Lanka too expressed interest in the fungal product.

Conclusion

Natural or augmented biological control of the coconut mite is here to stay. There has been a steady build-up in the diversity of natural enemy species, as well as their populations over the past few years in the regions infested with *A. guerreronis*. Although a suite of biological control agents remains desirable, the single currently available agent of *H. thompsonii* needs further thrust in the near future. Through a systematic research and development programme, adequate knowledge has been generated on the utility of *H. thompsonii* as a mycoacaricide for the coconut mite in India. This experience should be shared among all countries that are already affected by this pest, and with those that are facing the risk of this pest's invasion.

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Fusarium species: acaropathogenic fungi as potential control agents against coconut mite, *Aceria guerreronis*

Gunasingham Mikunthan & M. Manjunatha[1]

Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Jaffna, Sri Lanka, E-mail: gmikunthan@gmail.com, and [1]Department of Entomology, University of Agricultural Sciences, College of Agriculture, Shimoga 577201, Karnataka, India

Aceria guerreronis (Acari: Eriophyoidea) inflicts severe damage on the nuts of commercial coconut palms. Investigations were carried out in India and Sri Lanka to investigate the association of mycopathogens with *A. guerreronis.* Mite-infested nuts were collected, brought to the laboratory, and examined for the presence of fungi associated with *A. guerreronis.* Cadavers showing mycelial growth were surface sterilized and inoculated into potato dextrose media. *Fusarium* sp. was isolated from the cadavers. Biorationals such as Azadirachtin *Fusarium semitectum*, *Fusarium* sp. isolate GM15, *Lecanicillium lecanii*, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Trichoderma viride*, plus the regularly used pesticide Abamectin, were evaluated for their impact on *A. guerreronis*. The benign control experiment involved spraying equal amounts of distilled water. All treatments were applied to the crown area of the palms in the field and mites were counted by detaching nuts from the inflorescence prior to spraying as well as 7, 15, and 23 days after spraying. On day 23 after spraying, *Fusarium* sp. isolate GM 15 and *F. semitectum* were the most effective, with 94 and 79% mite population reduction relative to the water control, respectively. Azadirachtin (66%) was the next most effective, but Abamectin and *L. lecanii* were less effective. To establish an eco-friendly management of *A. guerreronis*, the two isolates of *Fusarium* sp. seem the best biorationals.

Key words: coconut mite, biorational, pathogenicity, *Fusarium*, *Aceria guerroronis*

The nut-infesting coconut mite, *Aceria guerreronis* Keifer, is a serious pest of coconut in Central and South America, Africa, and Asia. This mite penetrates between the upper and lower tepals and finally reaches the fruit surface covered by the perianth within a few weeks to a month after fertilization (Howard & Abreu, 1991). Feeding of the mites can cause physical damage. As newly formed tissues expand, the surface becomes necrotic and suberized. Uneven growth results in distortion and stunting of the coconut, leading to 15-40% reduction in copra yield. The labour requirement for dehusking has also been increased by *A.guerreronis.* Losses of 40-70% premature nuts have been reported. A loss of coir has also been reported in India to be as much as 40% (Nair, 2000). *Aceria guerreronis* can also kill coconut seedlings by feeding on growing tips (Aquino & Arruda, 1967).

Aceria guerreronis is a serious threat in coconut growing regions and like other invasive agricultural pests it is capable of dramatic population growth, causing the costs for control to be high. A wide range of chemicals has been employed to control the coconut mite over the past three decades, but the results have not been satisfactory. Coconut is traditionally grown by small farmers, who cannot afford repeated use of insecticides. As an alternative, biological control has been considered as a promising strategy against *A. guerreronis* (Moraes & Zacarias, 2002). In this study biorationals such as Azadiractin, *Fusarium semitectum*, *Fusarium* sp. isolate GM-15, *Lecanicillium lecanii*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Trichoderma viride*, and a standard check of Abamectin were evaluated for their impact on control of *A. guerreronis*.

MATERIALS AND METHODS

Tender nuts were collected from severely mite-affected coconut fields and inspected under stereomicroscope in the laboratory. The nuts were kept inside polythene bags to increase humidity, and fungal growth was examined. Slide mounts were prepared by transferring mite cadavers with a fine brush (size 0) and mounted in lacto-phenol cotton blue. Mycelial development and fungal characters were observed under 100× magnification.

Thin slices of meristematic tissue of coconut tender nuts were cut with a blade and the slices were placed separately in a Petri dish (90 mm diameter), with three layers of wet filter paper to increase humidity. Petri dishes were covered with black paper to prevent exposure to light.

Mites exhibiting mycelial growth were carefully lifted with a micro needle from the inner side of the perianth. The cadavers were surface-sterilized in 0.5% hypochlorite for approximately 2 min and then rinsed thrice in sterile deionized water. The mites were then placed directly on Sabouraud dextrose agar medium in 15-ml screw-capped culture tubes and incubated at 25 °C and L12:D12. Pure cultures of the fungus were prepared after three transfers at 72-h intervals from the original dish onto fresh dishes. Fungi were identified using a method by Booth (1971) and the keys of FUSKEY (http://res.agr.ca/brd/fusarium/home1.html).

An experiment was conducted to assess the effect of the mycopathogens and some biorational chemicals on *A. guerreronis*, with Abamectin as a control, on a bunch with pale

Table 1 Biorationals and their dosages used.

Treatments	Dosages
Azadirachtin (0.3 g/l)	3 ml / l of water
Fusarium semitectum	10^9 spores / ml
Fusarium sp. GM15	10^9 spores / ml
Abamectin (18 g/l)	1 ml / l of water
Lecanicillium lecanii	10^9 spores / ml
Beauveria bassiana	10^8 spores / ml
Metarhizium anisopliae	10^8 spores / ml
Trichoderma viride	10^6 spores / ml
Control	Distilled water alone

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Table 2 Efficacy of biorationals on the population of coconut mite, *Aceria guerreronis*.

Treatments	Pre-count no. mites/12.5 mm²	Population reduction (%) compared to water control			
		7 DAT	15 DAT	23 DAT	Mean
Fusarium sp. GM 15	125	61.3	64.9	93.9	73.3a
Fusarium semitectum	119	56.2	62.8	78.7	65.9a
Azadirachtin	120	41.2	43.5	66.4	50.4b
Abamectin	114	26.0	29.5	41.3	32.3c
Beauveria bassiana	112	25.0	32.2	40.1	32.4c
Metarhizium anisopliae	122	27.7	30.2	39.2	32.4c
Lecanicillium lecanii	129	14.5	32.6	34.2	27.1c
Trichoderma viride	115	13.5	28.2	31.3	24.3c

DAT, days after treatment

Means followed by the same letter were not significantly different (ANOVA followed by Duncan's Multiple Range Test; P>0.05)

yellow patches of damage (Table 1). *Fusarium semitectum* (ARSEF 7233) and *Fusarium* sp. GM15 (ARSEF 7381), which was isolated from the broad mite *Polyphagotarsonemus latus* (identified by M Geiser, PennState University, University Park, PA, USA), were used to evaluate the pathogenicity to coconut mites. Preliminary experiments revealed that these *Fusarium* spp. caused 93-95% mortality under controlled environmental conditions in the laboratory. In addition, *B. bassiana*, *M. anisopliae*, and an antagonistic fungus, *T. viride*, were evaluated. Yellow patches were used to indicate active and healthy populations of *A.guerreronis* underneath the perianth. Short hybrid palms of 10 years old with 2–6-month-old bunches were hand sprayed. The palms were labeled and the mites were counted prior to spraying. Treatments were replicated four times. The benign control involved spraying an amount of water on bunches equal to the volume used for the pesticides.

Samples were taken on day 7, 15, and 23 after spraying. One infested nut was collected from each palm for counting the mites. Total mite numbers were assessed on four places on the nut, from an area of 12.5 mm² marked by a cork borer at the meristematic tissue after removing the perianth.

RESULTS AND DISCUSSION

Fungal pathogens isolated from coconut mite cadavers were identified as *Fusarium* sp. The fungi were found infective on nymphs and adults. Diseased mites appeared moribund and were found entangled in fine silvery white mycelial strands. The association with other fungi, such as *M. anisopliae*, *B. bassiana*, and *Fusarium pallidoroseum*, has been recorded earlier in Kerala, India (Haq et al., 2000). In India the first isolation of *H. thompsonii* has been reported by Ramarethinam et al. (2000). In Sri Lanka, there were no reports so far, regarding the isolation of fungal pathogens from *A. guerreronis*, but Fernando (2000) claimed that *H. thompsonii* has been found on *Dolichotetranychus* sp., another mite infesting coconut.

Logistically the use of pathogens is likely to be more satisfactory than the use of predators and recent advances in mycopesticides make the development of an effective mycoacaricide a real possibility. It is expected that effective natural enemies of a pest will be found in the place of origin of the pest, where the pest and its enemies have been in contact for the longest time (Van Driesche & Bellows, 1996). Predatory mites have been reported by many workers, but this is the first record of *Fusarium* sp. from pest mites in Sri Lanka.

Although in a varying degree, all biorationals tested were found to express a significant suppressive effect on the coconut mite, compared to the control. The two *Fusarium* species proved to be superior in reducing the mite population: 23 days after treatment *F. semitectum* recorded 79% reduction compared to the water-treated control, *Fusarium* sp. GM15 94% (Table 2). Generally, micro-organisms produce a variety of secondary metabolites in culture medium and these metabolites have proven to be effective against a wide range of mites in fruits and vegetables crops.

Azadirachtin was the next best biorational to reduce coconut mites. In the form of Neem oil, this botanical pesticide is commonly recommended against coconut mite (Ramarethinam et al., 2000). Fernando et al. (2000) reported that a 2% Neem oil-garlic mixture caused highest reduction at 4 weeks after treatment, but these products also affected predatory mites up to 4 weeks after application. Treatments with Abamectin, *L. lecanii, M. anisopliae, B. bassiana*, and *T. harzianum* did not differ much from each other, but reduced the mite population, albeit less than Neem oil. The fungal entomopathogen *L. lecanii* has been reported by others to yield control of the pest mite in coconut groves (Ramarethinam et al., 2000; Gopal & Gupta, 2002).

In conclusion, the fungi *F. semitectum* and *Fusarium* sp. GM15 were found to be most effective in suppressing the population of coconut mites. Hence, developing a formulation using *F. semitectum* and *Fusarium* sp. GM15 seems to be the best choice for managing the coconut perianth mite, *A. guerreronis*.

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Agricultural Acarology: Pesticides and Biological Control

Biocontrol of phytophagous mites in Quebec apple orchards

Noubar J. Bostanian, Gaétan Racette & Jacques Lasnier[1]

Horticultural and Development Centre, Agriculture & Agri-Food Canada, 430 Gouin Blvd. St. Jean-sur-Richelieu, Quebec, Canada J3B 3E6. E-mail: bostaniannj@agr.gc.ca; and [1]Co-Lab R & D Inc, 655 Delorme, Granby, Quebec, Canada J2J 2H4

During the 1980s biocontrol of phytophagous mites was based on the mass rearing and inundative releases of predators in orchards. This approach was found to be unreliable and impractical on a large scale. Since 1990 a highly robust grower-friendly philosophy for biocontrol of phytophagous mites in orchards has been initiated, based on the conservation, re-colonization, and augmentation of several naturally occurring predacious mites in the field. The success of this approach is based on a comprehensive understanding of the toxicology of all pesticides used to manage arthropod pests and diseases of apple. This information is relayed by pamphlets to growers who prepare their own pest management programs, with the help of extension agents. A simple technique has also been developed to transfer pruned winter- and summer-wood from a donor orchard where biocontrol of mites has been established to a recipient orchard where biocontrol is in the process of being established.

Key words: Biocontrol, phytophagous mites, predacious mites, orchards, apples

In North America consumers are demanding high-quality fresh apples virtually free of any pesticide residues. Meanwhile, in addition to several diseases of apples, there are about 100 different species of arthropods that have been identified as direct or indirect pests of apple. Because of the extensive scientific information made available in recent years in a subject that can easily become overwhelming, we have focused our attention to biocontrol of phytophagous mites in Quebec (Canada) apple orchards, where we have been the principal researchers. We hope that this case history will generate enough interest to develop biocontrol programs in other crops.

HISTORY OF MITE BIOCONTROL ON APPLE IN QUEBEC

Spray and count era (1945-1970)

This was the era of 'laissez faire, laissez passer'. Prophylactic treatments based on the phenology of the apple tree were the order of the day. It had been brought about by the discovery of the insecticidal properties of DDT and the organophosphates (OP) just before World War II, and the carbamates in the 1950s. An exception to this approach was the 'modified' spray program developed and accepted by growers in Nova Scotia, Canada (Pickett et al., 1946). This program was based on the use of lead arsenate and Bordeaux mixture to manage arthropods and diseases of apple. It was modified for Quebec apple orchards, whereby the principal fungicide was glyodin and, in addition to the lead arsenate, several insecticides such as methoxychlor, ryania, nicotine sulphate, carbaryl, and phosalone were used whenever needed (LeRoux, 1960; Parent, 1975). This indiscriminate use of pesticides provoked insurmountable phytophagous mite problems, because of the accelerated development of resistance among the mites and the almost total elimination of predators and parasitoids in orchards.

Count and spray era (1970-1990)

The discovery of resistant strains of predacious mites by Downing & Arrand (1968) in British Columbia (Canada) and by Hoyt (1969) in Washington State (USA), ushered a new age in biocontrol of phytophagous mites in orchards in Pacific NW America. This was swiftly followed by the mass rearing, transport, and release of predacious mites in Michigan (USA), that had only insecticide-susceptible strains of predators (Croft & Barnes, 1972). Meanwhile in Quebec, a nominal action threshold of five motile forms of tetranychids during the growing season was established and acaricide treatments were rationalized to a pre-bloom treatment followed by mid-summer treatment based on the action threshold.

Biocontrol by inoculation

The late 1970s and early 1980s witnessed extensive research in biocontrol of phytophagous mites in Quebec apple orchards modelled after the studies of Wearing et al. (1978) in New Zealand and Croft & Hoyt (1983) in Michigan. The research was carried out in four phases. Phase 1 involved: (1) screening for naturally selected OP resistance, (2) mass rearing of predators, (3) check for maintenance of OP resistance, and (4) assess effects of other pesticides used in orchards to the selected strain of predator. Phase 2 involved an estimate of the number of predators and the most opportune time to release them in order to achieve biocontrol of phytophagous mites. Phase 3 involved the integration of techniques and know-how gained in the previous phases in an experimental block in an orchard. Phase 4 involved large-scale rearing of predators and validation of the program in a commercial context.

Phase 1

Naturally selected OP resistant *Neoseiulus fallacis* (Garman) (Phytoseiidae) (Fig. 1), from Dunham, Quebec, and from Vineland, Ontario, were crossed. Most members of this family (1) have a short generation (1 week from egg to adult), (2) are easy to rear on two-spotted spider mites (TSSM), *Tetranychus urticae* Koch (Tetranychidae), and pollen, and (3) do not fly, therefore they can be handled without having to be cooled or anaesthetized. The progeny was then reared in greenhouses according to Rock & Yeargan (1970). The progeny showed no mortality or behavioural changes following treatments with azinphos-methyl (Guthion® 50WP) and/or phosmet (Imidan® 50WP). Resistance was maintained by bi-weekly treatments of the mass-reared predators with phosmet. The mass-reared *N. fallacis* were also used to elucidate the effects of several pesticides to this predator (Bostanian & Belanger, 1985; Bostanian & Racette, 1997; Bostanian et al., 1984, 1985, 1998).

Phase 2

Small plot trials of 10 single-tree replicates showed that the most appropriate time to inoculate a plot was when the density of European red mite (ERM), *Panonychus ulmi* (Koch) (Tetranychidae), did not exceed 2.5 mobile forms per leaf in spring. Below that threshold, variations in ERM numbers within the block made ERM numbers statistically inaccurate. Furthermore, there would not be enough prey per leaf to sustain a predator release. On the other hand, above 2.5 ERM per leaf, a predator-prey ratio in favour of the predator was difficult to attain and sustain. The most practical release threshold, a compromise, among different parameters was estimated at 100 *N. fallacis* per tree at 2.2 motile tetranychids per leaf. At this threshold, biocontrol of these and other phytophagous mites within the same season was highly probable irrespective of whether the canopy of the trees touched or did not touch one another. Another small plot study showed that cyhexatin (Plictran® 50WP) at 140 g/ha could be used as a strategic treatment in biocontrol plots to tilt the predator-prey ratio in favour of the predator, whenever control of phytophagous mites by the predator seemed to be in difficulty. The cyhexatin treatment reduced the prey population, but it did not eradicate it completely. Based on these results, phase 3 was initiated in an immaculately weed-free plot of 100 dwarf trees, at the Agriculture and Agri-Food Canada research orchard at Frelighsburg, Quebec.

Figure 1 An adult *Neoseiulus fallacis* reared in a greenhouse feeding on an adult two-spotted spider mite (Photo: J. Lasnier).

Phase 3

Prior to the commencement of the study, the block was sampled systematically throughout the season in 1978 to note if any indigenous phytoseiid predators were present. None were found and in the spring of 1979, 10,000 *N. fallacis* were released in 100 dwarf apple trees. The predators were distributed on the scaffold limb section of the tree on leaves with host mites present. The leaves were stuck to the scaffold limb with double-sided sticky carpet tape.

The densities of phytophagous and predacious mites were estimated from early June to late September from 10 leaves per sample tree per sample date. In 1979, biocontrol of phytophagous mites in the experimental block was achieved by late June (Fig. 2A). The following year it was achieved by the end of July (Fig. 2B). In subsequent years, the ratio of *N. fallacis* to prey was monitored and an index described by Croft & McGroarty (1977) was used to verify whether biocontrol of phytophagous mites was taking place. Whenever required, cyhexatin was applied and additional predators were released to tilt the predator-prey ratio in favour of the predators and re-establish biocontrol of phytophagous mites.

Other arthropod pests were managed by the application of azinphos-methyl, pirimicarb (Pirimor® 50DF), and phosmet. Apple scab was managed by 'protective' and 'after infection' curative treatments. The following fungicides were applied whenever required: captan (Captan® 50WP), captafol (Difolatan® 480FL), dodine (Equal® 65WP), and dichlone (Phygon® XL). At harvest, every year (1979, 1980, 1981, and 1983) 3,000 apples were scored for injury caused by at least 10 species of arthropods and scab. With the exception of 1981 the mean percentage of damaged fruit was less than 5%. A cost analysis showed that the program was 34% less expensive than the 'count and spray' program (Bostanian & Coulombe, 1986).

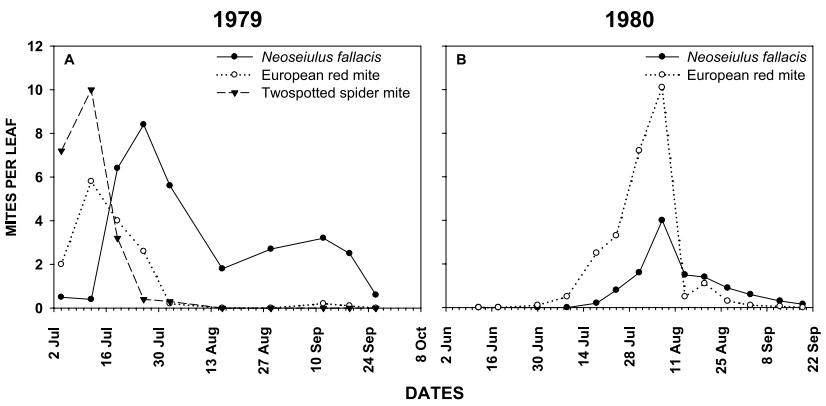

Figure 2 Predator-prey population levels (different stages of active forms only) per leaf in high-density apple orchard, Frelighsburg, Quebec, 1979 (A) and 1980 (B). Biocontrol in 1980 was achieved by late July following the maximum abundance of *Neoseiulus fallacis* (Garman) in the orchard (Courtesy of Bostanian & Coulombe 1986).

Phase 4

Two blocks of 1 ha each (1,000 dwarf trees per block) were selected in 1988 and 1989 at 'Pommeraie de Dunham Inc.' Dunham, Quebec. At the appropriate action threshold, approximately 100,000 predators were released in each plot and predator-prey ratios were monitored carefully. After two seasons, the study was considered a partial success, terminated, and shelved forever. It was an educational experience to the research team. For example, the action threshold (2.2 mites per leaf) to inoculate predators in an orchard was difficult to respect from a practical point of view. In 1988, just as the predators were to be released, adverse weather conditions forced the release date to be postponed by several days. Meanwhile, the trees had to be treated with a 'protectant' fungicide against apple scab. As the fungicide was known to have deleterious effects on the predator, a delay of 3 weeks was respected before the predators could be released. In the meantime, the prey population increased and, even after several inoculations, biocontrol of mites in the two blocks could not be achieved. The following year, the inoculations were carried out at the action threshold, and a strategic treatment of propargite (Omite® 50WP) was used to tilt the predator-prey ratio in favor of the predator. Biocontrol of phytophagous mites seemed to have been achieved when *N. fallacis* migration towards the ground cover jeopardized the results. Field observations confirmed reports in the literature that the apple rust mite (ARM), *Aculus schechtendali* (Nalepa) (Eriophyidae), was an alternate food for *N. fallacis.* However, we found *N. fallacis* to be restless predators and as the population of tetranychid mites decreased in the tree canopy, more and more *N. fallacis* searched for their preferred food, tetranychid mites, on the ground cover. This was because in commercial orchards, weed management was not as stringent as in the experimental small plots. Hence, if any weeds that harboured TSSM, had been located by the predators, then they would migrate and colonize it. Meanwhile, a virtually non-existent tetranychid mite population in the tree canopy allowed the few ARM (the least preferred food of *N. fallacis*) to increase to very high densities (2,000 ARM/leaf). Thus, while we had managed to control the tetranychids on the trees, ARM had now become a problem. The ARM could be managed with endosulfan (Thiodan® 50WP). However, endosulfan is toxic to *N. fallacis*. This scenario, along with the complex logistics required to inoculate orchards with *N. fallacis* (100,000/ha), indicated the impracticability of the approach in a commercial context and the program was shelved.

Conservation and augmentation (1990 to date)

Biocontrol of phytophagous mites from 1979 to 1989 was focused on inoculative releases of predators. Inspired by the interest shown by apple growers in biocontrol and some successes made by semi-organic apple growers in the late 1980s, we changed the research strategy from inoculation to conservation, re-colonization, and augmentation of natural predators in the early 1990s. Conservation and augmentation meant the creation of an environment that would conserve biocontrol agents and allow them to increase in numbers (DeBach, 1964). The extensive published and unpublished toxicological data we had along with the experience that we had acquired in recent years in biocontrol of mites, allowed us to create entire orchards that were friendly for the natural re-colonization and propagation of predacious mites from the perimeters of commercial orchards. A key factor for our success was the mosaic architecture of farmland between the South bank of the St. Lawrence basin and the US border, the principal apple growing region of Quebec. In this region we have orchards next to small forests, vineyards, small fruit gardens, prairies, corn fields, and vegetable plots: a highly diversified area, with fields that may be treated with pesticides, treated partially, or non-treated. Another factor that helped us was the availability of sterol inhibitor and strobulin fungicides, that were innocuous to predacious mites. This approach was initiated in less than 10 ha in 1991 and by 2006 it included over 80% of the orchard acreage.

The predacious mites are: *N. fallacis*, *Typhlodromus caudiglans* Schuster (Phytoseiidae), *Agistemus fleschneri* Summers (Stigmaeidae), *Balaustium* sp. (Erythraeidae) and sometimes a few *Anystis baccarum* (L.) (Anystidae). *Hyaliodes vitripennis* (Say) (Heteroptera: Miridae) has also been noted in a number of orchards. We are currently trying to understand the coexistence of these species in commercial orchards.

Orchards where biocontrol of mites has been well established are now being used as natural nurseries. We have developed techniques to harvest the complex of predacious mites from these nurseries and introduce them into orchards where biocontrol of phytophagous mites by natural re-colonization is being established. As the conservation and augmentation technique is based on a complex of predators, it is far more stable than the approach used in the early 1980s. Nevertheless, as in the program with 'inoculative releases' the growers have to follow a pesticide regime that is ecologically sound. In return, they save on acaricide costs and application time. To date grower interest has been keen because of the high cost of acaricides, resistance problems associated with phytophagous mites, and, last but not least, environmental concerns.

The procedure to transfer mites along with an exhaustive summary reporting the toxicity of most of the pesticides currently used in orchards to predacious mites was brought to the attention of every grower across Quebec and Canada by pamphlets (Lasnier et al., 2002, 2004).

Transfer of predacious mites from a donor to a recipient orchard

The recipient orchard block must be at least 0.2 ha and must provide sufficient phytophagous mites for the predacious mites to feed upon. If the phytophagous mite population in the recipient orchard is high, chemical controls should be used to reduce the mite population down to a manageable level prior to the introduction of predatory mites. In Quebec a 'tank mix' of 200 ml of clofentezine (Apollo® SC) with 65 l of dormant oil per ha in early spring (late tight cluster to early pink) has provided excellent early-season control of phytophagous mites until the predacious mites begin to recolonize the trees in mid season. Failure to reduce the pest populations to a manageable level may result in unsatisfactory control, possibly causing leaf bronzing.

Wood from winter and/or summer pruning is used to transfer predatory mites from a donor into a recipient orchard. Winter pruning should be carried out on 3-year-old or older wood, whereas summer pruning should be done on the annual growth and suckers. The amount of pruned wood collected and transferred to orchards depends upon the area of the recipient orchard, the population density of predacious mites on the pruned wood, and the size of trees within that orchard. In winter, 5-kg bundles of winter pruned

Table 1 Toxicity of certain pesticides to major predacious mites in apple orchards according to studies and observations carried out in Quebec.

	Active ingredients	Commercial names	Stigmaeidae	Phytoseiidae
Insecticides	Acetamiprid	Assail	-[1]	Non-toxic
	Azinphos-methyl	APM, Guthion, Sniper	Non-toxic	Non-toxic
	Bacillus thuringiensis	Dipel, Foray	Non-toxic	Non-toxic
	Imidacloprid	Admire	Non-toxic	Non-toxic
	Lambda-cyhalothrin	Matador	Non-toxic[2]	Non-toxic[2]
	Methomyl	Lannate	Toxic	Toxic
	Phosmet	Imidan	Non-toxic	Non-toxic
	Pirimicarb	Pirimor	Non-toxic	Non-toxic
	Tebufenozide	Confirm	-	Non-toxic
	Thiacloprid	Calypso	-	Non-toxic
	Thiamethoxam	Actara	-	Non-toxic
Acaricides	Clofentezine	Apollo	Toxic	Non-toxic
	Dicofol	Kelthane	Toxic	Non-toxic[3]
	Formetanate	Carzol	Toxic	Toxic
	Mineral oil	Superior oil	Non-toxic	Non-toxic
	Pyridaben	Pyramite	Toxic	Non-toxic
Fungicides	Captan	Captan, Maestro	Non-toxic	Non-toxic
	Dodine	Equal	Non-toxic	Toxic
	Flusilazole	Nustar	Non-toxic	Non-toxic
	Kresoxim-methyl	Sovran	Non-toxic	Non-toxic
	Mancozeb	Dithane, Manzate	Toxic	Toxic
	Mancozeb + Dinocap	Dikar	Toxic	Toxic
	Metiram	Polyram	Non-toxic	Non-toxic
	Myclobutanil	Nova	Non-toxic	Non-toxic
	Sulfur	Sulfur	Toxic	Toxic
	Trifloxystrobin	Flint	Non-toxic	-

[1]No data available; [2]Use up to 15 days after petal fall; [3]Non-toxic to certain predator strains.

wood should be placed vertically against the base of the tree trunks from bud break until petal fall to allow predatory mites to migrate into the orchard. Four to five bundles of winter pruned wood would be required for each standard size tree and 1-2 bundles would be required for every dwarf tree. In summer, the pruned wood from the donor orchard is distributed in the recipient orchard as follows: among dwarf trees 12-15 branches consisting of annual growth and suckers should be placed on the foliage of fruit bearing branches for 2-3 weeks. On standard trees, around 50 branches should be placed on the foliage of fruit bearing branches. The pruned wood should have 20-25 leaves and on average not less than one predator per leaf.

After the predacious mites have been released in an orchard, growers must use pesticides that have minimal effects on them. Predators that had survived pesticide treatments carried out in the donor orchard against insect pests and diseases are likely to be tolerant and resistant to these pesticides. Therefore, the owner of the recipient orchard should obtain the list of pesticides used in the donor orchard and use this information to plan his pest management strategies (Table 1). A comparison of the re-colonization of an orchard block by the transfer of pruned wood and natural re-colonisation is shown in Figure 3.

Finally, just as it is difficult to estimate the number of predators that may have been transferred from a donor to a recipient orchard, it is equally difficult to establish how long it would take to establish biocontrol in an orchard. It may require more than one season. Consequently, the population of phytophagous mites may be high in the first season but it eventually subsides to acceptable levels by the second or third season. Biocontrol is considered established when on average at least 10% of the leaves harbour a predator in early July.

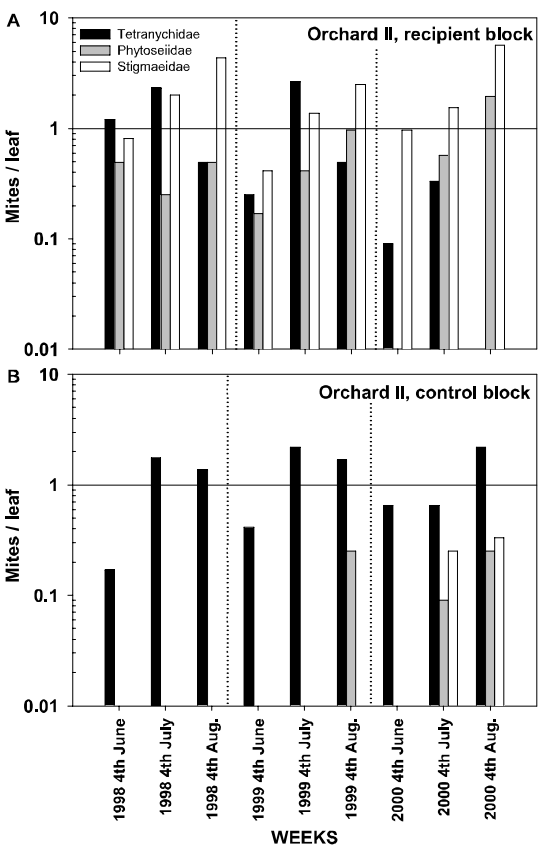

Figure 3 A comparison between a block that received winter-pruned wood (A) and its control block (B) that did not receive any winter-pruned wood. The winter-pruned wood was transferred into the release block during the second week of April in 1998 and again in 1999. Mites were counted once per month by brushing 50 leaves (five samples of 10 leaves) per block (Courtesy of Bostanian et al. 2005).

Conclusion

A highly robust, grower-friendly mite management philosophy is now in place across Quebec. It is based on the conservation, re-colonization, and augmentation of at least two dominant predacious mite species per season and often another 1-2 predacious species of minor importance every now and then. A key factor for the success of this program is based on a regular comprehensive evaluation and understanding of the toxicology of pesticides that may be applied in an orchard. This information is generated in confidence with the cooperation of the agro-chemical industry. At the appropriate time, the information becomes public and it is relayed in a suitable format to growers. Armed with this information and the cost of treatments, growers – with the help of their pest control advisors – design their own management programs and assume all risks.

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Side effects of pesticides on phytoseiid mites in French vineyards and orchards: laboratory and field trials

Serge Kreiter, Philippe Auger & Romain Bonafos[1]

UMR Centre de Biologie et de Gestion des Populations, Montpellier SupAgro/Institut National de la Recherche Agronomique, 2 Place Pierre Viala, 34060 Montpellier cedex 01, France. E-mail: kreiter@supagro.inra.fr; and [1]Centre de transfert, Montpellier SupAgro/Institut National de la Recherche Agronomique, Montpellier, France

Using the methodology of the French AFPP/CEB guideline no. 167 for testing side effects of pesticides on *Typhlodromus pyri*, we review the results obtained with laboratory trials for more than 120 pesticides. Most of the insecticides/acaricides tested were found to be toxic and most of the fungicides non-toxic or slightly toxic. These results provided a good indication of the toxicity assessed in field trials. To put this relation to the test, we carried out a more in-depth study on the fungicide mancozeb and its toxicity to phytoseiids in vineyard and orchard. Side effects of this fungicide were studied on *T. pyri* in the laboratory and in 4-year field trials in several grape crops. In vineyards where mancozeb had commonly been used over the years, this fungicide is generally slightly toxic. However, in plots where it had never been used, its effect on populations of *T. pyri* was more pronounced and varied from moderately toxic to toxic. Despite its intrinsic toxicity, populations were never eradicated. Laboratory results confirmed the field trial results. Even though toxicity of mancozeb is higher in lab trials, a significant correlation was established between field and laboratory results for all strains of *T. pyri*. It is striking to observe that – despite the intrinsic toxicity of not only mancozeb, but also pyrethroids and organophosphates – densities of *T. pyri* and *Amblyseius andersoni* in grape crops in the Region Midi-Pyrénées are commonly high. Our laboratory tests indeed showed that strains of these two species are resistant to deltamethrin, λ-cyhalothrin, and chlorpyriphos-ethyl, which probably explains their abundance in grape crops in this area.

Key words: Side effects, resistance, mancozeb, deltamethrin, λ-cyhalothrin, chlorpyriphos-ethyl, *Typhlodromus pyri*, *Amblyseius andersoni*

In vineyards and orchards in Western Europe, some species of Phytoseiidae are important predators of spider mites. These predators naturally occur on plants even in absence of prey because they can also feed on pollen (McMurtry & Croft, 1997). Such generalist predators must be preserved in agricultural crops by using pesticides that do not harm them. Knowledge of the side effects of pesticides on beneficial arthropods is thus of high importance for pest management (Croft, 1990). The aim of this article is to give an overview of activities of our research group at SupAgro-INRA, concerning two long-term studies and one more recently initiated study. This review serves to illustrate three advances: (1) a methodology for studying side effects, (2) the correlation between laboratory and field trials, and (3) the characterisation of pesticide resistance.

The French AFPP/CEB guideline no. 167 (Kreiter & Sentenac, 2004) is a sequential procedure for testing side effects of commercial products on *Typhlodromus pyri* Scheuten. Here, we summarize the methodology and provide a synthesis of the results obtained with the laboratory method (Kreiter & Sentenac, 1993, 2004). The correspondence between lab and field results was studied in more detail with respect to a commonly used fungicide that is toxic for phytoseiids in vineyards and orchards: mancozeb. Many studies have shown that the use of this dithiocarbamate in vineyards and orchards reduced the densities of various phytoseiids (James & Rayner, 1995; Bostanian et al., 1998). However, resistance to dithiocarbamates of one phytoseiid species has been demonstrated (Angeli & Ioriatti, 1994) and tolerance has been suspected for another species (Vettorello & Girolami, 1992). The aim of our study was to assess how the use of mancozeb in the past or the lack of it influenced the toxicity of mancozeb sprayings to *T. pyri*, the dominant species in French vineyards (Kreiter et al., 2000).

Long-term (4 years) field trials in grapevine plots at various sites were performed to determine whether mancozeb was less toxic to strains of this phytoseiid species in plots where it had been used in the past and whether it induced outbreaks of spider mites in plots where it had not been used in the past. Laboratory trials were carried out on *T. pyri* populations from this field experiment to assess the susceptibility of each strain to mancozeb. Apart from fungicides, such as mancozeb, phytoseiids in French vineyards and orchards are also exposed to many highly toxic insecticides. Field observations in the Region Midi-Pyrénées revealed that *T. pyri* and *Amblyseius andersoni* (Chant) could be found in reasonably high numbers in vineyards even though pyrethroids and organophosphates were used to control the yellow vine leaf-hopper, *Scaphoideus titanus* Ball. We tested the hypothesis that these high predator densities despite insecticide use were due to resistance to deltamethrin, λ-cyhalothrin, and chlorpyriphos-ethyl in populations of *T. pyri* and *A. andersoni* in South-West France.

MATERIAL AND METHODS

Description of the AFPP/CEB official guideline 167, part 'laboratory method'

Here, we only summarize the laboratory methods used. Details of these methods and of those used in the field are described by Kreiter & Sentenac (2004). To obtain the required quantity of females for testing, 50 deutonymphs of *T. pyri* were maintained for 6 days in artificial arenas (Overmeer & van Zon, 1982), and fed eggs of *Tetranychus urticae* Koch. Two days later, 50 males were introduced to these arenas to provide mating opportunities to the newly emerged females. Experiments were carried out on glass plates for testing residual and direct-contact effects of pes-

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ticides on *T. pyri* females (72-96 h old since mating). Each test arena consisted of two glass plates sprayed with the chemical compound, placed on wet tissue paper on top of black plastic. Each individual female (10/replicate) was isolated in a small area by means of strips of wet filer paper, serving as barriers preventing escape. Within this area, each female had access to a refuge consisting of a small piece of untreated black plastic. Pesticides were suspended in distilled water and applied using a Potter (1952) tower, producing a wet deposit of 1.5±0.2 mg/cm², equivalent to the wet deposit for a volume of 400 l/ha in vineyards. Each trial consisted of four replicates, each consisting of 10 females for the treatment and 10 for the control. Females to be tested were transferred to the glass plates, approx. 1 h after the spray residue had dried. Phytoseiids were fed with *T. urticae* eggs placed on a 0.5-mm-mesh cloth at each control. The bioassay arenas were kept in a climatic chamber at 23±1 °C, 70±10% r.h., and L16:D8 photoperiod. After treatment, female mortality and oviposition were checked on days 2, 5, 8, and 10; on day 10, surviving females were removed. Eggs, larvae, and protonymphs were counted at each control and on day 15 protonymphs were removed and their viability was evaluated. At the end of the trials (day 15), the 'global effect' (GE), ranging from 0 to 100%, was calculated according to the Baillod and Lenfant formula GE = 100 – (100 – M) × R1 × R2, where M is mortality (%) corrected for control mortality by Abbott's (1925) formula, R1 is the corrected fecundity index, and and R2 the corrected offspring survival index. R1, the corrected fecundity index, equals RT / RC, where RT is the fecundity in the treated group and RC is the fecundity in the control group (fecundity – or the number of surviving females between the 1st and the 10th day – being the sum of the numbers of eggs + larvae + nymphs observed between days 0 and 10, plus the numbers of nymphs removed in this period). R2, the corrected offspring survival index, equals OSIT (day 15) / OSIC (day 15), where the offspring survival index (OSI) in treated (OSIT) and control (OSIC) groups is expressed by the numbers of nymphs removed between days 0 and 10, divided by the numbers of eggs + larvae (on day 10) plus the numbers of nymphs removed in this period (Kreiter et al., 1998; Auger et al., 2004; Kreiter & Sentenac, 2004; Bonafos et al., 2008).

If mortality in the control exceeded 15% or if fecundity per female in the control was below 0.8 eggs/day during the test, the experiment was repeated. GE between 0-19% is considered to indicate neutral effects, whereas GE of 20-39%, 40-59%, 60-79%, and >80% indicate a slightly, moderately, clearly, and highly toxic pesticide, respectively. Here we present GE results obtained for more than 140 pesticides that are commercially available for use in vineyards and orchards.

Study of the side effects of mancozeb on *Typhlodromus pyri*

Field experiments

Trials were conducted in eight grape plots with various spraying programs located in Bordeaux, Champagne, Burgundy, and the river valleys of the Rhône and the Loire. Plots were investigated over the 1996-1999 period. Plots 1-4 were characterised by the common use of mancozeb (5,000-15,000 g/ha/year). In plots 5-8 this active ingredient had never been used. Each plot was split into two parts, one treated with mancozeb and one treated with folpet (folpet-treated subplot used as control as experiments took place in commercial plots). Folpet is a fungicide known to have no toxic effects on phytoseiid mites. The pesticides used to control other pests and diseases were neutral for *T. pyri* (Sentenac et al., 2002) and no acaricide was used during the 4-year experiment. The density of phytoseiid mites of each subplot was estimated with five (1996-97) and nine (1998-99) sample units consisting of five vine stocks. The same vine stocks were sampled for the 4-year period to assess the potential cumulative effect of mancozeb. Twenty leaves were sampled on the five vine stocks (4 leaves/vine) of each sample unit throughout the experiment. A total of 100 (1996–97) to 180 leaves (1998–99) per subplot were collected at each date. Four samples/year were collected in late spring, early summer, and late summer (growth stages BBCH 12-15, 65, 79-83, respectively; after Lorenz et al., 1994). Mites were collected using the leaf-washing method of Boller (1984) and counted and identified by means of a phase contrast microscope, using the key of Chant & Yoshida-Shaul (1987). Population residuals (RP) were calculated as the ratio between mean numbers of *T. pyri* in mancozeb-subplots and those of folpet-treated subplots and these were used to assess the relative impact of treatments.

Laboratory experiment

Phytoseiids were collected in six plots among the eight plots available at the end of the 4-year experiment (three from mancozeb, three from folpet-plots). They were reared separately and the 12 sub-populations were tested at the recommended field rate (1,400 g ai) registered for the control of downy mildew. The procedure used to assess side effects of mancozeb on strains followed guideline 167 (Kreiter & Sentenac, 2004). Two sets of three replicates of 10 females of *T. pyri* and glass support were treated with a solution of mancozeb (0.35 g ai/l) and with distilled water (control), respectively. Experimental conditions were 21±1 °C, 65±10% r.h., and L16:D8.

Statistical analysis

Statistical analyses of the field data were performed using each plot as a replicate (one replicate for mancozeb- and one for folpet-treated subplots). As mancozeb is known to have a delayed effect, densities of *T. pyri* at the end of the season were analysed. The effects (and interactions) of treatment (mancozeb vs. folpet), previous mancozeb use, year of experiment, and plot on phytoseiid densities were analysed with a mixed-model ANOVA. The same analysis was also performed with the populations in the next spring. A Student t-test (α = 5%) was used to analyse the ratios between the RP at the end of the growing season and at the beginning of the season in the following year, based on data obtained in the two types of plots (treated and untreated with mancozeb). The quantity of mancozeb/ha/year during the experiment in plots usually treated and never treated with mancozeb was compared with a Student t-test (α = 5%). A Kruskal-Wallis ANOVA was performed to compare the densities of *P. ulmi* during the 4-year period in mancozeb- and folpet-treated subplots in plots that were unsprayed or repeatedly sprayed with mancozeb. GE obtained in the laboratory experiment and the effect of mancozeb on three demographic parameters (female mortality, fecundity, offspring survival) were analysed with ANOVA followed by a Newman-Keuls test (α = 5%). Correlation between RP and GE was estimated to assess to what extent laboratory results reflect field observations.

Resistance of *Typhlodromus pyri* and *Amblyseius andersoni* populations to deltamethrin, λ-cyhalothrin, and chlorpyrifos-ethyl

Predatory mite populations

A susceptible population of *T. pyri* (TPs) was collected in an unsprayed area on *Rubus* sp. in Cévennes National Park. Moreover, a reference population of *A. andersoni* (AAs) was taken from an unsprayed apple orchard near Nîmes. Two populations of *T. pyri* (TP81-1, TP81-2) and two populations of *A. andersoni* (AA81-1, AA81-2) suspected to be resistant to pyrethroids (1) and to organophosphates (2), respectively, were collected in vineyards near Castelnau d'Auzan (County of Tarn, Region Midi-Pyrénées). Strains were kept in a climatic chamber at 21±1 °C, 70±10% r.h., and L16:D8 in artificial arenas (Overmeer & van Zon, 1982) and fed *T. urticae* eggs.

Insecticides tested

Deltamethrin (62.5 g/kg WP, recommended field rate [rfr] 0.0175 g/l), λ-cyhalothrin (100 g/l SC, rfr 0.1 g/l), and chlorpyriphos-ethyl (228 g/l SC, rfr 0.855 g/l).

Bioassays

The susceptibility of females (their exact age was unknown) of different strains was assessed. Five concentrations of each insecticide were tested. For *T. pyri*, tested concentrations ranged from 1.17×10^{-5} to 0.21 g/l for deltamethrin, 2×10^{-4} to 0.1 g/l for λ-cyhalothrin, and 1.71×10^{-3} to 0.855 g/l for chlorpyriphos-ethyl. For *A. andersoni*, they ranged from 10^{-4} to 2.65×10^{-2} g/l, 10^{-3} to 0.3 g/l, and 8.55×10^{-3} to 2.565 g/l, respectively. Both leaf disk (2.5 cm diameter, *Phaseolus vulgaris* L., cv. Contender) supports and phytoseiids were sprayed using a Potter tower. The disks were placed with the upper surface facing down in Petri dishes containing moistened cotton. After transferring females, Petri dishes were sprayed (76 kPa, 1.5 ml solution, 2.3 s) with a wet deposit of 1.5±0.2 mg/cm². For each concentration, eight leaf disks (8 replicates) were sprayed with insecticide and eight were sprayed with distilled water as a control. Following the treatment, 4-7 females were transferred onto each leaf disk, just after the deposit had dried. Eggs of *T. urticae* were added at the beginning of the bioassays to be used as a food source. Mortality was assessed 3 days after spraying. Bioassays were conducted in a climatic chamber at 21±1 °C, 70±10% r.h., and L16:D8.

Data analysis

For each dose, mortality was corrected by the Abbott formula and then analysed with Probit-Logit Analysis® software (Finney, 1971; Praxeme-CNRS, 1996). Significant differences between LC_{50} values were estimated using the 95% confidence limits. The F test was used to assess whether or not the slopes were significantly different. Resistance ratios >50% (RR_{50}) were obtained for each product and species by dividing the LC_{50} of the suspected resistant populations and that of the reference population. Resistance ratios >90% (RR_{90}) were obtained by the same relative measure applied to LC_{90}.

RESULTS AND DISCUSSION

General results obtained with the AFPP/CEB guideline 167, part 'laboratory method'

We tested more than 140 chemical products that are commercially available. For the fungicides, 70% of the products are neutral to slightly toxic, whereas for insecticides-acaricides, only 20% are in the 'neutral effect' category. Acaricides are often moderately toxic. Results obtained in the lab are correlated with those obtained in field experiments.

Side effects of mancozeb on *Typhlodromus pyri*

Field experiment

Compared to folpet, the effect of mancozeb on the end-summer populations of *T. pyri* was significant (F = 25.64, P = 0.0023) (Fig. 1). Densities of mites varied with the year of experiment (F = 4.99, P = 0.011), but were not influenced by the previous use of mancozeb (F = 3.24, P = 0.12) nor by plot (F = 1.426, P = 0.26). The *T. pyri* density was significantly influenced by interaction between the treatment and the previous use of mancozeb (F = 8.1, P = 0.029) and by the interaction between year of experiment and plot (F = 12.63, P<0.0001). There was no significant interaction between treatment and year of experiment (F = 2.86, P = 0.066), between treatment and plot (F = 2.27, P = 0.083), nor between previous use of mancozeb and year of experiment (F = 1.53, P = 0.24). Second-order interaction was not significant (F = 1.33, P = 0.29).

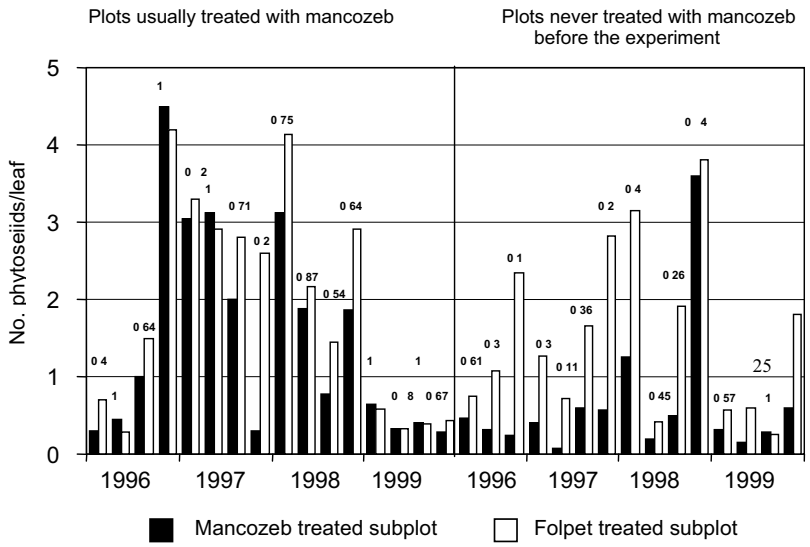

Figure 1 Densities of *Typhlodromus pyri* at the end of the season after repeated mancozeb treatments depending on previous mancozeb use. Numbers given in parentheses above columns indicate residual populations (RP) obtained in the various plots. RP = mean number of *T. pyri* in the mancozeb-treated subplot/mean number in the folpet-treated subplot.

According to our results, the effect of mancozeb sprays on the density of phytoseiids is significantly less pronounced in plots where this fungicide had been regularly used before the experiment. Detrimental effect is less frequent and the end-summer RP values were on average 2-fold lower in plots where mancozeb was not used before the experiment (Fig. 1). This difference cannot be attributed to variations in the amount applied during the experiment because the quantity of active ingredient per hectare was significantly higher (t = 3.01, P = 0.0054) in plots usually treated with mancozeb (10,740±750 vs. 7,470±780 g ai/ha/year). The more mancozeb is applied, the less *T. pyri* populations are affected.

According to the literature, *T. pyri* populations are reduced when the number of mancozeb applications is increased (Walker et al., 1989; Blümel et al., 2000a). Our results are consistent if we assume that a lower susceptibility to mancozeb of *T. pyri* populations, due to repeated exposure to this fungicide, has developed in plots where mancozeb was regularly used before the experiment. Based on their field results, Blümel et al. (2000b) also hypothesised that an increased tolerance towards mancozeb could explain the reduced detrimental effects on *T. pyri*. The toxicity of mancozeb to end-summer populations (resulting from sprayings made during the growing season), particularly in plots where it had not been used previously, was rather strong. Despite this rather strong mortality, mancozeb subplot spring populations of *T. pyri* of the following year often recovered to a level comparable to that recorded in folpet subplots (Fig. 2). The effect of mancozeb sprays was not significant on spring populations (F = 2.27, P = 0.18). In spring, the density of phytoseiid mites was not influenced by previous use of mancozeb (F = 0.07, P = 0.80) or by experimental plot (F = 1.87, P = 0.16). Year of experiment (F = 14.31, P = 0.0007), treatment, and experimental plot (F = 3.26, P = 0.043), and year of experiment*plot (F = 20.13, P<0.0001) had a significant effect on the density of *T. pyri*. The effect of treatment was not influenced by previous use of mancozeb (F = 0.02, P = 0.90) nor by year of experiment (F = 1.49, P = 0.26) and no interaction between previous use of mancozeb and year of experiment were observed (F = 0.145, P = 0.87).

Mancozeb applications ceased on late July or beginning of August. Even if mancozeb had a delayed effect (Zacharda & Hlucky, 1991), and mancozeb applications had a significant effect 4 weeks after the last treatment (Blümel et al., 2000a), *T. pyri* populations could have reproduced and developed with reduced effect during the time between the beginning of September and winter. This could reduce differences between population densities in the mancozeb- and folpet-treated subplots and explain partial or total recovery. On average, the ratios between spring and end-summer RP of the previous year obtained in plots commonly sprayed were 2-fold lower than in plots never sprayed with mancozeb before the experiment. Nevertheless, due to considerable variability, population recovery was not significantly different between the two management practices (t = 1.55, P = 0.14). The consequence of this population recovery could explain that the detrimental effect of mancozeb was not cumulative year by year, except in 1999 for spring populations in plots where mancozeb had never been used before. Nevertheless, in the same year the end-summer RP of the same plots were among the highest observed during the whole field experiment (Figs. 1, 2). Populations of *P. ulmi* were kept at very low levels during the whole experiment. This spider mite was only detected in 19% of samples, and, when detected, the average number of mites (adults and juveniles) per leaf was only 0.23±0.11. Moreover, *P. ulmi* densities were not significantly higher in mancozeb-treated than in folpet-treated subplots, regardless of the history of plant protection management and the year of experiment (H = 12.27, P = 0.66). No tetranychid outbreaks were detected in the 2 years following the end of the field experiment (2000 and 2001).

Laboratory experiment

Mancozeb toxicity to *T. pyri* was verified in lab tests and it was more pronounced than in field trials. On the toxicity scale of Kreiter & Sentenac (2004), mancozeb toxicity varied from moderately toxic to toxic. Overestimation of toxicity in a laboratory experiment is commonly observed and probably due to the fact that the experiment represents a worst-case situation (exposure to pesticide, stages of development, parameters measured). Moreover, Blümel et al. (2000a) have reported that, even after multiple applica-

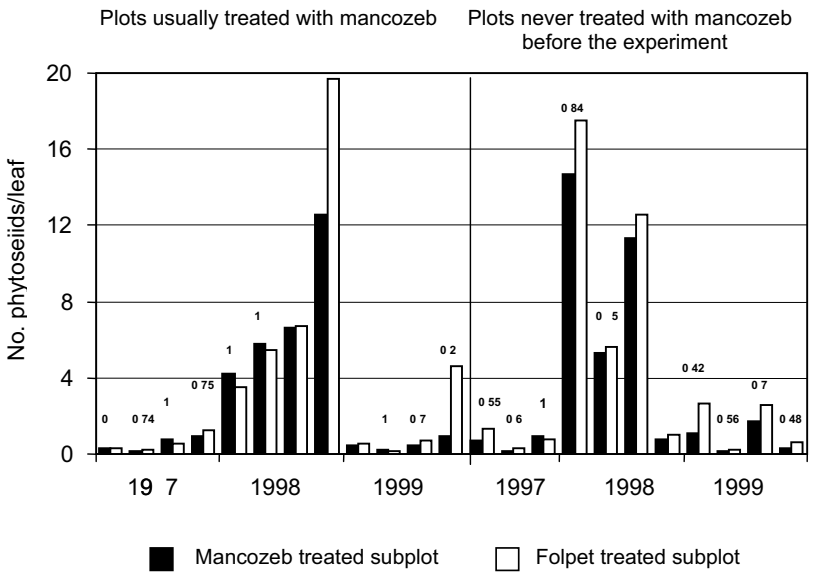

Figure 2 Densities of *Typhlodromus pyri* at the beginning of the season following mancozeb treatments applied the previous year depending on previous mancozeb use. Numbers given in parentheses above columns indicate the residual populations (RP) obtained in the various plots. RP = mean number of *T. pyri* in the mancozeb-treated subplot/mean number in the folpet-treated subplot.

tions, the effect of mancozeb was less pronounced in the field than in the laboratory where only a single spray was applied. Nevertheless, as in the field experiment, mancozeb toxicity was significantly more pronounced in *T. pyri* populations which were not usually in contact with this fungicide (F = 10.81, P = 0.0005) (Fig. 3). This result indicates that individuals collected in plots where mancozeb has been used for a long time developed tolerance to this fungicide. No significant difference was observed between mancozeb-treated and folpet-treated subplots, irrespective of pest management practices. Thus, a 4-year period of mancozeb or folpet application would not be sufficient to detect significant changes in mancozeb susceptibility of *T. pyri* populations (loss of tolerance to mancozeb in folpet-treated subplot from vineyards usually sprayed with mancozeb, or loss of susceptibility to mancozeb in mancozeb-treated subplots from plots in which this fungicide has never been used before). Mancozeb significantly affected female mortality (F = 5.48, P = 0.0037), potential fecundity (F = 6.49, P = 0.0015), and viability of female progeny (F = 8, P = 0.0004).

The laboratory method we used allowed us to demonstrate that this fungicide can affect female survival but also fecundity and survival of juveniles in mancozeb-treated females of *T. pyri*. Once again, these parameters are more affected in *T. pyri* populations originating from plots where mancozeb was not usually used and there was no difference between the two subplots of each plot. The reduction in susceptibility mainly resulted from reduced toxicity of mancozeb through female mortality (up to half reduced) and female fecundity (almost 2-fold higher) in populations usually in contact with this fungicide compared to populations never in contact. A moderate but significant correlation (r = 0.42, P = 0.004) was found between average end-summer RP and GE replicates of each population. The quite low coefficient of correlation could be explained by approximations in *T. pyri* RP estimations in the field experiment, by differences in amount of active ingredient sprayed between the plots (quality of spraying and volume/ha were also variable, resulting in variation in mancozeb exposure; alternatively, in the laboratory experiment, phytoseiids were exposed to a constant amount of mancozeb), or variations in GE estimation could also lead to moderate correlation between field and laboratory results because GE value is given with a confidence interval of 17.5% (Bonafos et al., 1999). It can be concluded that if mancozeb is considered for integrated pest control programs in plots where it has been used for a long time and where *T. pyri* is present, its use should be minimised in plots where it has never or rarely been used.

Resistance of *Typhlodromus pyri* and *Amblyseius andersoni* populations to deltamethrin, λ-cyhalothrin, and chlorpyrifos-ethyl

Deltamethrin

The TPs population (LC_{50} = 5×10^{-5} g/l; LC_{90} = 9×10^{-4} g/l) was significantly more susceptible than TP81-1 (LC_{50} = 7.9×10^{-2} g/l; LC_{90} = 3.4 g/l). RR_{50} and RR_{90} were 1,586 and 37,937, respectively. AAs (LC_{50} = 2.6×10^{-4} g/l) was 35× more susceptible than AA81-1 (LC_{50} = 9.2×10^{-3} g/l). RR_{90} was 55. RR_{90} was higher than RR_{50} for TP81-1 and AA81-1. It appeared that TPs was more affected by deltamethrin than AAs and that AA81-1 was more susceptible than TP81-1 (Table 1). The higher concentration of deltamethrin applied in French vineyards (1.75×10^{-2} g/l) corresponded to LC_{100} for TPs and AAs, to LC_{30} for TP81-1, and to LC_{65} for AA81-1 (Table 2).

λ-cyhalothrin

The TPs (LC_{50} = 3×10^{-4} g/l; LC_{90} = 3.5×10^{-3} g/l) was significantly more susceptible than TP81-1 (LC_{50} = 3.6×10^{-2} g/l; LC_{90} = 1.2 g/l). RR_{50} was 123, RR_{90} was 359. AAs was 7× more susceptible than AA81-1, and RR_{90} was 17. RR_{90} was higher than RR_{50} for TP81-1 and AA81-1. TPs was more susceptible than AAs, just like for AA81-1 and TP81-1 (Table 1). The higher concentration of λ-cyhalothrin applied in French vineyards (1.75×10^{-2} g/l) corresponded to LC_{100} for TPs and AAs, to LC_{65} for TP81-1, and to LC_{85} for AA81-1 (Table 2).

Chlorpyriphos-ethyl

The susceptibility between TPs (LC_{50} = 2.2×10^{-3} g/l; LC_{90} = 9.8×10^{-2} g/l) and TP81-2 (LC_{50} = 0.2 g/l; LC_{90} = 0.74 g/l) was significantly different. RR_{50} was 90, RR_{90} was 7.6. AAs was significantly more susceptible than AA81-2. LC_{50} values were 2.4×10^{-2} and 0.39 g/l, respectively, RR_{50} was 16, RR_{90} was 128 (Table 1). RR_{90} was higher than RR_{50} for AA81-2 and RR_{90} was lower than RR_{50} for TP81-2. It appeared that TPs was significantly less susceptible than AAs regarding LC_{50} and RR_{50} but

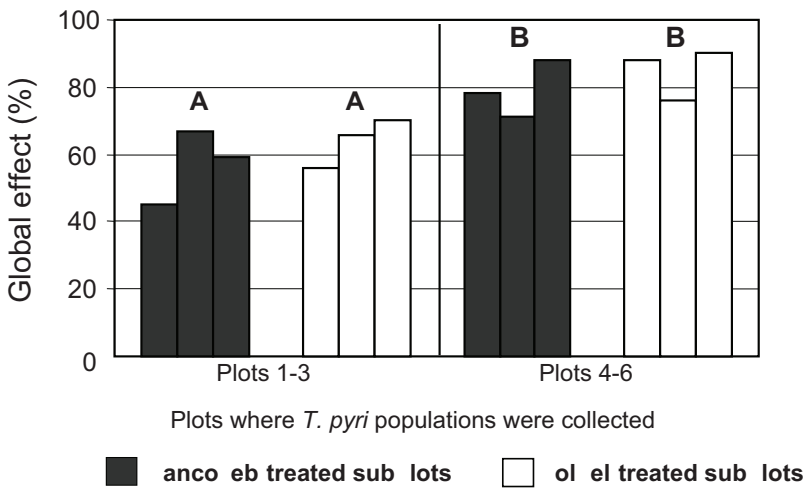

Figure 3 Global effect (GE) of mancozeb on various populations of *Typhlodromus pyri* when applied at 1,400 g ai/ha in the laboratory study depending on previous mancozeb use (means sharing the same letter are not significantly different; Newman-Keuls test, P>0.05).

Table 1 LC_{50}, LC_{90}, RR_{50}, and RR_{90} of various populations of *Typhlodromus pyri* and *Amblyseius andersoni* to deltamethrin, λ-cyhalothrin, and chlorpyriphos-ethyl.

Populations	n	Control mortality	Slope±SE[a]	LC_{50} (mg a.i.l^{-1})	95% fiducial interval	LC_{90} (mg a.i.l^{-1})	95% fiducial interval	RR_{50}	RR_{90}	χ^2	Probability
DELTAMETHRIN											
TPs	221	0	1.02±0.20a	5.0×10^{-5}	$3.0\text{-}8.0\times10^{-5}$	9.0×10^{-4}	$3.6\text{-}67.6\times10^{-4}$	-	-	2.20	0.53
TP81-1	228	0	0.78±0.22b	7.9×10^{-2}	$4.6\text{-}15\times10^{-2}$	3.4	0.81-424.2	1,586.4	37,937	3.29	0.34
AAs	218	0	3.11±0.37c	2.6×10^{-4}	$2.3\text{-}3.1\times10^{-4}$	6.8×10^{-4}	$5.0\text{-}9.4\times10^{-4}$	-	-	2.67	0.44
AA81-1	216	4.16	2.10±0.36d	9.2×10^{-3}	$7.1\text{-}11.3\times10^{-3}$	3.7×10^{-2}	$2.6\text{-}7.2\times10^{-2}$	35.53	55,10	282	0.41
λ-CYHALOTHRIN											
TPs	220	0	1.20±0.19a	3.0×10^{-4}	$1.6\text{-}4.6\times10^{-4}$	3.5×10^{-3}	$2.1\text{-}7.7\times10^{-3}$	-	-	7.44	0.05
TP81-1	249	0	0.83±0.13b	3.6×10^{-2}	$2.3\text{-}6.7\times10^{-2}$	1.2	0.42-9.5	122.63	358.91	0.52	0.91
AAs	214	0	3.08±0.36c	2.2×10^{-3}	$1.9\text{-}2.6\times10^{-3}$	5.9×10^{-3}	$4.7\text{-}8.2\times10^{-3}$	-	-	5.25	0.15
AA81-1	272	4.16	1.64±0.22d	1.6×10^{-2}	$1.0\text{-}2.2\times10^{-2}$	0.1	$7.2\text{-}10\times10^{-2}$	7.26	16.82	0.94	0.91
CHLORPYRIPHOS-ETHYL											
TPs	236	0	0.77±0.21a	2.2×10^{-3}	$0.56\text{-}3.9\times10^{-3}$	9.8×10^{-2}	$3.4\text{-}250\times10^{-2}$	-	-	2.65	0.44
TP81-1	237	2.08	2.25±0.25b	2.0×10^{-1}	$1.6\text{-}2.4\times10^{-1}$	7.4×10^{-1}	$5.5\text{-}11\times10^{-1}$	90.22	7.58	5.62	0.13
AAs	214	0	2.63±0.30c	2.4×10^{-2}	$2.0\text{-}3.0\times10^{-2}$	7.6×10^{-2}	$5.9\text{-}10\times10^{-2}$	-	-	0.85	0.83
AA81-1	203	0	0.91±0.18d	3.9×10^{-1}	$2.2\text{-}6.2\times10^{-1}$	9.7	3.9-68.1	16.35	127.76	1.55	0.67

[a]Slopes sharing different letters (within a single pesticide) are significantly different (Newman-Keuls test, P<0.05.

Table 2 Lethal concentration of different populations of *Typhlodromus pyri* and *Amblyseius andersoni* to 0.00175 g ai/l of deltamethrin (recommended field rate [rfr], for application to control Tortricoidea in vineyards), 0.0175 g ai/l λ-cyhalothrin (rfr for Tetranychidae), and 0.8125 g ai/l of chlorpyriphos-ethyl (rfr for Coccoidea).

	Deltamethrin 1.75×10^{-3} g ai/l	λ-cyhalothrin 1.75×10^{-2} g ai/l	Chlorpyriphos-ethyl 0.8125 g ai/l
TPs	LC_{100}	LC_{100}	LC_{98}
TP81-1	LC_{30}	LC_{65}	-
TP81-2	-	-	LC_{91}
AAs	LC_{100}	LC_{100}	LC_{100}
AA81-1	LC_{65}	LC_{85}	-
AA81-2	-	-	LC_{62}

the higher concentration of chlorpyriphos-ethyl applied in French vineyards (0.855 g/l) corresponded to LC_{98} for TPs and LC_{100} for AAs. TP81-2 was significantly more susceptible than AA81-2, and LC_{91} and LC_{62} were obtained for TPs and AAs, respectively, for a chlorpyriphos-ethyl concentration corresponding to the recommended field rate on Coccoidea (Table 2).

DISCUSSION

Our experiments on side effects of mancozeb on *T. pyri* in several grape crops showed a correlation between single-application trials in the laboratory and 4-year (multi-application) trials in the field. In vineyards where mancozeb had commonly been used over the years, this fungicide is generally only slightly toxic. However, in plots where it had never been used, its effect on populations of *T. pyri* was more pronounced and varied from moderately toxic to toxic. This indicates population/strain differences in mancozeb tolerance/resistance that depend on the field-specific history of mancozeb applications. According to the literature, mancozeb toxicity expression in phytoseiid mites depends on the species studied: mancozeb was responsible for high mortality in females of *Amblyseius victoriensis* (Womersley) but had no effect on females of *Typhlodromus doreenae* Schicha (James & Rayner, 1995). Other studies, using different methodologies, showed that mancozeb exhibited no effect on females of *Neoseiulus fallacis* (Garman) and sometimes caused female mortality in populations of *A. andersoni* but it reduced egg hatching, female fecundity, and affected offspring survival in *N. fallacis* and *A. andersoni* (Ioriatti et al., 1992; Bostanian et al., 1998). According to Baynon & Penman (1987), mancozeb does not cause significant mortality to adults or immatures of *T. pyri*. On the contrary, Zacharda & Hluchy (1991) have recorded mortality of females ranging from 50-90% depending on mancozeb concentration and Blümel et al. (2000a) have demonstrated that mancozeb reduced reproduction. Clearly, there is much variability on tolerance/resistance among species and populations. A survey indicating the frequency of mancozeb resistant phytoseiids throughout French wine-producing regions would be worthwhile to test this hypothesis in a more definitive way.

Resistance to deltamethrin and λ-cyhalothrin for TP81-1 and AA81-1, and to chlorpyriphos-ethyl for TP81-2 and AA81-2, was demonstrated in laboratory bioassays. In contrast, very high susceptibility to these active ingredients was demonstrated for *T. pyri* (TPs) and *A. andersoni* (AAs) from unsprayed locations (Bonafos et al., 2007). The resistance

level to deltamethrin, λ-cyhalothrin, and chlorpyriphos-ethyl obtained for each *T. pyri* and *A. andersoni* strains were moderately high to very high. *Typhlodromus pyri* appeared to be more resistant to deltamethrin and λ-cyhalothrin than *A. andersoni*, which was more resistant to chlorpyriphos-ethyl. However, it is difficult to conclude which species is more resistant to the compounds tested since the results were obtained in tests on a few strains only. Possibly, the resistance to deltamethrin of the predatory TP81-1 and AA81-1 also conferred resistance to λ-cyhalothrin and vice versa – similar to the cross-resistance between deltamethrin and cypermethrin shown by Markwich et al. (1990). It is possible that laboratory tests lead to higher mortality rates because experimental conditions maximize mortalities. Even with efficient equipment, the mortality rate of phytoseiids in grapevines would be lower due to refuges, mite development stages, and dispersal of mites to untreated plant surfaces (Duso, 1994; Croft et al., 1998). Phytoseiids TP81-1, TP81-2, AA81-1, and AA81-2, which were resistant to deltamethrin, λ-cyhalothrin, and chlorpyriphos-ethyl, would thus probably be less affected by these active ingredients in vineyards than in laboratory experiments.

Conclusion

Following the French AFPP/CEB guideline 167 (Kreiter & Sentenac, 2004), we applied a sequential procedure for testing side effects of commercial products on the generalist predatory mite, *T. pyri*. Our review of the results obtained since 1988 showed that there is a good correspondence between the results from lab and field trials. An in-depth study on the side effects of the fungicide mancozeb showed that this correspondence also holds when taking variability of *T. pyri* populations in the field into account. Moreover, in grape crops of Region Midi-Pyrénées, where *T. pyri* and *A. andersoni* were found to be abundant despite applications of pyrethroids and organophosphates, laboratory tests revealed that strains of these phytoseiids were resistant to at least some of these pesticides. These results are widely published in scientific journals and grower bulletins and appear to be accepted by the growers, as can be concluded from their apparent product choice.

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Pesticide side-effects on predatory mites: the role of trophic interactions

Alberto Pozzebon & Carlo Duso
Department of Environmental Agronomy and Crop Science, University of Padova, Viale dell'Università 16 35020 Legnaro, Padova, Italy. E-mail: alberto.pozzebon@unipd.it

The control of spider mites in protected crops is often critical due to the development of mite resistance to acaricides. Studies of the side-effects of pesticides on beneficial mites are required in order to improve integrated spider mite management. Pesticide side-effects on predatory mites of the family Phytoseiidae can be studied at the field or laboratory level. In the field, phytoseiid abundance can be related to the presence of prey and/or alternative foods, which can affect the response of predatory mites to pesticides. We investigated whether pesticide side-effects on phytoseiids may be mediated by trophic interactions. In the laboratory we evaluated the effects of two insecticides (derived from pyrethrins and *Beauveria bassiana*) on the predatory mite *Phytoseiulus persimilis* by testing the same under various exposure conditions (leaf residues, treated prey, topical application). The effects of these insecticides on *P. persimilis* were higher when the predators were fed with treated prey. Furthermore, we investigated the effects of pyrethrins on the predatory mite *Amblyseius andersoni* on vines with low or high incidence of the plant pathogenic fungus *Plasmopara viticola* (grape downy mildew, GDM). GDM mediated interactions between pyrethrins and *A. andersoni* since it is alternative food for this predatory mite. The implications of these studies for the development of toxicological methods and integrated pest management are discussed.

Key words: Pesticide side-effects, Phytoseiidae, Tetranychidae, *Amblyseius andersoni*, *Phytoseiulus persimilis*, *Tetranychus urticae*, pyrethrins, *Beauveria bassiana*, grape downy mildew, alternative food, IPM

The study of pesticide side-effects on the natural enemies of arthropod pests has attracted many researchers in the last two decades. Comparative studies have been carried out on various beneficial organisms, providing important data on the impact of several pesticides on agro-ecosystems (Hassan et al., 1983; Sterk et al., 1999). Among the natural enemies tested are the predatory mites *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius andersoni* (Chant) (both Phytoseiidae).

Phytoseiulus persimilis is a key predator of the two-spotted spider mite, *Tetranychus urticae* Koch, a serious pest of vegetables, ornamentals, and annual crops all over the world (Helle & Sabelis, 1985a). This predator occurs naturally in the Mediterranean region, but it has also been released on crops for the purpose of biological control. This practice has spread in recent decades due to resistance of *T. urticae* to several acaricides. The activity of *P. persimilis* can be hindered by the use of pesticides needed against pests or pathogens that lack a biocontrol agent. Even in organic farming, pesticides of botanical origin are frequently used. Therefore, knowledge of the selectivity of organic or 'botanical' pesticides towards *P. persimilis* is required to obtain successful biological control.

Amblyseius andersoni is an important predator of the European red spider mite, *Panonychus ulmi* (Koch), in fruit orchards and vineyards in European and north-American regions (Helle & Sabelis, 1985b; Ivancich Gambaro, 1986; McMurtry & Croft, 1997). Therefore, the impact of pesticides on this species has long been the subject of studies (e.g., Hassan et al., 1983, 1988; Sterk et al., 1999).

Different methods of assessing pesticide side-effects have been proposed for both *P. persimilis* and *A. andersoni* (Overmeer & van Zon, 1982; Hassan, 1985; Bakker et al, 1989; Candolfi et al., 2001). Most laboratory methods are designed to evaluate the effects of pesticides by exposing predatory mites to pesticide residues. However, it should be stressed that predatory mites may also be exposed topically and/or by ingestion when the prey is contaminated. A few studies have been carried out considering all the potential exposure modes and, in particular, the impact of ingesting pesticide-treated prey (e.g., Zhang & Sanderson, 1990).

In this study we have assessed the effects of two pesticides (based on pyrethrins or on *Beauveria bassiana*), frequently used to control aphids and whiteflies, on a susceptible strain of *P. persimilis*. In the laboratory, the predator was exposed to these pesticides in various ways: through topical application, fresh leaf residues, or through ingestion of its prey, *T. urticae*. Additional studies were carried out to investigate whether food availability can mediate the effects of pesticides on *A. andersoni*. We investigated the effects of pyrethrins on *A. andersoni* populations under field conditions, in particular on vines with low or high incidence of the plant pathogenic fungus *Plasmopara viticola* (Berk. & Curtis ex de Bary) Berlese & De Toni (grape downy mildew, GDM). This pathogenic fungus represents alternative food for *A. andersoni* (Duso et al., 2003; Pozzebon & Duso, 2008).

MATERIALS AND METHODS

Effects of pesticides on *Phytoseiulus persimilis* in the laboratory

Stock cultures

The strain of *P. persimilis* used in our trials was collected in Sardinia, Italy (near S. Teodoro, Nuoro) in the summer of 2003 from pesticide-free beans. This strain was reared in the laboratory of the Department of Environmental Agronomy and Crop Science, University of Padova (Italy) on beans infested by *T. urticae* at 25±1 °C and 70±10% r.h.

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Table 1 Experimental design to evaluate the effects of different modes of exposure of *Phytoseiulus persimilis* females to pyrethrins solution and *Beauveria bassiana* conidia suspension. Symbols indicate applications of pesticides (+) or of distilled water (-).

Treatments	Mode of exposure	Substrate	Prey	Predator
Control		-	-	-
Leaves (L)	leaf residues	+	-	-
P. persimilis (PP)	topical application	-	-	+
T. urticae (TU)	treated prey	-	+	-
L + PP		+	-	+
L + TU		+	+	-
PP + TU		-	+	+
L + PP + TU		+	+	+

Toxicological tests

We exposed *P. persimilis* females to pesticides by immersing in the pesticide solution: (a) predators, (b) substrate (leaves), or (c) prey (*T. urticae*). All combinations of these modes of exposure are included in the experiment (Table 1). In the 'worst case' situation, *P. persimilis* females were immersed in the pesticide solution, then transferred on treated leaves and fed with *T. urticae* previously treated with the same pesticide. Following pesticide application, the predators were reared in experimental cages similar to those described by Dennehy et al. (1993) containing a detached bean leaf as a substrate for predators and prey (*T. urticae*) (Duso et al., 2008).

Topical applications to predators or prey were conducted by the micro-immersion bioassay (Dennehy et al., 1993; Castagnoli et al., 2005; Duso et al., 2008). Mites were drawn into a small pipette tip one at a time, after which the test solution was applied, immersing the mites for 30 s. The mites were ejected from the pipette, dried on filter paper, and then transferred to experimental cages using a fine brush. One specimen of *P. persimilis* was transferred per cage. In specific treatments, leaves were treated by immersing them in the pesticide solution for 30 s. These leaves were then included in experimental cages after pesticide solutions had dried up.

Toxicological tests were conducted using young ovipositing females of *P. persimilis* collected from rearing units (25±1 °C, 70±10% r.h., and L16:D8 photoperiod). Predators were placed in experimental cages where their prey (adult *T. urticae* females) had previously been situated according to the experimental design. Every 48 h, 20-30 *T. urticae* females were added to the experimental cages. At least six replicates of five females were used for each pesticide tested. Pesticide toxicity was evaluated 72 h after application with pyrethrins and 144 h after application with *B. bassiana* solution. Mites were considered dead if they were unable to react when gently prodded with a fine brush. Maximally 10 surviving females per treatment were selected randomly and isolated individually into new cages to assess their fecundity for an 8-day period. The eggs laid were checked daily for hatching until day 5 after the first egg hatched in the control treatments. All trials were conducted under controlled climate conditions at 25±1 °C, 70±10% r.h., and L16:D8 photoperiod.

Pesticides used

The following commercial pesticides were tested on *P. persimilis*: Naturalis® (*B. bassiana* JW-1, ATCC 74040, 2.3×10^7 conidia/ml) (Intrachem Bio Italia, Grassobbio-BG) at 150 ml/hl (10.8 g a.i./hl) and Biopiren plus® (pyrethrins, 20 g a.i./l) (Intrachem Bio Italia) at 200 ml/hl (4 g a.i./hl). Each pesticide was used at the maximum concentration suggested for field applications. Distilled water was used as a control in all trials. Pyrethrins act as contact insecticides and are effective against various homopterans (e.g., aphids and leafhoppers). They have a knock-down effect and low persistence in the environment. The entomopathogenic fungus *B. bassiana* is also effective after contact to various arthropods. Relative humidity rates exceeding 60% and presence of water favour spore germination. Infection starts 24-36 h after contact with spores. Arthropods can survive for a few days after inoculation.

Interactions among *Amblyseius andersoni*, pyrethrins, and GDM in vineyards

The effects of interactions between pyrethrins application and GDM infection on *A. andersoni* colonization were evaluated in a vineyard applying a 2×2 factorial experiment. The factors involved, each with two levels, were: pyrethrins application (yes/no) and GDM foliar symptom incidence (high/low). The experiment was carried out on grapevines of the Merlot variety, in a vineyard located at Spresiano (Treviso, Veneto, Italy) during the growing season of 2005. In a preliminary survey the vineyard was readily colonized by *A. andersoni*. The experimental design ('split-plot') consisted of two blocks each with two plots of 18 vines for each GDM level. This was obtained by selecting vines showing serious or negligible GDM foliar symptoms. Each plot was sub-divided into a subplot of 9 vines per insecticide application level. The pyrethrins solution (150 ml/hl) was sprayed on 26 August. Samples were taken before insecticide application and 3, 7, and 15 days afterwards, by randomly removing 36 leaves per treatment (2 leaves per vine taken from the mid part of shoots). Mite densities were assessed in the laboratory using a dissecting microscope. Leaf symptom density was estimated using a 1-cm^2 scale printed transparent.

Data analysis

We used one-way ANOVA to estimate the effects of different modes of pesticide exposure on *P. persimilis* survival, fecundity, and egg-hatching. Treatment means were separated applying Tukey-Kramer test ($\alpha = 0.05$). Data on survival were angular transformed, whereas data on fecundity were $\sqrt{(x+1)}$ transformed, prior to the analyses, in order to approximate the ANOVA assumptions. Mortality was calculated applying Abbott's formula (1925). Data on phytoseiid abundance and GDM foliar symptom density in the experimental vineyard were analyzed by a repeated measures two-ways ANOVA (Proc GLM of SAS; SAS Institute, 1999) applying the option REGWQ of SAS to compare each factor effect per sampling date ($\alpha = 0.05$). Moreover, a t-test was applied to examine differences in least-square means for the effect of 'insecticide*GDM' interaction. The 'block*GDM' interaction was considered as an error term for the GDM effect. Data were log (y+1) transformed prior to the analysis to meet ANOVA assumptions.

RESULTS

Effects of pyrethrins on *Phytoseiulus persimilis*

The corrected mortality rates of *P. persimilis* in treatments characterised by pesticide exposure ranged from 59 to 100%. The lowest values were observed when pesticides were applied following a single mode of exposure and the highest when different modes of exposure were combined (Fig. 1). Statistical analysis applied to *P. persimilis* survival rates showed significant effects of all the modes of exposure to pyrethrins ($F_{7,42}$ = 51.73, P<0.001). Survival observed in the control was significantly higher than that in treatments involving a single mode of exposure (Fig. 1). Further differences were observed between the latter treatments and those using multiple modes of exposure where no females survived.

Fecundity of surviving *P. persimilis* females was higher in the control than under the pesticide treatments (Fig. 2). Additional differences emerged among the latter since predators exposed to pesticide-treated prey exhibited lower fecundity ($F_{3,15}$ = 16.30, P<0.001). No differences among treatments were observed regarding egg-hatching rates ($F_{3,17}$ = 2.46, P = 0.093, Fig. 2).

Effects of *Beauveria bassiana* on *Phytoseiulus persimilis*

Regarding the impact of *B. bassiana*, the corrected mortality ranged from 35 to 60% with lower values for treatments involving a single mode of exposure (Fig. 3). Exposure to *B. bassiana* had a significant effect on *P. persimilis* survival ($F_{7,39}$ = 7.98, P<0.001). Most treatments caused a decrease in survival rate in comparison with the control. However, survival rates were not significantly different between the control and the treatment characterised by topical exposure (predator micro-immersion) (Fig. 3).

Fecundity of *P. persimilis* proved to be significantly affected by *B. bassiana* applications ($F_{7,63}$ = 36.45, P<0.001). A greater effect on *P. persimilis* fecundity was observed when combined with treated prey and treated substrate (Fig. 4). Egg-hatching rates were not affected by *B. bassiana* applications, with the exception of the treatment where the three modes of exposure were combined ($F_{7,63}$ = 6.19, P<0.001; Fig. 4).

Interactions among *Amblyseius andersoni*, pyrethrins, and GDM in vineyards

During the experiment, GDM foliar symptom densities were higher on plots selected at the beginning for high GDM symptom levels ($F_{1,138}$ = 227.65, P<0.001) (Table 2). GDM symptoms were comparable in plots with a different insecticide application ($F_{1,138}$ = 3.20, P = 0.07). Regarding phytoseiids, a significant effect of pyrethrins application was observed on *A. andersoni* abundance ($F_{1,138}$ = 22.18, P<0.001) (Table 2). A significant 'time*insecticide' interaction indicates variation in phytoseiid abundance during the experiment because of the insecticide effect ($F_{1,414}$ = 10.12, P<0.001). In fact, phytoseiid densities were similar among treatments on the sampling date before pesticide application, whereas on subsequent dates they were higher on unsprayed treatments (Table 2). Moreover, phytoseiid abundance was positively affected by GDM foliar symptoms ($F_{1,138}$ = 170.79, P = 0.049). The interaction 'time*GDM' was significant ($F_{1,414}$ = 18.12, P = 0.002). *Amblyseius andersoni* densities were similar among GDM treatments on the date before pyrethrins applications, whereas differences increased during the experiment (Table 2). The significant interaction 'insecticide*GDM' ($F_{1,138}$ = 6.10, P = 0.014) indicates that the impact of pyrethrins on *A. andersoni* abundance depended

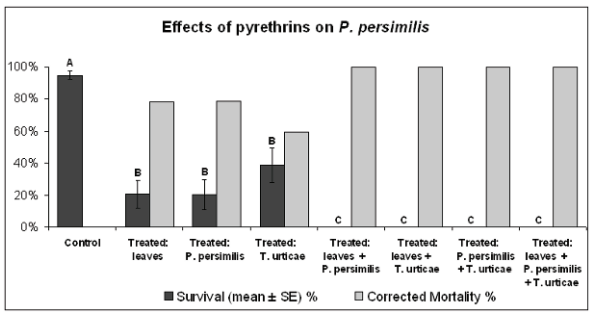

Figure 1 Survival and Abbott-corrected mortality of *Phytoseiulus persimilis* as affected by different modes of exposure of *P. persimilis* females to solutions of pyrethrins. Means followed by different letters indicate significant differences according to the Tukey-Kramer test (P<0.05).

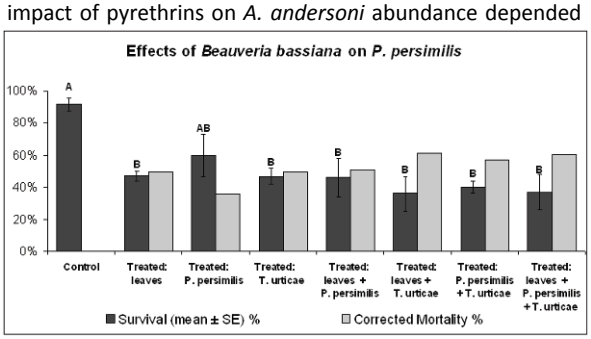

Figure 3 Survival and Abbott-corrected mortality of *Phytoseiulus persimilis* as affected by different modes of exposure of *P. persimilis* females to *Beauveria bassiana* conidia suspension. Means followed by different letters indicate significant differences according to the Tukey-Kramer test (P<0.05).

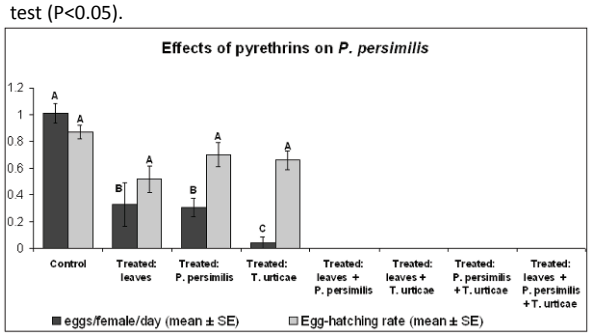

Figure 2 Fecundity and egg-hatching of *Phytoseiulus persimilis*, as affected by different modes of exposure to pyrethrins solution. Means followed by different letters indicate significant differences according to the Tukey-Kramer test (P<0.05).

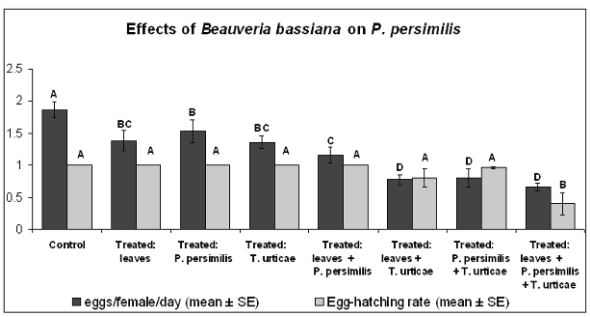

Figure 4 Fecundity and egg-hatching of *Phytoseiulus persimilis*, as affected by different modes of exposure to *Beauveria bassiana* conidia suspension. Means followed by different letters indicate significantly differences at the Tukey-Kramer test (P<0.05).

Table 2 Population dynamics of *Amblyseius andersoni* and Grape downy mildew (GDM) incidence observed during the experiment. Treatments: '+' indicates pyrethrins application or high GDM symptom incidence, '–' indicates absence of pyrethrins application or low GDM symptom incidence. Means followed by different letters indicate significant differences according to the t-test (P<0.05).

Treatments	T-1	T+3	T+7	T+15
Amblyseius andersoni				
Pyrethrins – GDM +	0.722±0.19	1.611±0.35a	1.111±0.18a	1.306±0.24a
Pyrethrins – GDM –	0.5±0.15	0.667±0.19b	0.556±0.10b	0.43±0.11c
Pyrethrins + GDM +	0.778±0.17	0.722±0.04c	0.556±0.16b	0.806±0.16b
Pyrethrins + GDM –	0.583±0.12	0.722±0.08c	0.25±0.09c	0.081±0.06d
Grape downy mildew foliar symptoms				
Pyrethrins – GDM +	3.75±0.75a	5.278±0.68a	6.222±1.34a	6.639±0.89a
Pyrethrins – GDM –	0.222±0.13b	0±0b	0.167±0.09b	0.222±0.13b
Pyrethrins + GDM +	3.25±1.02a	4.306±1.01a	6.722±1.06a	3.778±0.67a
Pyrethrins + GDM –	0±0b	0.056±0.06b	0.417±0.18b	0.167±0.12b

on GDM symptom levels (Table 2). In particular, phytoseiids were seen to re-colonize vines, especially those treated with pyrethrins and infected by GDM. On the other hand, phytoseiid densities remained at low levels on treated vines with negligible GDM symptoms (Table 2).

DISCUSSION

Pyrethrins proved to be highly detrimental to *P. persimilis* females when different modes of exposure were combined. Lower mortality rates were found when treatments were done with a single mode of exposure. Since *P. persimilis* can only survive by preying actively upon tetranychids and must repeatedly disperse to find its prey, the probability of being exposed to pesticides on treated crops is high because of the multiple ways in which they may get in contact. The *P. persimilis* strain used in the experiments had not been subject earlier to pyrethrins, and can thus be considered susceptible to pesticides. It is likely that this strain has low chances of survival in commercial and organic farms. However, we also obtained maximal mortality when *P. persimilis* strains collected in conventional farms (pesticide-treated) were exposed both directly (predator immersion) and indirectly (leaf immersion) to pyrethrins (Duso et al., unpubl.). The fecundity of surviving females was significantly affected by pyrethrins application. Moreover, the effect of the pesticide was higher when the predator was offered treated prey.

A few experiments on the effects of pyrethrins on phytoseiids have been reported in the literature. Hassan (1982) found complete pyrethrin-induced mortality in three strains of *P. persimilis* when juveniles were exposed to direct sprays and ingested treated prey or when adult females were exposed to leaf residues on potted plants. Because Hassan (1982) used a different test method (potted plants) and did not report the concentration of active ingredients in the pyrethrin formulate, it is not possible to compare his data with our results. In other laboratory studies involving the phytoseiid mite *Neoseiulus californicus* (McGregor), pyrethrins applied directly (predator immersion) and indirectly (leaf immersion) caused 81% mortality and reduced fecundity by about 50% (Castagnoli et al., 2005). Our experiments revealed that *P. persimilis* exposed to pyrethrins through a single mode of exposure had some chance of survival. Moreover, we found that a single mode of exposure to pyrethrins allows for the reproduction of *P. persimilis*, albeit at a reduced level. Among the modes of exposure used, the greatest effect on fecundity was observed when treated prey was provided to phytoseiids.

The moderate detrimental effect of *B. bassiana* on *P. persimilis* survival was independent of the different modes of exposure. The impact of *B. bassiana* on *P. persimilis* fecundity and egg-hatching was more relevant when predators were also exposed to treated prey.

Our results stress that exposure to contaminated prey has a relevant effect on *P. persimilis*. In other studies, interactions between pesticides and *P. persimilis* have been evaluated by using different modes of exposure (e.g., residual or ingestion of treated prey). Greater toxic effects were found when acaricide-treated prey were provided to the predators, confirming the trends that emerged from our data (Kim et al., 2002). Zhang & Sanderson (1990) exposed *P. persimilis* to abamectin residues on leaves or to contaminated prey. The effects on survival were significant only in the latter case, while those on reproduction were fairly similar (about 53%). In another study, where *P. persimilis* was exposed to endosulfan by leaf residues or pesticide-treated prey, only the former appeared to be detrimental for the predator (Blümel et al., 1993).

The present study may have implications for the protocol of toxicological tests, since these are usually performed by adding untreated prey to *P. persimilis* on a treated substrate (Oomen et al., 1988; Bakker & Calis, 1989; Blümel et al., 1993). Usually, in biocontrol strategies, *P. persimilis* is released some days after pesticide application in order to reduce the impact of pesticide residues. Hence, the three modes of contamination examined in the present work have a reduced chance to occur simultaneously. However, in south-European regions, *P. persimilis* can naturally occur on vegetables infested by its prey and both get potentially treated with pesticides applied for the control of other pests. Our simulation can be useful to predict the impact of pesticides in these situations and to improve integrated pest management strategies. Local pesticide applications can decrease the risks associated to multiple modes of exposure. This practice is widely considered in organic farming in order to decrease pesticide side-effects on beneficials. This can apply especially to pyrethrins since they have knock-down effects on several arthropods, but also low persistence. If applications are performed locally, phytoseiids may partially escape from detrimental effects of pyrethrins due to the reduced probability of coming into contact with pesticide residues. This advantage may be higher for generalist phytoseiids than for specialists, because the generalists maintain a population even on plants without the target prey.

The second experiment deals with the effects of fungi in mediating interactions between pesticides and predators. GDM mycelium is an alternative food for many generalist

phytoseiids, including *A. andersoni* (Pozzebon & Duso, 2008). The presence of GDM foliar symptoms promoted the post-treatment colonization of *A. andersoni.* Meanwhile phytoseiid densities remained at low levels on vines treated with pyrethrins and with a low incidence of downy mildew. The mechanisms involved could be related to a mid-range attraction of phytoseiids induced by the presence of GDM symptoms. In a previous study on fungicide side-effects on phytoseiids, the presence of GDM foliar symptoms was associated with a misestimation of the impact of pesticides, since the higher population levels (in the control plots) were associated to severe GDM symptoms (Duso et al., 2005). On the other hand, the availability of alternative food compensates for the detrimental effects of pesticide application. Our experiments suggest that, under realistic situations, the interactions among pesticides, food source, and predators should be considered as part of the ecosystem's response to agricultural practices. Further experiments are required to gain understanding of these complex interactions.

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Integrating pesticides and biocontrol of mites in agricultural systems

Frank G. Zalom & Francisco Javier Sáenz-de-Cabezón Irigaray
Department of Entomology, University of California, One Shields Avenue, Davis, CA 95616, USA.
E-mail: fgzalom@ucdavis.edu

Integration of a biocontrol agent into most agricultural systems will not be successful unless the natural enemy can survive the pesticides used in that crop system. Knowledge of pesticide selectivity to beneficial arthropods is important to their utility in IPM programs. Phytoseiid mites are effective as biocontrol agents in California's fruit crop systems. *Galendromus occidentalis* and *Phytoseiulus persimilis* are among the most important – the first species is native to the western US, the latter has become established in coastal California. Our previous research has shown that pyrethroids, in particular, affect the abundance of *G. occidentalis* in almond through both contact and residual activity. Harmful residues existed for over a year on bark, and for 5 months on treated leaves. Several new acaricides have been registered for use in the US since 2000. Abamectin, although registered for many sites prior to these other chemicals, has enjoyed dramatically increased use as replacement for the older products. Understanding the acute and side effects of these new products is important to their successful integration into conservation and augmentation programs. Our recent research was devoted to characterizing the direct and residual effects of a number of new acaricides on survival, fecundity and fertility of *G. occidentalis* and *P. persimilis*. Total effect on adult female reproductive potential was calculated and categories for persistence were assigned following IOBC guidelines. Of these acaricides, etoxizole and fenpyroximate had the greatest total effects on both species. Abamectin had the least total effect on *G. occidentalis,* and acequinocyl had the least total effect on *P. persimilis*.

Key words: Phytoseiidae, *Galendromus occidentalis, Phytoseiulus persimilis,* selectivity, side effects, abamectin, acequinocyl, etoxazole, fenpyroximate

Integrated pest control is based on an understanding of the necessary interrelatedness of pesticides and natural enemies. Integration of a biocontrol agent into most agricultural systems will not be successful unless the natural enemy can survive the pesticides used in that crop system due to disruption of trophic relationships. Selectivity may be achieved by a pesticide's specific activity towards a narrow range of taxa, or by limiting pesticide exposure of a biocontrol agent in its natural environment while killing its host or prey. Knowledge of insecticide and acaricide selectivity to beneficial arthropods is important to their utility in integrated pest management (IPM) programs.

Predatory mites in the family Phytoseiidae are effective as biocontrol agents in California's fruit crop systems. *Galendromus occidentalis* (Nesbitt) and *Phytoseiulus persimilis* Athias-Henriot are among the most important of these phytoseiids. *Galendromus occidentalis* is native to the western U.S., and *P. persimilis* has become established following augmentative releases in coastal California. Both species are reared commercially for augmentation by several insectaries, although conservation and enhancement of the naturally occurring populations are most desirable.

In the early 1980s, Marjorie Hoy and others introduced the concept of Integrated Mite Management (IMM), a component of an overall IPM program, into almond and grape production in California (e.g., Hoy, 1985; Hoy et al., 1982; Zalom et al., 1984). Major practices comprising the IMM system included using least disruptive insecticides for control of pest species, monitoring for spider mites, monitoring for predators (including phytoseiids), use of economic thresholds, considering predator-prey ratios before applying pesticides, applying lower than label rates of selective acaricides to balance predator-prey ratios, and employing releases of pesticide-resistant *G. occidentalis* when augmentation was needed.

In the 1980s, California tree and vine growers relied primarily on azinphosmethyl and other organophosphates for insect control, and a high degree of tolerance to organophosphates was present in many native *G. occidentalis* populations. "Selective" acaricides available at that time included propargite, fenbutatin-oxide and cyhexatin which could be applied at lower than label rates to reduce densities of spider mites relative to predators. Propargite remained the major acaricide used by California growers for over 20 years, in spite of increasing regulatory pressure which limited its use due to increased re-entry and harvest intervals. Spider mite management had become rather predictable in this system, but the introduction of new insecticides with the potential to disrupt the balance of predators and phytophagous mites created the need to reconsider all existing IPM programs.

Research on integrating biological and chemical controls in the Zalom laboratory at UC Davis through the late 1990s examined the effects of insecticides on *G. occidentalis* in the almond, grape and strawberry production systems. Pyrethroids, in particular, were shown to severely affect the abundance of this predator, much as they had affected other phytoseiids in fruit and vine crops elsewhere in the world (e.g., van de Vrie, 1985; Schruft, 1985). In a study by Bentley et al. (1987) on almonds conducted in three regions of California (Kern, Fresno and Butte Counties), harmful effects of the pyrethroid 'permethrin' were shown to exist in almond orchards for over a year. Figure 1 illustrates the densities of *Tetranychus urticae* Koch present for the remainder of the season following applications of azinphosmethyl, carbaryl or permethrin, and the apparent residual effect of the permethrin application the following spring.

A possible mechanism for phytophagous mite resurgence during the year following their application was shown by Zalom et al. (2001) to be persistent bark residues of both per-

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methrin and esfenvalerate. In that study, permethrin or esfenvalerate were applied to dormant trees that had never been treated previously. Field samples of treated twigs were cut from the trees in February (after the dormant spraying), in July, and in August (one month after hull-split spraying), and subjected to both residue analysis and bioassays of female *G. occidentalis.* Results of that study indicated that total permethrin residue per 4 cm length of twig decreased from 1.72 ± 0.49 ng/mm² following application, to 0.40 ± 0.27 and 0.26 ± 0.18 following hullsplit in July and harvest in late August, respectively. Female *G. occidentalis* survival after 48 h when placed with *T. urticae* on twigs collected at harvest was 48.1%. Results of that study further indicated that esfenvalerate residue per 4 cm length of twig decreased from 0.84 ± 0.12 ng/mm² following application, to 0.28 ± 0.08 and 0.26 ± 0.18 following hullsplit in July and harvest in late August, respectively. Female *G. occidentalis* survival after 48 h when placed with *T. urticae* on twigs collected at harvest was 8.4%. Although pyrethroid residues declined considerably in the months following their application, the remaining residues were sufficient to cause significant mortality to *G. occidentalis* for almost 7 months after their application.

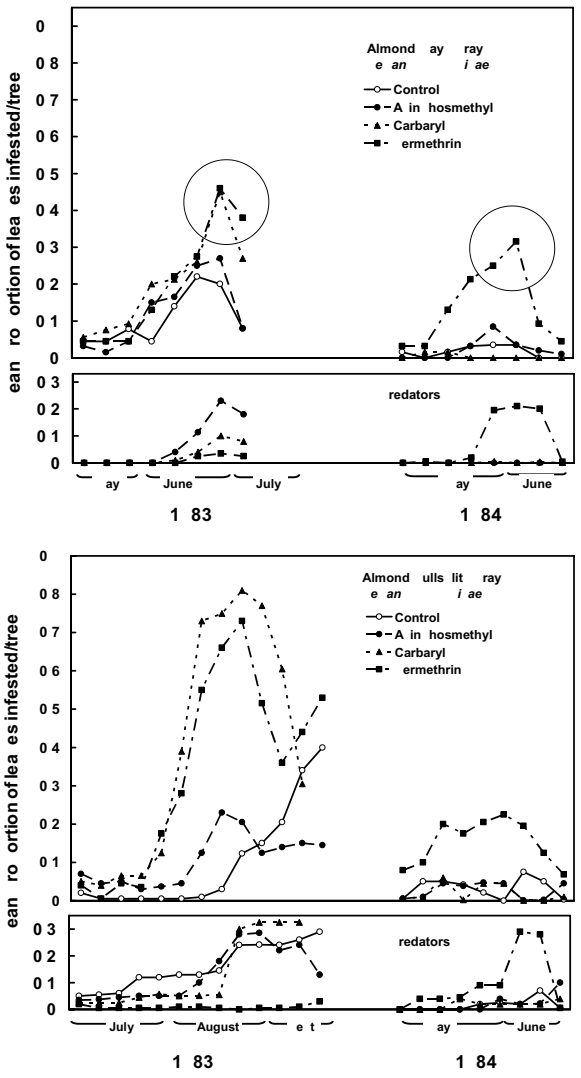

Figure 1 Mean proportion of almond leaves infested with *Tetranychus urticae* and *Galendromus occidentalis* active stages during the season, following a May spray (top), or a July hullsplit spray, and in May and June of the following year.

Walsh et al. (1998) followed the bark residue study by exposing *G. occidentalis* females to a range of esfenvalerate and permethrin residues on almond leaf disks from trees sprayed in mid-June to evaluate sub-lethal and residual effects. Their study found that permethrin residues of only 1.56% of the field rate resulted in significantly ($P<0.05$) greater mortality and repellency, and lower fecundity than on untreated control leaves. Esfenvalerate residues of only 0.195% of the field rate resulted in significantly ($P<0.05$) greater mortality and repellency, while residues of 0.76% resulted in lower fecundity.

The use of pyrethroid insecticides has become dominant to organophosphates as dormant sprays in California orchards since the time of these studies, largely due to concern for organophosphate runoff into surface water from winter rains. The hydrophobic properties of pyrethroids and their propensity to bind to organic matter has made them preferred to organophosphates as dormant sprays by regulatory agencies. Their use has also increased in season on tree crops and a number of other crops such as strawberries, although organophosphates continue to be used in these systems. Newer insecticides such as neonicotinoids and insect growth regulators are currently being introduced. Clearly, introduction of new insecticides with the potential to disrupt the balance of predators and phytophagous mites creates the need to continually reconsider IMM and IPM programs.

Several new acaricides have been registered for use in the US since 2000, representing different chemical classes than propargite, dicofol, and fenbutatin oxide which had been widely used acaricides during the prior two decades. These new chemicals include etoxazole, spiromesifen, fenpyroximate, pyridaben, hexythiazox, bifenazate, and acequinocyl. Abamectin, although registered for many sites prior to these other chemicals, has enjoyed dramatically increased use as applications of the older products declined. Understanding the acute and side effects of these products is important to their successful integration into conservation and augmentation programs where they may be used to suppress outbreak populations or mite species that are not effectively targeted by biocontrol agents. Recently, the Zalom lab has initiated research to characterize the direct and residual effects of a number of new acaricides on survival, fecundity and fertility of *G. occidentalis* and *P. persimilis*. Our studies have largely concentrated on acaricides representing distinctly different primary target sites of action. The chemicals we have chosen to study initially were etoxazole, a mite growth regulator that has an unknown mode of action; spiromesifen, an inhibitor of lipid synthesis; fenpyroximate, a site I electron transport inhibitor; bifenazate, a neuroactive chemical with an unknown mode of action; acequinocyl, a site III electron transport inhibitor; and abamectin, a chloride channel activator (GABA agonist).

It is not sufficient to state that a pesticide is harsh on natural enemies, rather it is most useful to characterize their specific activity against individual biocontrol agents. There are a number of ways to measure pesticide toxicity. The most common way is to measure acute toxicity by determining the percent mortality resulting from exposure of the biocontrol agent after some set period of time, often 24 or 48 h. This can be done by direct exposure (treating the target agent), or by indirect exposure (treating the surface the target agent will come in contact with or treating its prey before allowing them to feed). Another common method is to measure the LD50 or LC50, the lethal concentration of the pesticide that

kills half of the target agents that are exposed. Less commonly are side effects measured. Side effects include those which do not necessarily kill those individuals exposed to the pesticide, but rather affect their fecundity, ability of eggs to hatch (fertility or sterility), and immature development. Data concerning the direct and side impacts of a pesticide on a target agent permits the calculation of total effects, a more complete assessment of toxicity. Few studies measure the persistence of the pesticide against a target agent by allowing residues of the pesticide to be exposed in the field for varying lengths of time before introduction of the target agent to the previously treated surface. Knowledge of the direct and side effects of a pesticide's residue over time is important for both conservation and augmentation programs. Behavioral modification of the target agent and its ultimate impact on predation or parasitism is seldom studied.

Perhaps because it has been available longer than the other chemicals we evaluated, considerable literature exists on impacts of abamectin on phytoseiids. This is particularly true of effects of abamectin on *P. persimilis* because of the importance of this species in protected cultures. Among those studies, Cote et al. (2002) reported no mortality after 24 h of exposure. Zhang & Sanderson (1990) found that exposure to residues following a 4 ppm application reduced egg-laying by as much as 50%, and that feeding on treated *T. urticae* reduced egg production. Oomen et al. (1991), Malezieux et al. (1992), and Shipp et al. (2000) reported no significant effect on survival from exposure to residues.

Several recent studies report effects of bifenazate on phytoseiids. For example, Dekeyser et al. (1996) found that bifenazate was harmless to *G. occidentalis*. James (2002) reported bifenazate at the full field rate was moderately toxic to *G. occidentalis*, *N. fallacis*, *A. andersoni,* and 2 coccinellid beetle species in laboratory bioassays, with mortality ranging from 37-81%. That study also reported bifenazate to be less toxic at half and quarter rates, producing mortality of 0-44% and 0-11%, respectively.

Kim & Seo (2001) and Kim & Yoo (2002) reported studies on side effects of bifenazate, acequinocyl and etoxazole on *Neoseiulus (Amblyseius) womersleyi* (Schicha) and *P. persimilis*, respectively. In their direct-contact bioassays, acequinocyl and bifenazate did not affect survival, fecundity or fertility of either species, but etoxazole affected immature development.

Hereafter, we summarize our studies to date on the direct and side effects of acaricides on *G. occidentalis* and *P. persimilis* and implications for conservation and augmentation.

MATERIALS AND METHODS

The lethal doses of six acaricides were determined in bioassays of adult female *P. persimilis*, green lacewing larvae (*Chrysoperla carnea*), and minute pirate bug adults (*Orius tristicolor*). The acaricides evaluated were abamectin (Agrimek 0.15EC, Syngenta Crop Protection, Greensboro, NC, USA), bifenazate (Acramite 50WS, Chemtura, Middlebury, CT, USA), hexythiazox (Savey 50DF, Gowan, Yuma, AZ, USA), cinnamic aldehyde (Valero, Mycotech, Butte, MT, USA), etoxizole (Zeal, Valent U.S.A., Walnut Creek, CA, USA), and pyridaben (Pyramite, BASF, Research Triangle Park, NC, USA). For the *P. persimilis* bioassays, adult females were transferred to 2.5 cm diameter plastic Petri dish arenas to which *T. urticae* eggs were added as a food source. All interior surfaces were treated with the acaricide, and the *P. persimilis* females were treated on host leaf surfaces before being transferred to the arenas. Each arena contained four predators, and each acaricide treatment and rate was repeated 5 × for each trial. The trials were repeated 3 × at weekly intervals using previously untreated predators and Petri dishes, and these 3 repetitions were considered replicates for statistical analysis. Untreated Petri dishes containing *P. persimilis* females and *T. urticae* eggs were maintained as controls. Survival was assessed after 72 h. The Petri dish bioassays for *C. carnea* larvae and *O. tristicolor* adults were conducted in a similar manner.

All pesticides were applied to the Petri dishes at a series of five or six doses bracketing the label rate to exceeding the label rate until some predators were observed to survive. Mortality data were subjected to probit analysis using the POLO-PC program (LeOra Software, Irvine, CA, USA) to obtain the LD50 values. LD50 data are presented as percentage of the labeled field rate for strawberries in California.

Contact and residual bioassays with *Galendromus occidentalis*

A *G. occidentalis* colony was maintained in growth chambers at 24±1 °C, 75-85% r.h. and L16:D8 photoperiod on detached cotton leaves infested with mixed stages of *T. urticae.* The leaves were obtained from a *T. urticae* colony on cotton seedlings in a greenhouse. The predators were reared on detached cotton leaves infested with mixed stages of *T. urticae*. The original source of the predator colony was Biotactics (Riverside, CA, USA) which had been periodically infused with field collected predators from California's San Joaquin Valley. The active ingredients tested, their trade names, formulations and concentrations applied are listed in Table 2, and include acequinocyl (Kanemite 15SC, Arysta, San Francisco, CA, USA), bifenazate, etoxazole, spiromesifen (Oberon 2SC, Bayer Cropscience, Research Triangle Park, NC, USA), and fenpyroximate (Fujimite, 5EC, Nichino America, Wilmington, DE, USA). All of the concentrations are within the range labeled for field use on California almonds.

Each bioassay unit consisted of a 20 mm diameter green bean leaf disk cut with a cork borer from previously untreated leaves removed from plants raised from seed in our greenhouse. The disks were placed on wet filter paper inside a 90 mm diameter Petri dish. The dish cover had three 6-mm diameter holes to prevent excessive humidity. Bioassays were conducted at 27±1 °C, 50-60% r.h. and L16:D8 photoperiod.

One randomly selected adult *G. occidentalis* female was placed onto each treated leaf disk using a fine camel hair brush. As food for the predator, *T. urticae* active stages and eggs were transferred to each leaf disk. Survival and fecundity were recorded daily for 3 days, and fertility was determined 6 days after being placed on the surface. There were twenty replicates of each acaricide treatment and control.

Each chemical evaluated was mixed with distilled water to achieve a solution of the desired concentration. Acaricides were applied using a 200-ml hand sprayer held 30 cm away from the leaf disks resulting in a 10.6±0.53 µl/cm^2 deposit. Control leaf disks were sprayed in the same manner with distilled water.

For the contact bioassays (Sáenz-de-Cabezón & Zalom 2006), thirty randomly selected adult *G. occidentalis* females were placed onto a detached bean leaf and directly exposed to an acaricide solution by spraying the leaf disk surface as described previously. Each of these treated adult *G. occidentalis* females were placed separately onto each of twenty untreated leaf disks. Active stages and eggs of *T. urticae*

Table 1 Field rate (%) that killed 50% of the target agents 72 h after treatment and exposure to leaf surface residues during that time.

		LD50 expressed as % of field rate		
Chemical	Concentration (ppm)	*P. persimilis*	*C. carnea*	*Orius* sp.
Abamectin	11.27	0.54	3.9	0.12
Bifenazate	227.69	87.2	>100	60
Hexythiazox	112.65	>100	>100	100
Cinnamic aldehyde	2000.00	0.79	5.9	2.5
Etoxizole	53.93	>100	>100	5.7
Pyridaben	179.76	0.10	>100	0.06

Table 2 *Galendromus occidentalis* survival, fecundity and fertility resulting from exposure of adult females to direct contact spray with label rates of five acaricides.

Active ingredient	Concentration (ppm)	% Survival[1]	Total eggs/female[1]	Fertility (% hatch)[1]	E (%)
Control	-	100a	12.4±0.8a	100a	-
Acequinocyl	62.50	100a	9.2±0.6b	96.0±4.9a	28.5
Bifenazate	24.12	100a	9.4±0.5b	92.3±3.4a	30.2
Etoxazole	158.00	98.3±2.2a	9.4±0.7b	0b	100
Spiromesifen	112.75	98.3±2.2a	8.6±0.5b	96.1±4.0a	34.0
Fenpyroximate	76.20	0b	0c	0b	100

[1]Means followed by the same letter do not differ significantly at P<0.05 by LSD.

Table 3 *Galendromus occidentalis* survival, fecundity and fertility resulting from exposure of adult females to leaf surface residues of label rates of five acaricides.

Active ingredient	Concentration (ppm)	% Survival[1]	Total eggs/female[1]	Fertility (% hatch)[1]	E (%)
Control	-	98.3±2.2a	11.2±1.0a	100a	-
Acequinocyl	62.50	93.4±3.0a	9.6±0.5a	92.2±4.9a	25.1
Bifenazate	24.12	95.1±2.7a	9.6±0.9a	96.0±4.0a	20.1
Etoxazole	158.00	93.4±3.0a	9.0±0.5a	0b	100
Spiromesifen	112.75	91.7±3.2a	5.0±0.7b	92.6±4.3a	61.7
Fenpyroximate	76.20	0b	0c	0b	100

[1]Means followed by the same letter do not differ significantly at P<0.05 by LSD.

were transferred to each leaf disk as food for the predator. Survival and fecundity were recorded daily for 3 days and fertility was determined 6 days after treatment. There were twenty replicates of each acaricide treatment and control.

For the residual bioassays, the acaricide solution was applied to the leaf disk surface as described previously, and the treated leaf disks were air-dried for at least 5 min after spraying. Adult *G. occidentalis* females were placed separately onto each of twenty treated leaf disks. Active stages and eggs of *T. urticae* were transferred to each leaf disk as food. Survival and fecundity were recorded daily for 3 days and fertility was determined 6 days after treatment. There were twenty replicates of each acaricide treatment and control.

Statistical analysis

Fecundity and fertility were analyzed by ANOVA with means separated by LSD (P<0.05) (SPSS 2003). Total effects of pesticides (E) values were calculated according to Overmeer & Van Zon (1982) by adjusting fertility-corrected values to the reproductive value using the equation: E (%) = 100% – (100% – M) × R, where M = Abbott-corrected mortality (Abbott 1925), and R = reproduction per treated female (eggs/female × % fertility) / reproduction per untreated female.

RESULTS

Acaricide dose responses of *Phytoseiulus persimilis*, *Chrysoperla carnea*, and *Orius tristicolor*

The LD50 toxicity values indicate that all of the miticides tested are not very toxic to *C. carnea* larvae at the label rate for strawberries, as exposure to any of the products did not result in >50% mortality (Table 1). Abamectin and pyridaben exposure caused 50% mortality of *O. tristicolor* at concentrations of 1/8th and 1/16th of the label rate, respectively. Pyridaben produced 50% mortality of exposed *P. persimilis* females at 1/10th of the label rate, while abamectin and cinnamic aldehyde resulted in 50% mortality of *P. persimilis* females at a bit over half and 80% of the respective label rates. These results concern only acute toxicity from direct contact exposure to the chemicals, and do not predict side effects, if any.

Contact and residual bioassays with *Galendromus occidentalis*

Of the five acaricides evaluated for side effects following exposure of adult female *G. occidentalis* by direct contact (Table 2) and to leaf surface residues (Table 3), fenpyroximate was the only chemical that resulted in acute mortality. All of the exposed females were killed by both direct contact and exposure to residues. Eggs produced by the surviving females were significantly (P<0.05) reduced compared to untreated females, following exposure to acequinocyl, bifenazate, etoxazole and spiromesefen by direct contact, with reduction in fecundity ranging from 24-30%. Eggs produced by females following exposure to leaf surface residues of acequinocyl, bifenazate and etoxazole were not significantly reduced, with reduction in fecundity ranging from 14-20%. Eggs produced by females exposed to leaf surface residues of spiromesefen were significantly (P<0.05) reduced, however, by about 55%. Egg hatch was not significantly reduced relative to the control for eggs produced by females exposed to acequinocyl, bifenazate, or spiromesefen, but none of the eggs produced by females exposed to etoxazole hatched (Tables 2 and 3).

Total effects E of the five acaricides on *G. occidentalis* females varied greatly (Table 4). Using the IOBC classification for total effects of pesticides as a measure (Sterk et al.,

Table 4 Total effects of five acaricides following exposure of *Galendromus occidentalis* adult females to direct contact spray or leaf surface residues of label rates of five acaricides with IOBC classification.

	Direct contact spray		Leaf surface residues		
Chemical	E	IOBC class	E	IOBC class	Apparent cause
Acequinocyl	28.5	Harmless (1)	25.1	Harmless (1)	
Bifenazate	30.2	Harmless (1)	20.1	Harmless (1)	
Spiromesifen	34.0	Harmless (1)	61.7	Slightly harmful (2)	Reduced fecundity
Etoxazole	100	Harmful (4)	100	Harmful (4)	Increased sterility
Fenpyroximate	100	Harmful (4)	100	Harmful (4)	Direct mortality

1999), acequinocyl and bifenazate would be considered 'harmless', with E-values of 30 or lower for exposure by either direct contact or to leaf surface residues. Spiromesefen would be considered 'harmless' when exposed by direct contact and 'slightly harmful' when exposed to leaf surface residues, with reduced fecundity being the primary side effect noted. Etoxazole and fenpyroximate would both be considered 'harmful', etoxazole because of its extreme impact on egg hatch and fenpyroximate because of its acute impact on survival of *G. occidentalis* females.

DISCUSSION

Tetranychus spp. mites can be significant pests of open field and protected crops. They are typically under acceptable natural control unless induced by climatic or horticultural factors, or released from natural biocontrol by intervention with agricultural chemicals. Understanding practical methods of integrating biological and chemical controls is essential to implementation of Integrated Mite Management programs in crops where pesticide intervention is routinely used by farmers for control of key target insects or pest mite species. For many years, producers of orchard and small fruit crops in the western United States and elsewhere in the world relied on organophosphates for control of key insect pests and acaricides such as propargite, fenbutatin oxide and cyhexatin for controlling pest mites. IPM systems were developed which incorporated the judicious use of these products, allowing economical plant production while attempting to minimize their unintended impacts.

The shift from use of these well-researched products, driven largely by regulatory impacts and development of pest resistance, to new insecticides and acaricides with different modes of action has presented a challenge. Pyrethroid insecticides largely replaced organophosphates in many cropping systems because they were effective against key insect pests and were inexpensive relative to other alternatives. Use of pyrethroids in many cropping systems resulted in serious outbreaks of tetranychid mites (e.g., van de Vrie, 1985; Schruft, 1985). Our California studies on perennial crops documented that pyrethroids not only produced mite outbreaks during the season in which they were used, but also in the following season (Bentley et al., 1987), and that residues persisted on treated bark and leaves for many months (Walsh et al., 1998; Zalom et al., 2001).

New acaricides introduced into California have helped to control outbreaks of tetranychid mites. Unfortunately, their integration with previously established biocontrol systems using Phytoseiidae within an IPM framework was not studied prior to registration. Our research indicates that the measures used to identify toxicity can impact conclusions as to compatibility with phytoseiids. Measuring direct phytoseiid mortality resulting from acaricide application is but one measure of potential effect. Measuring side effects of contact and residual exposure such as reduced fecundity and fertility are necessary to more completely asses potential impact. The results of our studies presented in this paper support those observations of other researchers including Zhang & Sanderson (1990), who documented side effects from exposure to abamectin, and Kim & Seo (2001) and Kim & Yoo (2002), who documented side effects from exposure to bifenazate, acequinocyl and etoxazole. Both direct mortality and side effects must be considered when developing future IMM and IPM systems.

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The impact of sulfur on biological control of spider mites in Washington State vineyards and hop yards

David G. James & Deirdre Prischmann*

Department of Entomology, Washington State University, Irrigated Agriculture Research and Extension Center, 24106 North Bunn Road, Prosser, WA 99350, USA. E-mail: david_james@wsu.edu; and *United States Department of Agriculture, Agricultural Research Service, Northern Plains Area, North Central Agricultural Research Laboratory, 2923 Medary Ave., Brookings, SD 57006 USA

Data collected from field studies and experiments conducted in Washington State vineyards and hop yards during 2001-2005 indicate that the multiple use of sulfur as a tool to manage powdery mildew, has an adverse impact on biological control of spider mites. In vineyards there appears to be a positive correlation between the frequency of sulfur applications and spider mite population densities. Multiple (>4) applications of sulfur can result in spider mite population outbreaks requiring control. Similarly, in replicated vineyard and hop yard experiments, multiple applications of sulfur increased spider mite populations. Conversely, predatory mite (Phytoseiidae) populations are smaller in sulfur-exposed hops and grapes than in non-exposed plants, and it is likely that suppression of phytoseiid populations by multiple applications of sulfur contributes to spider mite population outbreaks in Washington State hops and grapes.

Key words: Side effect, *Tetranychus macdanieli*, powdery mildew

Spider mites (Acari: Tetranychidae) are global crop pests that often infest grapes and hops. They ingest leaf cell contents, thus reducing plant photosynthesis (Park & Lee, 2002), and potentially decrease fruit quality and yield (Shruft, 1985; Flaherty & Wilson, 1999). Spider mites are a classic example of a secondary, or induced, pest that exhibits population outbreaks when pesticides intended to reduce primary pest densities also kill natural enemies (Huffaker et al., 1970; McMurtry et al., 1970). Several arthropods feed on spider mites, notably predaceous mites (Acari: Phytoseiidae), *Stethorus* spp. (Coleoptera: Coccinellidae), and generalist macropredators (Hemiptera, Neuroptera, Thysanoptera) (McMurtry et al., 1970; Flaherty & Wilson, 1999).

Wine grapes and hops in Washington State (USA) are commonly sprayed with sulfur and synthetic fungicides to control fungal pests, primarily powdery mildew. Sulfur is moderately to be highly toxic to spider mites in laboratory assays (Blümel & Hausdorf, 2002; Guichou et al., 2002). Laboratory bioassays on *Tetranychus urticae* from Washington showed low toxicity, but caused up to 50% reduction in adult longevity with a consequent reduction in fecundity (Price & James, 2007). Although sulfur applications can suppress pest mite populations in agricultural fields (Croft, 1990), densities often increase after applications cease (van de Vrie et al., 1972; Hanna et al., 1997). Pesticides can also impact spider mite densities indirectly, via negative effects on spider mite natural enemies or altering plant quality (McMurtry et al., 1970; van de Vrie et al., 1972; Schruft, 1985). Sulfur can have harmful effects on phytoseiid mites in agricultural fields (Croft & Brown, 1975; Hanna et al., 1997) and in laboratory tests (Kreiter et al., 1998; James, 2001). Although laboratory experiments are useful for assessing some aspects of pesticide toxicity, field tests are more desirable because pesticide deposition and degradation, residue toxicity and repellency, sublethal effects on population structure, or potential interactions between compounds may differ under field conditions (Croft, 1990).

This paper summarizes a number of separate field studies and experiments aimed at determining the impact of sulfur on biological control of spider mites in Washington State vineyards and hop yards.

MATERIALS AND METHODS

Surveys of spider mite and predatory mite populations in Washington State vineyards with high, low, or no pesticide inputs

High-input (fungicides, herbicides, acaricides, insecticides), low-input (fungicides, herbicides), and no-input (no pesticides) vineyards were monitored monthly for spider mites and predatory mites (Phytoseiidae) during spring-autumn 1999-2002 – see James et al. (2002) and Prischmann et al. (2005a) for full details of methodology.

Field experiment on the impact of sulfur and chlorpyrifos on spider mites and their natural enemies in a vineyard

The impact of sulfur and chlorpyrifos on spider mites (primarily *Tetranychus macdanieli*) and their natural enemies was monitored in a replicated open field experiment conducted in an abandoned vineyard in Washington State – see Prischmann et al. (2005b) for full details of methodology.

Field experiment on the impact of sulfur on spider mites and their natural enemies in a hop yard

The effect of four fungicide treatment regimes on spider mite populations in hops were tested: (1) control (water only); (2) micronized sulfur (Microthiol Disperss) at 6.73 kg/ha (3) stylet oil at 1% by volume, and (4) synthetic fungicides (Quintec at 461 g/ha, Rally 40W at 461 g/ha, and Flint 50WG

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Table 1 Fungicides and amount of water applied by date to hops in a fungicide trial, WSU-Prosser, 2005.

	11-May	18-May	24-May	1-Jun	7-Jun	14-Jun	23-Jun	29-Jun	6-Jul	20-Jul	3-Aug
Water	X	X	X	X	X	X	X	X	X	X	X
Sulfur	X	X	X	X	X	X	X	X	Rally	Flint	Quintec
Stylet oil	X	X	X	X	X	X	X	X	Rally	Flint	Quintec
Synthetic	Quintec	-	Rally	-	Flint	-	Quintec	-	Rally	Flint	Quintec
Litres/ha	748	748	748	748	748	748	906	906	1513	1513	1513

X, application made; -, no application on that date.

at 9.9 g/ha). Table 1 shows the spray dates, treatments, and the amount of water that was applied through the season. Treatments were applied with a Stihl powered backpack sprayer. A randomized complete block design with four replicates of six hills each was established in a block of Willamette hops in a subsurface drip-irrigated hop yard at the WSU-Prosser research center. Leaf samples were collected at about 2-week intervals from 9 May to 15 August. Ten leaves from about 3 m high were collected from each plot for a total of 40 leaves per treatment per date. Twospotted spider mite (*T. urticae*) motiles and eggs, predatory mites [*Galendromus occidentalis*, all stages], and predatory insects [*Stethorus* spp.(all stages combined), *Orius* spp. (adults and nymphs), lady beetles (all stages), and green lacewings (larvae and eggs)] were counted under a binocular microscope. Analysis of Variance and Duncan's Multple Range Test determined the statistically significant differences between treatment means.

RESULTS

Surveys of mite populations in Washington State vineyards with high, low, or no pesticide inputs

In the earliest surveys (1999-2000), spider mite populations were generally small in high-input and no-input vineyards and in 1999 they did not differ significantly. In 2000, significantly larger infestations occurred in pesticide-treated vineyards, with the greatest population (up to 14 mites/leaf) seen in a vineyard which received 13 applications of sulfur, well above the average of four applications used in Washington vineyards in 1999 (Anon., 2000). The mite population in this vineyard developed only after cessation of sulfur sprays in July. Phytoseiid populations in this vineyard were very small throughout the season (Fig. 1).

Later surveys (2001-2002) were more extensive with 47 vineyards surveyed in the first year and 29 in the second year. Information on pesticide use in these vineyards during 2001-2002 is shown in Table 2. Mean mite (pest and beneficial) densities recorded during the survey are provided in Table 3. *Tetranychus mcdanieli* was the most abundant spider mite, followed by *T. urticae*. *Galendromus occidentalis* was the most common specialist phytoseiid, and was collected in all site types. *Galendromus flumenis* was the most common generalist phytoseiid mite, followed by *Typhlodromus pyri*, although the former was only found in unmanaged sites. Densities of fungivorous tydeid mites and pest tenuipalpid mites were highest in unmanaged sites, whereas predatory anystid, bdellid, and stigmaeid mites were rarely found on sampled vines.

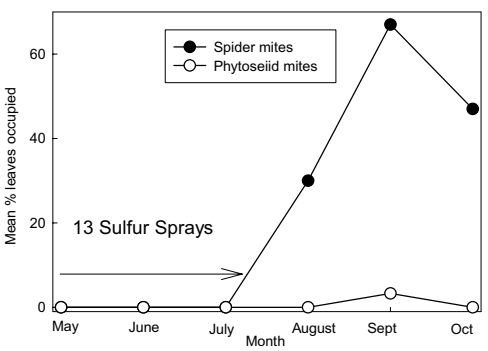

Figure 1 Seasonal abundance of spider mites and phytoseiid mites in a sulfur-treated vineyard in 2000.

Spider mite densities significantly increased throughout the season. Chemical input significantly altered spider mite densities in a manner that was consistent within and between years. High-input sites had significantly higher densities of spider mites compared to unmanaged sites ($P<0.001$), although there were no significant differences between low-input and unmanaged sites ($P = 0.13$; Fig. 2A) or high- and low-input sites ($P = 0.17$).

At the majority of sites, specialist phytoseiid mite densities increased in August and September. However, densities of specialist predators did not significantly differ due to year or management regime, or due to the interactive effects of these variables with one another or with time. Generalist phytoseiid mite densities remained relatively constant from June to September. Management regime significantly altered densities of generalists; this effect was consistent through time within years, and did not significantly differ between years. Unmanaged sites had significantly higher densities of generalist phytoseiid mites compared to low- and high-input sites (low: $P = 0.04$; high: $P<0.001$), although differences were not significant between low- and high-input sites ($P = 0.73$).

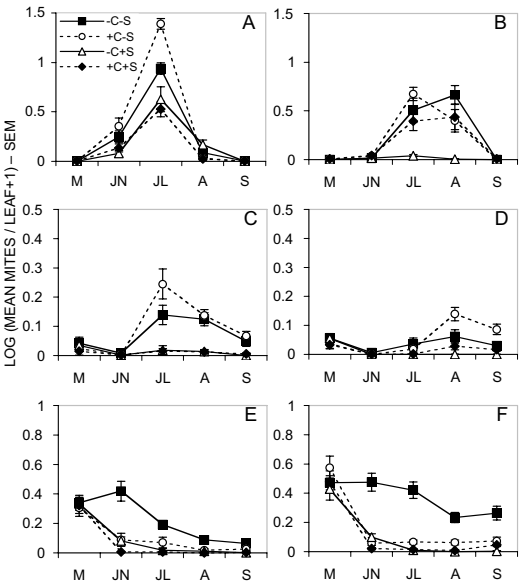

Figure 2 Mite densities (presented as log (means/leaf +1) ± SEM) from leaf sampling. (A, B) Spider mites, (C, D) specialist phytoseiid mites, (E,F) generalist phytoseiid mites; (A, C, E) A replicates, (B, D, F) B replicates. -C-S, control; +C-S, chlorpyrifos only; -C+S, sulfur only; +C+S, chlorpyrifos and sulfur.

Table 2 Pesticide information received from vineyards sampled in 2001 and 2002.

Active ingredient	Product/trade name(s)	Use[1]	Average applications/vineyard[2]			
			2001	2001	2002	2002
			Low	High	Low	High
Abamectin	Agri-Mek	A	0	0.07	0	0
Azoxystrobin	Abound	F	0.33	0.07	0	0.33
Bacillus subtilis	Serenade	F	0	0.13	0	0
Buprofezin	Applaud	I	0	0	0	0.11
Calcium polysulfide	Lime Sulfur, Sulforix	F[3]	0	0.47	0	0
Carbaryl	Sevin	I	0	0.2	0	0
Chlorpyrifos	Lorsban	I	0	0.33	0	0.44
Dicofol	Kelthane	A	0	0.2	0	0
Dimethoate	Digon	A I	0	0.27	0	0.22
Diuron	Direx	H	0	0.07	0	0
Fenarimol	Rubigan	F	0.56	1.27	0.50	1.22
Fenhexamid	Elevate	F	0.11	0	0	0
Glyphosate	Roundup	H	0.67	0.73	0	0.56
Harpin protein	Messenger	F I	0	0	0	0.22
Imidacloprid	Admire, Provado	I	0	0.93	0	0.33
Iprodione	Rovral	F	0.22	0	0.50	0
Kresoxim-methyl	Sovran	F	0.33	0.20	0	0
Malathion	Maltox	A I	0	0.07	0	0
Myclobutanil	Rally	F	0.67	0.40	1.50	0.44
Norflurazon	Solicam	H	0	0.07	0	0.22
Oils, mineral/petroleum	JMS stylet oil, Gemini supreme oil	F[4]	0.56	1.07	0	0.78
Oxyfluorfen	Goal	H	0.67	0.80	0	0.56
Paraquat	Gramoxone	H	0.89	0.93	0	0.89
Phosmet	Imidan	I	0	0	0	0.22
Potassium bicarbonate	Kaligreen	F	0.22	0	0	0
Potassium salts	M-Pede	A	0	0.20	0	0
Propargite	Omite	A	0	0	0	0.22
Simazine	Princep	H	0	0.13	0	0
Sulfur	Golden dew, Microthiol, Thiolux	F[3]	3.11	4.20	3.50	3.00
Tebuconazole	Elite	F	0.11	0.20	0.50	0
Trifloxystrobin	Flint	F	0.22	0.53	0.50	0.44
Triflumizole	Procure	F	0.67	0.53	0	0.56
Trifluralin	Trust	H	0	0	0	0.11

[1]A, acaricide, H, herbicide, I, insecticide, F, fungicide; [2]2001 data from 9 of 11 low sites, 15 of 20 high sites; 2002 data from 2 of 7 low sites, 9 of 14 high sites; [3]Primarily used as a fungicide, but has some acaricidal/insecticidal properties (Morrell & Schreiber, 1998); [4]Often used as an insecticide (Morrell & Schreiber, 1998).

Table 3 Mean mite densities (no. mites/leaf ± SEM) from leaf sampling in 2001 and 2002 according to chemical input.

	Unmanaged		Low input		High input	
	2001	2002	2001	2002	2001	2002
Anystidae[1]	0.005±0.003	0.002±0.002	0	0	0	0
Bdellidae	0	0.002±0.002	0	0	0	0.003±0.003
Phytoseiidae[2]	0.72±0.32	0.88±0.28	0.45±0.28	0.13±0.08	0.20±0.05	0.17±0.04
GENERALISTS	0.32±0.11	0.77±0.29	0.28±0.28	0.09±0.08	0.01±0.01	0.01±0.01
A. andersoni	0.02±0.01	0	0	0	0.003±0.003	0
A. ovatus	0.001±0.001	0	0	0	0	0
G. flumenis	0.25±0.11	0.52±0.26	0	0	0	0
M. citri	0	0	0	0	0.01±0.01	0
M. pomi	0	0.08±0.08	0	0	0	0
T. caudiglans	0.004±0.004	0.03±0.03	0	0	0	0
T. pyri	0.05±0.04	0.08±0.06	0.28±0.28	0.08±0.08	0	0
SPECIALISTS	0.40±0.28	0.11±0.06	0.18±0.05	0.05±0.02	0.12±0.03	0.16±0.04
G. occidentalis	0.40±0.28	0.11±0.06	0.18±0.05	0.05±0.22	0.12±0.03	0.16±0.04
N. fallacis	0	0	0	0	0.005±0.004	0
UNKNOWN	0.001±0.001	0.05±0.03	0.01±0.01	0.003±0.003	0.003±0.002	0.01±0.01
Stigmaeidae	0	0.02±0.02	0	0	0	0.003±0.003
Tenuipalpidae	0.39±0.26	0	0	0	0.003±0.003	0
Tetranychidae[3]	0.51±0.37	0.98±0.62	6.22±6.54	2.41±1.06	6.54±2.42	10.57±2.41
P. ulmi	0.001±0.001	0	0.03±0.02	0.05±0.03	0.001±0.001	0.006±0.005
T. mcdanieli	0.47±0.37	0.91±0.63	5.71±5.01	0.11±0.11	2.88±1.02	5.72±2.00
T. urticae	0.04±0.03	0.07±0.06	0.48±0.21	2.24±1.11	2.25±1.64	4.84±1.76
Tydeidae	3.06±1.55	5.04±1.75	0.58±0.47	0.53±0.22	0.17±0.08	1.97±1.52

[1]*Anystis agilis* Banks; [2]Generalists: *Amblyseius andersoni* Chant, *A. ovatus* (Garman), *Galendromus flumenis* (Chant), *Metaseiulus citri* (Garman et McGregor), *M. pomi* (Parrott et al.), *Typhlodromus caudiglans* (Schuster), and *T. pyri* Scheuten; specialists: *G. occidentalis* (Nesbitt), *Neoseiulus fallacis* (Garman). After McMurtry & Croft (1997); [3]*Panonychus ulmi* (Koch), *Tetranychus mcdanieli* McGregor, *T. urticae* Koch.

Table 4 Mean mite densities (no. mites/leaf ± SEM) from leaf sampling from June to September according to chemical treatment.

	-C-S control	+C-S chlorpyrifos only	-C+S sulfur only	+C+S chlorpyrifos + sulfur
Phytoseiidae	1.251±0.123	0.432±0.059	0.088±0.018	0.059±0.009
A. andersoni[a]	0.011±0.005	0	0.001±0.001	0.001±0.001
G. flumenis[a]	0.061±0.020	0.024±0.006	0.001±0.001	0.001±0.001
G. occidentalis[b]	0.159±0.026	0.267±0.051	0.011±0.006	0.026±0.006
M. citri[a]	0.004±0.002	0.005±0.003	0	0.001±0.001
M. pomi[a]	0	0.001±0.001	0	0
T. caudiglans[a]	0.918±0.131	0.096±0.029	0.073±0.018	0.027±0.006
T. pyri[a]	0.098±0.035	0.039±0.014	0.003±0.002	0.004±0.002
All generalist spp.	1.092±0.129	0.165±0.032	0.078±0.018	0.033±0.007
All specialist spp.	0.159±0.026	0.267±0.051	0.011±0.006	0.026±0.006
Stigmaeidae	0.022±0.012	0.019±0.006	0	0.001±0.001
Tetranychidae	2.303±0.449	4.259±1.004	0.809±0.355	1.099±0.245
T. mcdanieli	2.295±0.449	4.257±1.004	0.781±0.355	1.068±0.246
T. urticae	0.008±0.005	0.001±0.001	0.003±0.002	0.021±0.012
Unknown spp.	0	0.001±0.001	0.025±0.014	0.013±0.005
Tydeidae	0.112±0.020	0.139±0.040	0.001±0.001	0.004±0.002

[a]Generalists: *A. andersoni*, *G. flumenis*, *M. citri*, *M. pomi*, *T. caudiglans*, *T. pyri*; [b]Specialists: *G. occidentalis.*

These data suggest that low pesticide input (fungicide and herbicide) may be as disruptive to biological control of mites as high pesticide input (fungicides, herbicides, acaricides, insecticides). Since most synthetic fungicides and herbicides have relatively low toxicity to phytoseiids, sulfur appears to be a component of the low-input pesticide regime with the most potential as a disruptant to biological control of spider mites in Washington grapes.

Field experiment on the impact of sulfur on spider mites and their natural enemies in a vineyard

Tetranychus mcdanieli was the most abundant spider mite (SM) in all treatments, followed by *T. urticae* (Table 4). SM densities were low early in the season, peaked in summer, and then declined in the fall. Chlorpyrifos increased but sulfur decreased SM densities. In each replicate the dynamics driving the significant chlorpyrifos*sulfur interactions appeared to be different (Fig. 2A, B). In replicate A, chlorpyrifos dramatically increased SM densities, but only in the absence of sulfur, whereas sulfur by itself or with chlorpyrifos depressed SM densities. In contrast, in the B replicate, sulfur by itself strongly suppressed SM densities; the insecticide mediated this effect, and although chlorpyrifos-alone increased SM densities, the increase was not dramatic (Fig. 2B).

Galendromus occidentalis was the only specialist phytoseiid mite (SPM) occurring on our study plants (Table 4). Overall, specialists were largely absent in the early season, increased in summer, and declined in the fall, leading to a significant time effect. For SPM, there was no statistically significant interaction between the two chemicals. In both replicates, sulfur clearly impacted SPM dynamics, with very strong suppression in either treatment receiving sulfur (Fig. 2C, D). Suppression of SPM by sulfur became pronounced after June, leading to significant sulfur*time interactions in both replicates.

A guild of generalist phytoseiid mites (GPM) occurred in our study plots, with densities of *Typhlodromus caudiglans* highest in the control treatment, and densities of *T. pyri* highest in the chlorpyrifos only treatment (Table 1). Overall, there was no consistent pattern of seasonal dynamics for generalists, leading to a non-significant time effect. Consistently in either of the two replicates, both chemicals strongly suppressed GPM densities, leading to strong main effects for both chlorpyrifos and sulfur (Fig. 2E, F).

Field experiment on the impact of sulfur on spider mites and their natural enemies in a hop yard

The synthetic fungicide treated plots had significantly more motile spider mites than the other treatments on 6 June and 5 July but by 19 July the stylet oil and sulfur treatments had the most (Table 5). On 1 August, the sulfur plots had significantly more spider mites than the other treatments, with stylet oil in second place. The numbers in the sulfur plots fell on 15 August, while the stylet oil numbers increased. Over the whole season, the stylet oil and sulfur treatments had significantly more spider mites than the synthetic and control treatments (Table 5). Twospotted spider mite egg numbers generally paralleled those of the motiles, especially on the last three sample dates. The stylet oil and sulfur treatments had significantly more spider mite eggs for the season than the other two treatments (Table 5). Predatory mite abundance was consistently low in the sulfur plots, especially late in the season when the predatory mite numbers increased in all the treatments except the sulfur (Table 5).

DISCUSSION

Mite fauna surveys conducted during 1999-2002 indicated that low pesticide input (fungicides, herbicide) management regimes in Washington vineyards, resulted in similar-sized spider mite populations as found in high pesticide input (fungicides, herbicides, acaricides, insecticides) management regimes. Similarly, phytoseiid mite populations were small under both types of management. Low spider mite populations and large populations of phytoseiids were only characteristic of unsprayed vineyards.

Comparing mite populations of managed vineyards with different pesticide regimes can isolate effects of pesticides in a more homogeneous setting by reducing variability between sites. We hypothesized that there would be fewer predators and more pests in managed vineyards exposed to insecticides/acaricides. However, despite a trend towards lower spider mite populations in low-input vineyards, there were no significant differences in mite densities between low- and high-input vineyards. Sulfur was applied in 90% of the managed vineyards, and is known to have negative effects on susceptible spider mites (Auger et al., 1999; Price & James, 2007), phytoseiid mites (James & Rayner, 1995; James,

Table 5 Mean number of mites per leaf or per cone on hops treated with fungicides, Drip-irrigated hopyard, 2005. Each per leaf mean had 40 observations except the season means each had 240 observations.

	Treatment	9 May	6 June	5 July	19 July	1 August	15 August	All season
T. urticae motiles	Stylet oil	0	0.05b	20.90b	293.40a	190.40b	334.60a	120.00a
	Sulfur	0	0.03b	28.30b	281.20a	298.40a	53.00b	95.20a
	Synthetic	0	0.95a	112.90a	41.80b	111.50bc	118.40b	56.00b
	Untreated	0	0b	36.90b	45.40b	71.20c	92.90b	35.50b
T. urticae eggs	Stylet oil	0	0.53b	127.20a	402.20a	295.30ab	168.60a	142.60a
	Sulfur	0	0.03b	255.50a	299.40a	424.50a	29.10b	146.50a
	Synthetic	0	2.05a	144.60a	55.60b	170.20b	109.80a	72.90b
	Untreated	0	0b	116.80a	75.50b	171.20b	157.80a	77.50b
Phytoseiidae	Stylet oil	0	0.03a	0.38a	1.58a	1.08ab	6.38b	1.34b
	Sulfur	0	0a	0.15a	0b	0.20b	0.18c	0.08c
	Synthetic	0	0.03a	0.05a	0.30b	2.13a	7.20b	1.39b
	Untreated	0	0a	0.23a	0.75ab	1.53ab	17.00a	2.79a

Means followed by different letters within a group of mites and within a date are significantly different (Duncan's Multiple Range test, $P<0.05$).

2001b), and tydeid mites (Hanna et al., 1997; English-Loeb et al., 1999). We tested this hypothesis in replicated field experiments designed to clarify how use of the broad-spectrum insecticide chlorpyrifos and sulfur impacts predator-prey relationships within managed vineyards. An early chlorpyrifos application consistently lowered densities of generalist phytoseiid mites and resulted in higher spider mite and specialist phytoseiid mite densities (Prischmann et al., 2005b). Multiple sulfur applications, alone and in combination with chlorpyrifos, virtually eliminated generalist and specialist phytoseiid mites, and suppressed spider mites. These experimental results support the notion that sulfur limits generalist phytoseiid mite populations within managed vineyards, and may be responsible for similar spider mite and specialist phytoseiid mite densities in low- and high-input sites. However, in our vineyard survey, sulfur use did not exclude specialist phytoseiid mites from managed sites, and spider mite densities tended to be higher in high-input sites. This discrepancy could be due to resistant spider mite and specialist phytoseiid mite populations within managed vineyards, and/or broad-spectrum insecticide use ameliorating harmful effects of sulfur on pest mites. Spider mites, and to a lesser extent phytoseiid mites, have developed resistance to several pesticides (Croft, 1990), whereas sulfur-resistant strains of *G. occidentalis* have been developed in the laboratory through artificial selection (Hoy, 1984) and are found naturally in some California vineyards (Hoy & Standow, 1981).

Grapevines in southcentral Washington can support high densities of generalist phytoseiid mites. These predators are important spider mite control agents in several perennial crops (Collyer, 1964; McMurtry, 1992; Nyrop et al., 1998), including grapes (Duso et al., 1991; James, 2001a, Prischmann et al., 2005c). However, these predators were virtually absent from managed vineyards, which appears likely due to the widespread use of sulfur for disease management.

The data reported here for hops along with data obtained in a similar trial in Corvallis (OR, USA) conducted by Dr D. Gent (pers. comm.) indicate that multiple applications of sulfur or oil during spring also result in inflation of spider mite populations on this crop during summer. During the period of weekly sulfur and stylet oil applications, very few mites were present. However, immediately following cessation of sulfur and oil applications, spider mite numbers increased dramatically in these plots, reaching levels 4-5 times greater than in the synthetic fungicide and untreated plots. Suppression of predatory mites and in the sulfur and oil plots was at least part of the reason. We know from laboratory bioassays (unpubl.) that *G. occidentalis*, the major predatory mite species present in this trial, suffers when exposed to multiple applications of sulfur, despite being relatively unaffected by a single spray application. The eight applications of sulfur experienced by *G. occidentalis* populations in the sulfur plots was sufficient to prevent virtually any occurrence of this important spider mite predator in these plots for the whole season. In contrast, spider mite populations in the plots treated with synthetic fungicides for the season were only slightly larger than in the untreated plots. Synthetic fungicides significantly depressed predatory mite populations by about 50% but clearly did not remove the biocontrol benefits of this predator as sulfur did. Curiously, stylet oil had a similar (to synthetic fungicides) muted effect on predatory mites, but in this case spider mites rapidly increased to high levels like under the sulfur treatment. Perhaps the functionality of predatory mites was impaired in some way by stylet oil.

The evidence presented here is indicative of a significant interfering effect of multiple applications of sulfur on biological control of spider mites in hops and grapes in Washington State. In order to improve spider mite biocontrol in hops, grapes, and other perennial crops, it is important to ensure that disease management is not based on multiple applications of sulfur. The use of less toxic fungicides appears to be just as important as using selective insecticides/miticides in enabling conservation biological control of mites to work as efficiently as possible.

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Impact of new pesticide chemistry on acarine communities in apple orchards

Raul T. Villanueva[1] & James F. Walgenbach[2]

[1]Department of Plant Pathology, NC State University, Don Ellis Laboratories, 1320 Varsity Drive, Raleigh, NC 27695, USA; present address: Texas AgriLife Extension, Texas A&M University, 2401 East Highway 83, Weslaco, TX 78596, USA. E-mail: rtvillanueva@ag.tamu.edu; and [2]Mountain Horticultural Crops Research and Extension Center, NCSU, 455 Research Drive, Fletcher, NC 28732, USA

Changes in the acarine community of several apple orchards due to adoption of new arthropod pest control practices were examined over a 4-year period in western North Carolina, USA. At each of nine study sites, a reduced-risk integrated pest management (IPM) program was compared to a conventional IPM plot. In addition to pheromone-based mating disruption, reduced-risk plots also included one or more of the following new insecticides: the nicotinoids imidacloprid, thiamethoxam, acetamiprid, and thiacloprid; the oxadiazine indoxacarb; the biological insecticide spinosad; and the insect growth regulators methoxyfenozide, novaluron, and pyriproxyfen. Conventional plots were treated with broad-spectrum organophosphate, carbamate, and pyrethroid insecticides. The most common phytophagous mite was *Panonychus ulmi,* and the most prevalent predators were the phytoseiid *Neoseiulus fallacis* (>99%) and the stigmaeid *Agistemus fleschneri*. Results showed that phytophagous mite outbreaks did not occur in the reduced-risk plots, and acaricides were applied 15 and 7 times in the conventional and reduced risk treatments, respectively, throughout the four-year study. Phytoseiid and stigmaeid predatory mites were more abundant in reduced-risk vs. conventional plots. High numbers of the mite-eating thrips, *Scolothrips sexmaculatus*, were noticed in one orchard in 2004 and they were more abundant in reduced-risk vs. conventional plots. In addition, crop protectant aluminum silicate clay (Surround® WP) negatively affected phytoseiid mites compared to a control involving a water spray.

Key words: Reduced risk insecticides, Tetranychidae, Phytoseiidae, IPM, *Panonychus ulmi*, *Neoseiulus fallacies*, *Agistemus fleschneri*, *Scolothrips sexmaculatus*

Organophosphate (OP) insecticides have been the cornerstones of apple pest management programs in the USA for almost 40 years. They provide excellent control of most direct insect pests and, despite their broad-spectrum activity, are of relatively low toxicity to many important natural enemies, particularly mite predators (Croft, 1990). However, implementation of the Food Quality Protection Act (Cash et al., 2003), which created more stringent pesticide tolerance levels that placed greater emphasis on the safety of children, is resulting in the loss of OP insecticides to the apple industry. Consequently, there is a need to develop programs that rely on alternative pest management tactics.

In recent years several new management tools classified as 'reduced-risk' (RR) have become available to the apple industry. In general, these tools are characterized as having a relatively narrow spectrum of pest activity, friendly environmental profiles, and lower toxicity to non-target organisms compared with broad-spectrum OPs (Cash et al. 2003). Examples include pheromones for mating disruption, 'new chemistry' insecticides (e.g., insect growth regulators, neonicotinoids, and oxadiazines), and naturally occurring minerals such as kaolin. Although there is a relatively broad knowledge base on the efficacy of these products against key direct pests, there is limited knowledge on their effects on secondary pests and natural enemies.

European red mite (ERM), *Panonychus ulmi* (Koch), and apple rust mite (ARM), *Aculus schlechtendali* (Nalepa), are the two most common phytophagous mite species on apple in North Carolina. ERM is by far the most damaging of the two, whereas ARM is of minor importance and on occasions even beneficial, because it can serve as alternative prey for phytoseiids and stigmaeids (Villanueva & Harmsen, 1998). The phytoseiid mite *Neoseiulus fallacis* (Garman) has long been recognized as an important predator of ERM in the apple production region of western North Carolina (Farrier et al., 1980; Shaffer & Rock, 1983). Avoidance of certain insecticides known to upset predator-prey relationships has been important in maintaining successful biological control of ERM. For instance, carbamate, organochlorine, and pyrethroid insecticides are notorious for causing mite flare-ups due to toxicity to natural enemies, hormoligosis, or dispersal, or for causing repellent effects on phytophagous and predatory mites (Dittrich et al., 1974; Penman et al., 1981; Bostanian et al., 1985; Childers et al., 2001).

As the North Carolina apple industry transitions to pest management programs that rely on new reduced-risk pest control methods, it is important to understand how these new pest control tools affect acarine communities. Although many of the 'new chemistry' insecticides are more target-specific compared with OPs, some are known to have effects on phytophagous or phytoseiid mites that may adversely affect ERM biological control programs. For instance, among the neonicotinoid insecticides, imidacloprid can increase the fecundity of two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (James & Price, 2002), and acetamiprid has been observed to increase spider mite populations in pears after repeated applications (Beers et al., 2005). Whereas the insect growth regulator novaluron did not affect TSSM (Hilton & Van Buskirk, 2002), this insecticide can cause ERM flare-ups and repel phytoseiids after repeated applications in apples (RT Villanueva & JF Walgenbach, unpubl.). In addition, spinosad (a spinosyn) is toxic to TSSM and *N. fallacis*, but it does not affect ERM (Villanueva & Walgenbach, 2006).

As a precursor to the adoption of new pest management programs by tree fruit growers in the eastern USA, seven Land Grant universities undertook a cooperative project supported by the USDA Risk Avoidance and Mitigation Program (RAMP), from 2002-2005 to evaluate new reduced-risk pest management program on apples. The goal of this multistate project was to compare the economics, efficacy, and

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Table 1 Products used in reduced-risk and conventionally managed blocks of apples during a 4-year RAMP project in NC, USA, along with the no. of orchards that used the different products and product rate ranges. There were nine, eight, nine, and eight study sites in 2002, 2003, 2004, and 2005, respectively. '-' indicates that the product was not used because it was not registered at the time of the study. Isomate products were used as slow release twisting ties.

Insecticide class	Common name	Trade name	Reduced Risk				Conventional				Rate range
			2002	2003	2004	2005	2002	2003	2004	2005	(ha^{-1})
nicotinoids	thiamethoxam	Actara 25WDG	9	8	9	8	9	9	8	6	280-350 g
	acetamiprid	Assail 70WP	-	2	4	4	-	1	2	2	140-218 g
	thiaclopid	Calypso 4SC	-	-	6	4	-	-	1	4	220-290 ml
	imidacloprid	Provado 1.6F	1	6	0	6	2	3	2	1	150-440 ml
insect growth reg.	pyriproxyfen	Esteem 35WP	9	1	0	1	0	0	0	1	140-280 g
	methoxyfenozide	Intrepid 2F	8	7	8	8	3	6	7	6	561-980 g
	novaluron	Rimon 0.83EC	-	-	-	3	-	-	-	0	1,400 g
	indoxacarb	Avaunt 30WDG	7	5	8	7	0	1	0	1	315-421 g
biological based	spinosad	SpinTor 2SC	5	3	3	2	0	0	1	1	370 ml
pheromone	pheromone tie	Isomate Rosso	3	2	1	1	0	0	0	0	370 ties
		Isomate C+	3	3	2	0	0	0	0	0	990 ties
		Isomate CTT	0	0	1	2	0	0	0	0	495 ties
		Isomate M100	1	2	0	1	0	0	0	0	248 ties
	pheromone spray	OFM –spray 3M	6	4	3	5	0	1	0	1	50-180 ml
		Checkmate F	-	-	4	1	-	-	0	1	70-100 ml
		CM Spray	0	0	0	0	0	1	0	0	730 ml
organophosphate	azinphosmethyl	Guthion 50WP	4	0	0	1	9	8	9	7	1,680-3,370 g
	phosmet	Imidan70WP	0	0	0	0	8	7	7	3	2,690-4,490 g
	chlorpyrifos	Lorsban 4E	0	0	0	0	4	4	4	2	2,340 ml
	dimethoate	Dimethoate 4EC	0	0	0	0	1	2	2	0	2,340-4,680 ml
organochlorine	endosulfan	Endosulfan 50WP	0	0	0	0	2	1	2	0	2,250 g
carbamates	carbaryl	Sevin 4F	9	8	9	8	9	8	9	8	1,680-2,810 g
pyrethroids	esfenvalerate	Asana XL	1	0	0	0	6	5	4	3	140-702 g
	fenpropathrin	Danitol 2.4EC	0	0	0	0	1	2	1	0	1,170 ml
	permethrin	Pounce 3.2EC	0	0	0	0	0	0	2	1	590 ml
acaricides	bifenazate	Acramite 50W	0	1	0	1	1	0	0	0	1,120 g
	abamectin	Agrimek 0.15EC	0	0	0	0	1	1	0	0	730 ml
	clofentezine	Apollo SC	0	0	0	0	0	0	1	0	281 g
	pyridaben	Pyramite 65WP	0	1	0	0	2	1	2	1	309-463 g
	etoxazole	Zeal 72WD	0	0	1	0	0	0	0	1	210 g
others	kaolin	Surround WP	1	1	1	1	1	1	1	1	11,000 g

changes in the arthropod community when transitioning from conventional OP-based to RR-IPM programs. Studies were conducted in commercial orchards at multiple sites in each state. Reported here are results of the response of the apple acarine community in western North Carolina to RR-IPM programs.

MATERIALS AND METHODS

RAMP study in North Carolina

Study sites were located in the three counties Henderson, Lincoln, and Polk, with six, one, and two test sites, respectively. At each site, a RR-IPM program ranging from 2 to 5 ha was compared to an adjacent conventionally managed (organophosphate based) block of equal or greater acreage. To evaluate different reduced-risk management approaches, the selection of insecticides and mating disruption programs varied among orchards (Villanueva & Walgenbach, 2003, 2004, 2005b, 2006b). Decisions on insecticide and acaricide applications in RR programs were made by RAMP project leaders, whereas in conventional blocks decisions were made by growers or their consultants. Table 1 presents the products used, the number of orchards that used these products, and the range of insecticide rates used.

Mites assessed included ERM, TSSM, ARM, predacious phytoseiid and stigmaeid mites, and mite-eating insects (thrips, *Stethorus* sp., and cecidomyiids). Mite densities were estimated by passing five leaves per tree from 10 locations per treatment through a mite brushing machine. Each orchard was treated as a replicate, and orchard-mean numbers were transformed using $\sqrt{(x+0.05)}$ before analysis by two-way ANOVA. Results are presented as back transformed values. Also, a total of 1,150 phytoseiids and 54 stigmaeids were mounted in permanent slides for identification.

Additional mite counts were made at two RR blocks in 2002 and 2005 where predacious stigmaeid mites were observed in high numbers. Female ERM and motile forms of phytoseiids and stigmaeids were monitored from 10 July to 18 September 2002 on five leaves per tree, from six trees per treatment, and from 15 June to 17 August 2005 on 10 leaves per tree, from four trees per treatment. Sample leaves were observed under a stereomicroscope in the laboratory. Comparisons were made between the RR and conventional treatments and data were processed as above. Also, in 2004 populations of the predatory thrips, *Scolothrips sexmaculatus* (Pergande) (Thysanoptera: Thripidae), were recorded in one orchard in unusually high densities.

Surround® effects on mites

This study was conducted in a 'Golden Delicious' apple orchard in Henderson County, NC, where mite populations were monitored in treatments sprayed with various application intervals of the kaolin product Surround® targeted for the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). Treatments consisted of Surround WP (28.0 kg/ha) sprayed at a volume of 935 l/ha at 7- and 14-d intervals, and a non-treated control. All plots were sprayed using

Table 2 Mean (± SEM) number of *Panonychus ulmi* females, phytoseiid and stigmaeid motiles per leaf in a reduced-risk (RR) and conventional IPM managed orchard. '*' indicates significant differences between treatments within the same species and date (P<0.05; df = 1,11 in 2002, or 1,7 in 2005).

Year	Date	*P. ulmi* females		Phytoseiid motiles		Stigmaeid motiles	
		RR	Conventional	RR	Conventional	RR	Conventional
2002	10-Jul	8.0±2.3	5.0±1.8	0.9±0.1	0.7±0.2	0.2±0.1	0.1±2.3
	24-Jul	0	0	0.4±0.1	0.3±0.1	0	0
	7-Aug	0	0	0.6±0.2	0.2±0.1	0.4±0.2*	0
	22-Aug	0	0	0.8±0.2*	0.3±0.1	0.9±0.3*	0
	4-Sep	0	0	0.6±0.3	0.5±0.2	1.3±0.4*	0.05±0.05
	18-Sep	0	0	0.7±0.2	0.4±0.1	2.8±1.0*	0.2±0.1
2005	15-Jun	0.5±0.2	0	0	0	0.1±0.1	0.1±0.1
	23-Jun	1.1±0.4*	0.2±0.1	0.1±0.1	0.1±0.1	0	0.0
	28-Jun	0	0.1±0.1	0.2±0.1*	0.0	0	0.1±0.1
	6-Jul	0	0.1±0.1	0.3±0.1	0.4±0.2	0	0.1±0.1
	13-Jul	1.1±0.2	1.3±0.3	0.8±0.5	0.5±0.1	0	0
	20-Jul	3.5±1.9	1.9±0.9	1.0±0.5	0.7±0.3	0.1±0.1	0
	28-Jul	9.8±3.5	6.4±3.8	3.8±0.2	3.3±0.7	0.5±0.3	0.1±0.1
	4-Aug	1.5±0.6	3.3±1.6	6.0±0.9*	3.7±0.3	0.3±0.3	0.2±0.1
	10-Aug	0.3±0.1	0.5±0.2	3.8±1.0	2.3±0.6	0.1±0.1	0.1±0.1
	17-Aug	0	0.1±0.1	0.6±0.2	1.3±0.6	0	0.1±0.1

an air blast sprayer. Plots consisted of three trees and treatments were replicated three times in a randomized complete block design. Weekly applications of Surround were made on 8, 15, 22, and 29 July, and 5, 12, 19, and 26 August; 14-d interval applications were made on 15 and 29 July, and 12 August. Weekly mite counts were made from 12 July to 22 August on five leaves/tree by removing leaves, returning them to the laboratory, and observing leaves under a stereomicroscope. Motile phytoseiid mites were counted on both sides of the leaves, whereas ARM was counted on 4 cm^2 along the mid-vein on each side of the leaf. Mean mite counts were transformed using $\sqrt{(x+0.05)}$, before two-way ANOVA, and separation of means by LSD (P<0.05) (StatSoft, 2000). Results are presented as back transformed values.

RESULTS

RAMP study in North Carolina

There were similar numbers of ERM in RR and conventional treatments throughout the duration of the study (Fig. 1). TSSM tallies were insignificant. The highest ERM counts were observed in the conventional treatment in 2004, but differences between treatments were not significant. In 2002, 2004, and 2005 more phytoseiids were observed in RR vs. conventional treatments, with differences being significant in June 2004 ($F_{1,17}$ = 8.7, P = 0.01) and July 2005 ($F_{1,17}$ = 5.3, P = 0.04) (Fig. 1). Similarly, stigmaeids were more abundant in the RR treatment in 2004 and 2005, although these differences were not significant. Mean numbers of ARM were similar in both treatments from 2003-2005.

In the single orchard with high numbers of stigmaeid mites, significantly more phytoseiid motiles were observed in the RR treatment on 22 August in 2002 ($F_{1,11}$ = 14.3, P = 0.01), 28 July ($F_{1,7}$ = 12.7, P = 0.03), and 4 August ($F_{1,7}$ = 19.3, P = 0.02) in 2005 (Table 2). A similar trend was observed with the stigmaeid populations in 2002, with significantly more stigmaeids observed in the RR vs. conventional treatment on 7 August ($F_{1,11}$ = 11.2, P = 0.02), 22 August ($F_{1,11}$ = 9.9, P = 0.03), 4 September ($F_{1,11}$ = 17.2, P = 0.008), and 18 September 2002 ($F_{1,11}$ = 18.6, P = 0.02) (Table 2). In 2005, stigmaeid populations were very low compared to 2002. The highest count in 2005 was on 28 July with only 0.5±0.3 and

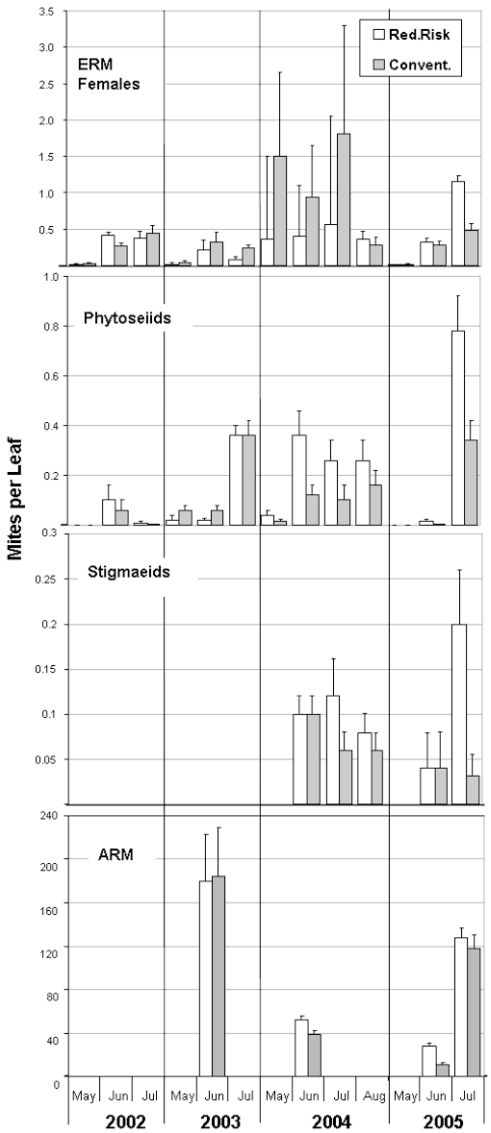

Figure 1 Mean (+ SEM) number of *Panonychus ulmi* females, phytoseiid, stigmaeids, and *Aculus schlechtendali* motiles per leaf in reduced risk and conventional (organophosphate-based) apple orchards in North Carolina. Significance in a two-way ANOVA is indicated by '*' for each sample date (P<0.05).

0.1±0.1 (mean ± SEM) motiles in the RR and conventional plots, respectively ($F_{1,7}$ = 6.86, P = 0.07). Phytoseiids identified from permanent slides were predominately *N. fallacis* (99%), and all stigmaeids were *Agistemus fleschneri* Summers. With the exception of one orchard in 2004, populations of mite-eating thrips *S. sexmaculatus* were observed in very low numbers. At this site there were significantly more *S. sexmaculatus* in the RR block (8.4±2.2 thrips per 10 leaves) than in the conventional block (4.8±0.8) ($F_{1,19}$ = 5.81, P = 0.03).

Surround® effects on mites

On the sample dates in this study, ERM were not detected, but phytoseiid populations were abundant (Fig. 2A). Phytoseiid mites were significantly more abundant in the control compared with the weekly and biweekly Surround treatments on 22 July ($F_{2,8}$ = 14.1, P = 0.015) and 9 August ($F_{2,8}$ = 15.6, P = 0.012). There were no significant differences in phytoseiid numbers between the weekly and biweekly Surround sprays. Although not significantly different, cumulative number of ARM were lower in Surround vs. control treatments (Fig. 2B).

DISCUSSION

During the 4-year RAMP project in NC, the organophosphate azinphosmethyl was applied one to five times per season in conventionally managed blocks. Due to inadvertent errors of the growers, azinphosmethyl was applied once each in four RR blocks in 2002 and one block in 2005 (Table 1). In addition, acaricides were used 11 times in the conventional and 4 times on the RR orchards. The reduced acaricide use in RR blocks may be related to the greater specificity of 'new chemistry' insecticides and pheromones applied for mating disruption, because these were less harmful to non-target organisms compared with the broad-spectrum insecticides used in the conventional program.

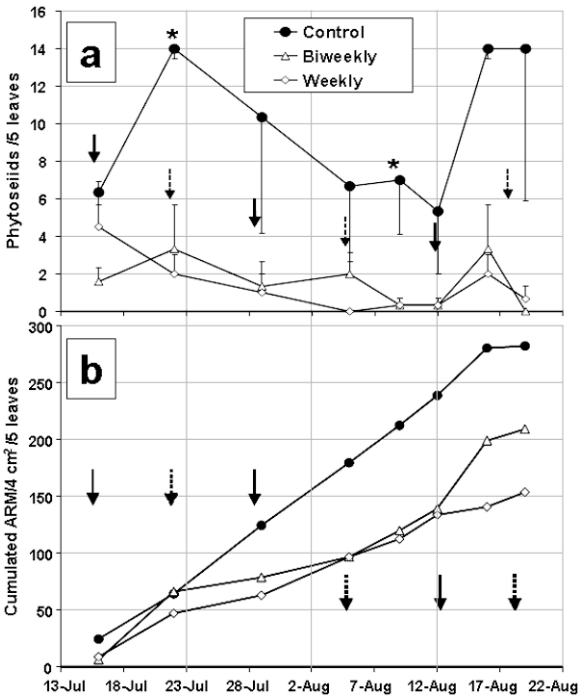

Figure 2 (A) Mean (± SEM) number of phytoseiid motiles and (B) cumulative number of *Aculus schlechtendali* found in an orchard treated with weekly or biweekly Surround® (28 kg 935 l^{-1} ha^{-1}). Solid and dash arrows indicate weekly Surround sprays. Solid arrows indicate biweekly sprays. '*' denotes significant differences (two-way ANOVA followed by Fisher's LSD, P<0.05).

European red mite flare-ups did not occur in the RR or conventional treatments during the 4-year study; ERM populations were of similar density in both programs, with few significant differences detected between treatments (Fig. 1). In apple orchards of the Pacific Northwest USA, epigeal mites appeared to be less affected by broad-spectrum insecticides compared with RR programs (Epstein et al., 2000). In addition, when an alternative IPM program based on oils and *Bacillus thuringiensis* was compared with a conventional OP-based program in West Virginia, USA, the alternative program showed lower levels of ERM, aphids, and leafhoppers (Biggs et al., 2000). In the RAMP program in NC, foliar phytoseiid and stigmaeid mites were present in higher numbers in the RR programs than in conventional treatments (Fig. 1). This was particularly evident in the single orchard where stigmaeids were found in significantly higher numbers in August and September 2002 (Table 2). Although a similar trend was observed in 2005, populations were low and further conclusions cannot be drawn from the data set. Among the other cooperating universities in this project, a similar pattern for natural enemies of spider mites was observed in Pennsylvania (DJ Biddinger, pers. comm.).

Neither of the pest management programs affected ARM. Mite-eating insects such as *Stethorus* sp. and cecidomyiid larvae were found in very low numbers, but *S. sexmaculatus* was very conspicuous in one orchard in 2004, and their numbers were higher in the RR compared with the conventional block. Although this high *S. sexmaculatus* population may have been related to phenological and/or climatological factor(s) rather than insecticide programs, natural enemies of aphids (anthocorids, cecidomyiids, coccinellids, chrysopids, and syrphids) were also more abundant in most RR orchards compared with conventional orchards (Walgenbach & Villanueva, 2007). Hence, it seems reasonable to assume that broad-spectrum insecticides more severely affected these predatory insects than did the narrow-spectrum insecticides used in the RR treatments.

ERM was not detected in the Surround experiment, but this absence was apparently due to extremely low numbers in this orchard rather than an effect of Surround, because mites were not detected in the control treatment. However, previous studies have shown that phytophagous mites were often high in kaolin-treated apple orchards (Knight et al., 2001). In our RAMP orchards from 2002-2005, there was one orchard where Surround was used in both RR and conventional blocks, and ERM populations were abundant in both treatments and required one to two acaricide sprays in the conventional block and at least one in the RR block. This abundant population of ERM was probably due to the endemic nature of ERM populations, but the Surround applications may have contributed to these high densities due to adverse effects on phytoseiids. Our results showed that phytoseiid numbers were reduced more than twofold in Surround vs. the water-treated control (Fig. 2a), although Bostanian & Racette (2008) observed no effects of Surround on phytoseiids under laboratory conditions. ARM populations were detected in lower numbers in the Surround vs. control plots (Fig. 2b), which is consistent with studies conducted by Bostanian & Racette (2008), that found signifi-

cantly low numbers of ARM in Surround treated vs. insecticide treated field plots. Surround is widely used for organic apple production in NC (Villanueva & Walgenbach, 2007), and its use is expected to increase as organic apple acreage increases in response to market demands.

Conclusions on the beneficial effects of RR products on non-target arthropods may be speculative at this time due to the nature of this project, where combinations of reduced risk products and/or insecticides were used in both the RR and conventional blocks (i.e., acetamiprid, methoxyfenozide, Surround). However, the absence of broad-spectrum organophosphate insecticides in the RR program may serve as an indicator that transition to 'new chemistry' insecticides in NC apple orchards will lead to high numbers of natural enemies, less intense fluctuations of spider mite populations, and reduction in the use of acaricides.

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Effect of monocrotophos and the acaropathogen, *Fusarium semitectum*, on the broad mite, *Polyphagotarsonemus latus*, and its predator *Amblyseius ovalis* in the field

Gunasingham Mikunthan & M. Manjunatha[1]

Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Jaffna, Sri Lanka.
E-mail: gmikunthan@gmail.com; and [1]Department of Entomology, University of Agricultural Sciences, College of Agriculture, Shimoga 577201, Karnataka, India

Chili crops suffer from damage by the broad mite, *Polyphagotarsonemus latus*, and they also harbour a predatory mite, *Amblysius ovalis*, as a potential control agent. To understand the change in the population of these mites when applying the acaricide monocrotophos and an acaropathogenic fungus, *Fusarium semitectum*, experiments were conducted in India during *Kharif* (Sept-Jan) and summer (March-June). Monocrotophos (0.05 and 0.025%) and *F. semitectum* (formulated in oil emulsion and dust water; 10^8 spores/ml) were sprayed in the field on the chili variety 'Byadagi', commonly used in Karnataka (India). Mite populations were estimated at 15-day intervals, starting 30 days after planting the chili crop. Dry chili yield was used for comparison of the treatments. Overall mite population estimation indicated that *F. semitectum* was effective to suppress broad mites, either alone or in combination with monocrotophos. The combination of *F. semitectum* with the recommended dose of monocrotophos (0.05%) best suppressed *P. latus*, whereas it did not affect *A. ovalis* numbers. Dust-water formulation of *F. semitectum* in combination with 0.05% monocrotophos suppressed broad mites better than the one mixed with a sub-lethal dose (0.025%) of the toxicant. The predatory mite population was not affected by *F. semitectum* nor by monocrotophos. Oil-emulsion formulation was as effective as the combination of dust water formulation and monocrotophos in both seasons. Considering the chili dry yield of all treatments, the fungal formulation sprayed in combination with monocrotophos (1.06 t/ha) gave the best control followed by the monocrotophos alone treatment (0.78 t/ha). Oil-emulsion formulation obtained the highest benefit-cost ratio (6:1) which suggests that the application of the oil formulation against the mites is feasible.

Key words: Monocrotophos, *Fusarium semitectum*, chili, *Polyphagotarsonemus latus*, *Amblyseius ovalis*

Chili pepper (*Capsicum annuum* L.) suffers from the characteristic downward-leaf-curl symptom, which is caused by the broad mite, a tiny tarsonemid [*Polyphagotarsonemus latus* (Banks)] (Kulkarni, 1922). This broad mite is polyphagous, frequently encountered, and widely distributed in the tropics and subtropics. Their feeding activities give rise to leaf curl complex symptoms. They infest plants throughout the year in major chili growing areas of Karnataka (India) and their infestation rate is very high compared to other pests (Hosmani, 1993). A complete failure of chili crops due to severe attack of *P. latus* has been reported by several authors.

Although many new acaricides have been evaluated against mites in chili, these newer chemicals are hardly used due to their high cost, non-availability and low toxicity, possibly due to the development of (cross-) resistance of the pest mites. Combinations of methods have been in practice to manage pests like mites. Insecticides often fail to control mites due to rapid development of resistance against the chemicals and/or low level of toxicity against the target organisms. Microbial acaricides have proven to be ideal candidates to control mites under controlled humid conditions. However, under field conditions this requirement of high relative humidity may not always be met. Insect pathogens are generally safe to humans and other non-target species. Hence, they can be used in harmony with other control tactics (Rombach et al., 1986). Few natural enemies of broad mites have been reported for chili crops. These natural enemies are not effective enough to prevent the fast build-up of broad mites especially during the peak growth phases of chili.

To evaluate the effect on broad mites and associated predatory mites under field conditions, the present study focuses on the combined use of an acaropathogen, *Fusarium semitectum* (ARSEF 7233), locally isolated from diseased cadavers of broad mites, and an acaricide, monocrotophos, or neem seed kernel extract, in chili-based cropping systems.

MATERIALS AND METHODS

Two experiments were conducted in the fields of the Zonal Agricultural Research Station (ZARS), Navile, Shimoga, India, during autumn (*Kharif*; September-January) 2003 and summer 2004. During *Kharif* 2003, two trials were conducted to evaluate the pathogenicity of fungi. Early transplanting of chili is a recommended practice to escape infestation of thrips and broad mites (Anonymous, 1995). However, to ensure a reasonable pest load in our experiments, chili was transplanted late in the two seasons.

Nine treatments consisting of combinations of *F. semitectum* formulations were tested in a randomized complete block design. Treatments were: (1) oil-emulsion formulation of *Fusarium* sp., (2) dust-water formulation of *Fusarium* sp. (DWF), (3) DWF + monocrotophos (0.05%), (4) DWF + monocrotophos (0.025%), (5) monocrotophos (0.05%), (6) DWF + 5% neem seed kernal extract (NSKE), (7) 5% NSKE, (8) water spray, and (9) no spray. Treatments were replicated thrice, treatment plots were 4.5 × 3.5 m.

Farm yard manure and inorganic fertilizer (NPK = 100:50:50 kg/ha) were applied according to general recommendations. One-month-old chili seedlings of the variety Byadgi were planted at a density of one plant per 90 × 60 cm. Treatments were bordered by two rows of hybrid maize with a spacing of 60 cm to prevent spore movement. Generally recommended practices of crop management were followed.

Two kinds of formulations were chosen to evaluate the pathogenicity of the fungi. A dust-water formulation was prepared from a 4-week-old fungal culture grown on rice, ground to a grain size of ≤0.2 mm, shade dried and dissolved in a volume of distilled water, and filtered through cheese cloth. Spore concentration was 10^8 per ml. For the second formulation, *Fusarium* sp. grown on rice grains was first ground and immediately suspended in sterile distilled water. Then, the suspension was adjusted to a final concentration

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of 10^8 spores/ml. The formulation was prepared by mixing the fungal suspension with 3% sunflower oil (refined) and emulsifier (Triton-X-100). Three sprayings were undertaken, each at 35, 50, and 70 days after transplanting (DAT) during evening hours just before sunset, using a knapsack sprayer at 500 l/ha. In case the treatment involved fungus formulation in combination with monocrotophos, the chemical was sprayed 3 days prior to fungal formulation application to prevent the inhibitory effect of the chemical on the fungus as suggested by Ganassi et al. (2000).

Neem seed kernel powder was soaked overnight in water and the following day it was filtered using cheese cloth. The supernatant (NSKE) was used for spraying at 2 kg/acre.

Populations of *P. latus* and *A. ovalis* were estimated at 15-day intervals after 30 DAT, following methods in Borah (1987). Crop damage was assessed at 40 and 100 DAT.

Polyphagotarsonemus latus

Five young leaves were collected from each of 10 randomly selected plants and the number of active stages and quiescent larvae per cm^2 of leaf area was recorded under a stereomicroscope (20×).

Amblyseius ovalis

Ten terminal leaves per plant were picked randomly and immediately after picking each leaf was held against the light and the minute rapidly moving, whitish or transparant phytoseiids (identified as *A. ovalis*) were recorded on each of the two leaf sides. A 10× hand lens was also used when needed. Ten plants selected randomly from the plot were examined during each observation.

Damage index

Leaf curl incidence was estimated by a damage index, reflecting the degree of foliage injury by *P. latus* (Borah, 1987). It was calculated by multiplying the damage score with the number of plants in that damage category and the sum of the products for each category was divided by the total number of plants in the experiment. Ten plants were rated using the following scale:

Score	Extent of damage
0	No symptom (healthy)
1	1-25% of leaves of a plant showing downward curling
2	26-50% of leaves curling; moderately damaged
3	51-75% of leaves curling; heavily damaged, malformation of growing points, reduction in plant height
4	>75% of leaves curling; severe to complete destruction of growing points, drastic reduction of plant height, defoliation and severe malformation

Scoring was done twice, at 40 and 100 DAT, and damage index was calculated. Parameters like plant height, fruit length, plant dry weight, and chili dry weight were recorded. The data thus obtained were subjected to ANOVA, if necessary after square root transformation.

RESULTS AND DISCUSSION

The two field experiments (*Kharif* 2003 and summer 2004) demonstrated the efficacy of the fungal formulations against broad mite. During the two seasons, an early moderate build-up of broad mites at 30 DAT on chili plants prior to first spraying (taking place at 35 DAT) was recorded, due to the broad mites already present on the plant material used for transplanting (Tables 1 and 2). The initial pest load was higher in the summer season (Table 2) than in autumn (Table 1).

As the crop growth advanced during *Kharif*, the mite population declined in plots where fungal formulations were sprayed. These populations reached strikingly lower numbers than in the NSKE and water-treated plots representing the control treatment (Table 1), indicating a clear effect of the fungus on the broad mites. The rate of reduction in the number of active stages of mites was strongest in plots sprayed with the fungus in oil formulation and dust water formulation in combination with monocrotophos (0.05%). Several workers also found this combined effect when they sprayed entomopathogenic fungi along with pesticides, as the chemicals readily cause mortality of the pests due to their quick knock-down effect.

The first spraying of fungal formulation at 35 DAT reduced the population of broad mites significantly during *Kharif* 2003. The efficacy of the mycopathogen was probably promoted by the high humidity (>80% r.h.) and the relatively low day temperatures (25 °C) in this season. The considerable rainfall in the second half of August might be the main environmental factor conducive to the mycopathogen. At 60 DAT, the spraying of fungal formulation had an effect in reducing the number of active stages of mites that was more pronounced than in the other treatments, except in NSKE-treated plots.

The influence of weather parameters on the efficacy of the mycopathogen was evident from the results of the field experiment conducted during summer 2004. Mite populations recorded at 30 and 45 DAT in the plots sprayed with fungal formulations showed no significant reduction in the population after the first spraying (35 DAT). This may be due to the slower action of the mycopathogen under dry weather conditions. Rainfall in the first half of May might have caused the mycopathogen to reduce the number of mites at 60 DAT.

The oil-emulsion formulation showed significant reduction of mites on chili and was equally effective compared to the combination of dust water formulation and monocrotophos during each of the two seasons. It yielded next-to-best yield of plant biomass (81 g) and dry chili (0.43 t/ha) when compared to dust water formulation of the fungus in combination with monocrotophos (98 g and 0.51 t/ha, respectively) during *Kharif* season (Table 3). During summer the oil formulation gave a similar effect, yielded equal plant biomass (80 g) but a third best, dry chili yield (0.76 t/ha). Considering the dry chili yield of all treatments, fungal formulation sprayed in combination with monocrotophos (1.07 t/ha) was better than the plots treated with monocrotophos alone (0.77 t/ha) (Table 3). Oil-emulsion formulation gave the highest benefit-cost ratio of 6:1, which is in support of the feasibility of the oil-based formulation against broad mites. The additive effect of the oil-based formulation of the mycopathogen was clearer in *Kharif* than in the summer season as the oil prevents fungus spores from being washed away.

Many biocontrol workers aim to develop fungal formulations by mixing with various oils to improve efficacy at low humidity. Laboratory work on *Metarhizium* and *Beauveria* conidia formulated in oil has provided evidence of infection at low humidities (Ramoska, 1984). Smith (1994) notified that *Paecilomyces fumosoroseus* conidia sprayed in oil (ShelsolT : rape seed oil = 7:3) and in two emulsions (1 and 10% Cadacide emulsifiable oil in water) killed 80-100% of whiteflies (*Bemisia tabaci*) under dry conditions (45-100%

Table 1 Effect of *Fusarium* sp. formulations on *Polyphagotarsonemus latus* and *Amblyseius ovalis* in chili (*kharif* 2003).

Treatments	Mean number of active stages of mites (*P. latus*/cm², *A. ovalis*/10 leaves/plant)											
	30 DAT		45 DAT		60 DAT		75 DAT		90 DAT		105 DAT	
	P. latus	*A. ovalis*	*P. latus*	*A. ovalis*	*P. latus*	*A. ovalis*	*P. latus*	*A. ovalis*	*P. latus*	*A. ovalis*	*P. latus*	*A. ovalis*
Oil-Emulsion formulation of *Fusarium* sp.	6.6	4.6	4.2b	5.3	4.0c	4.2	2.2d	2.1b	1.6c	0.8c	0.5d	0.2b
Dust-Water formulation of *Fusarium* (DWF)	6.3	4.3	4.2b	4.7	3.8c	4.0	2.0d	2.0b	1.5c	0.7c	0.3d	0.3b
DWF + Monocrotophos (0.05%)	6.2	4.2	3.1c	4.8	1.7e	4.3	0.8f	2.0b	0.2e	0.6cd	0.1e	0.2b
DWF+ Monocrotophos (0.025%)	6.2	4.7	4.2b	4.6	2.4d	3.8	1.5e	1.9b	0.7d	0.8c	0.4d	0.2b
Monocrotophos (0.05%)	5.7	4.6	3.1c	5.3	1.7e	3.8	0.7f	2.0b	0.5de	0.5e	0.2d	0.1c
DWF + 5% NSKE	6.6	4.3	6.1a	4.9	4.1c	3.7	3.6c	1.8b	3.1b	0.3e	2.4c	0.0c
5% NSKE	6.7	4.3	6.2a	4.8	7.4b	4.1	7.1b	1.6b	11.7a	0.4d	9.5b	0.1c
Water spray	6.4	4.0	6.7a	5.2	8.0ab	3.9	8.7a	2.7b	11.6a	1.3b	12.9a	0.9b
No spray (control)	6.4	4.1	6.7a	5.3	8.4a	4.8	8.7a	4.6a	12.6a	4.2a	13.2a	1.4a
F test	ns	ns	*	ns	*	ns	*	*	*	*	*	*

DAT, days after transplanting; *, P<0.05; ns, not significant; NSKE, neem seed kernel extract.

Means within a column followed by the same letters do not differ statistically (ANOVA followed by Duncan's Multiple Range Test; P>0.05).

Table 2 Effect of *Fusarium* sp. formulations on *Polyphagotarsonemus latus* and *Amblyseius ovalis* in chili (summer 2004).

Treatments	Mean number of active stages of mites (*P. latus*/cm², *A. ovalis*/5 leaves/plant)											
	30 DAT		45 DAT		60 DAT		75 DAT		90 DAT		105 DAT	
	P. latus	*A. ovalis*	*P. latus*	*A. ovalis*	*P. latus*	*A. ovalis*	*P. latus*	*A. ovalis*	*P. latus*	*A. ovalis*	*P. latus*	*A. ovalis*
Oil-Emulsion formulation of *Fusarium* sp.	12.4	1.8	10.7c	3.2	6.2d	4.4	6.2d	2.8b	3.1c	0.4b	1.4d	0.2b
DWF + Monocrotophos (0.05%)	12.9	2.1	9.6c	3.0	7.4c	4.9	7.4c	3.6b	1.2d	0.9b	1.0d	0.2b
Monocrotophos (0.05%)	13.4	1.9	6.4d	2.9	5.3e	4.3	5.3e	3.4b	1.6d	0.7b	1.4d	0.1b
DWF + 5% NSKE	13.0	2.0	10.4c	2.8	6.1d	5.0	6.1d	2.6b	3.3c	0.8b	4.1c	0.2b
5% NSKE	13.2	1.7	13.6b	2.9	14.2b	4.2	14.2b	2.9b	8.3b	0.6b	5.8bc	0.3b
Water spray	13.3	2.2	15.2a	3.1	15.7ab	5.0	15.7ab	2.6b	9.2b	0.8b	5.3b	0.4b
No spray (control)	12.4	2.1	14.7ab	3.0	16.3a	6.3	16.3a	4.8a	11.4a	2.9a	9.6a	2.5a
F test	ns	ns	*	ns	*	ns	*	*	*	*	*	*

DAT, days after transplanting; *, P<0.05; ns, not significant; NSKE, neem seed kernel extract.

Means within a column followed by the same letters do not differ statistically (ANOVA followed by Duncan's Multiple Range Test; P>0.05).

Table 3 Impact of application of *Fusarium* sp. formulations and monocrotophos on chili plant parameters and damage index during *kharif* 2003 and summer 2004.

Treatments	Mean chili plant dry weight (g)		Mean chili yield (t/ha)		Damage Index *Kharif* 2003		Summer 2004	
	Kharif	Summer	*Kharif*	Summer	40 DAT	100 DAT	40 DAT	100 DAT
Oil-emulsion formulation of *Fusarium* sp.	81.00b	80.33b	0.43b	0.76b	1.50	1.1c	1.5	0.3b
Dust-water formulation of *Fusarium* sp. (DWF)	76.33c	76.81b	0.41b	0.72b	1.75	1.0c	1.49	0.3b
DWF + monocrotophos (0.05%)	98.33a	80.00b	0.51a	1.05a	1.80	1.2d	1.6	0.2b
DWF + monocrotophos (0.025%)	74.67cd	78.56c	0.41b	0.65b	1.70	1.0c	1.6	0.2b
Monocrotophos (0.05%)	73.67d	90.00a	0.33c	0.77b	1.6	1.2c	1.7	0.2b
DWF + 5% NSKE	75.67c	80.33b	0.28d	0.65bc	1.70	1.1c	1.6	0.2b
5% NSKE	60.33e	75.00c	0.25de	0.46cd	1.60	1.3b	1.4	0.3b
Water spray	50.67e	56.67d	0.25de	0.37d	1.50	1.96ab	1.6	0.1b
No spray (control)	41.67e	43.00e	0.24e	0.30d	1.70	2.0a	1.5	1.4a
F Test	*	*	*	*	ns	*	ns	*

DAT, days after transplanting; *, P<0.05; ns, not significant; NSKE, neem seed kernel extract.
Means within a column followed by the same letters do not differ statistically (ANOVA followed by Duncan's Multiple Range Test; P>0.05).

r.h. during day and night), but killed none when sprayed on 0.1% aqueous Tween 80. Spore survival on the plant surface during dry conditions is another criterion that probably enhanced the efficacy of oil formulations. Conidia of *M. flavoviridae* survived longer on foliage in sprays of oil and oil-in-water emulsions than in water, presumably because the oil component gave greater protection from environmental stresses (Jenkins & Thomas, 1996). Curtis et al. (2003) also showed conidia of *Verticillium lecanii* on leaves germinated at 70% r.h. (ambient) when formulated in oil.

Dust water formulation of *F. semitectum* in combination with the recommended dose of monocrotophos (0.05%) suppressed the population of mites compared to DWF mixed with a sub-lethal dose (0.025%), resulting in significantly higher chili yield: 0.51 vs. 0.41 t/ha, respectively (Table 3). Combining the sub-lethal dose of the insecticide with the fungus was effective in suppressing the broad mite population compared with the oil-emulsion formulation. This effect is based on the principle that the lower dose of the toxicant weakens the pest – possibly slightly increasing the LT_{50} value –, ultimately favoring subsequent fungal infection, e.g., so that it establishes more easily under adverse conditions (Burges, 1998). Initial establishment of the inoculum in the field is a criterion for successful mycosis on the host for any entomopathogenic fungus. This in turn allows reducing the number of chemical sprays.

Neem seed kernel extract did not reduce the population of broad mites significantly in each of the two seasons. A combined effect of NSKE and the fungus was not evident from the sampling estimates, but the combination obtained a higher benefit-cost ratio than NSKE alone. Spraying NSKE and water did not suppress the broad mite population.

Fluctuations in the population size of *P. latus* were associated with those of *A. ovalis*. We have no reason to think that the predatory mite population was negatively affected by *F. semitectum*, other than by the effect of this fungus on reducing the number of its prey.

There was no reason to think that *F. semitectum* was pathogenic to the chili plant, based on estimates of plant height and fruit length, despite the fact that *F. semitectum* belongs to a complex group of fungi and other species of *Fusarium* are well known as plant pathogens causing wilt disease on solanaceous plants and other crops. Height of the plant and length of the fruit were not significantly different among treatments. However, some phytotonic effect was observed in *F. semitectum*-treated plants, which needs further investigations.

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Compatibility of pesticides with the acaropathogenic fungus, *Fusarium semitectum*

Gunasingham Mikunthan & M. Manjunatha[1]
Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna, Jaffna, Sri Lanka.
E-mail: gmikunthan@gmail.com; and [1]Department of Entomology, University of Agricultural Sciences, College of Agriculture, Shimoga 577201, Karnataka, India

Acaropathogenic microbes should be compatible with other control methods to be able to effectively utilize them against crop pests. This study aimed to assess whether different pesticides used in the chili ecosystem are compatible with *Fusarium semitectum*, a potential mycopathogen naturally infective to the active stages of the broad mite, *Polyphagotarsonemus latus*. A selection of pesticides easily available to farmers in local stores were prepared at 50, 100, 500 and 1,000 ppm. *F. semitectum* was inoculated on agar plates and the inhibitory effect on radial mycelial growth compared to a water-treated control was assessed Fungicides and insecticides were moderately inhibitory and equally toxic (on average, 41 and 37%, respectively) to *F. semitectum*, but dicofol, the one acaricide tested, was less toxic (27%). Out of nine fungicides carbendazim and benomyl were detrimental (54.5 and 53.3% inhibition). Conversely, copper oxychloride and sulphur were comparatively harmless to *F. semitectum*. Our results underline that compatibility of pesticides with the *F. semitectum* should be considered when selecting pesticides for use in addition to *F. semitectum* to control broad mites and mite pests in general.

Key words: Compatibility, *Fusarium semitectum,* pesticides, suppression, acaropathogenic fungus

Naturally occurring microbes are known to suppress mite pests in crops under suitable environmental conditions. Such a natural balance is often interfered by the non-selective application of pesticides. Ignorance on the interference by these pesticides may affect the potential role of biological control agents. Pesticides may be the only option available to farmers for the management of pests of chili plants (*Capsicum annuum* L.). Indiscriminate use of pesticides, however, can negatively affect naturally occurring mycopathogens, thereby promoting non-target or even target pests. *Fusarium semitectum* was isolated from active stages of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae), and it has been found to be a potential agent against this mite.

As a component of integrated pest management (IPM) acaropathogenic fungi should be compatible with other methods for their effective utilization. Acaropathogenic fungi, in general, tolerate a wide range of hydrogen ion concentrations (pH 5-10) with the optimum being pH 7 (Gustaffson, 1965; Humber, 1973). Their susceptibility to chemical pesticides varies widely between isolates and it should be possible to find suitable isolates for use in an IPM program. Fungi may be selected and even formulated with chemicals to obtain a synergistic or additive action (Burges, 1998). Many workers demonstrated the importance of proper selection of fungicides to prevent interference with natural epizootics of acaropathogenic fungi. An attempt was made to assess whether pesticides used in chili crops are compatible with *F. semitectum.*

MATERIALS AND METHODS

After *F. semitectum* was isolated for the first time from *P. latus* and *Scirtothrips dorsalis* (Hood) (Thysanoptera: Thripidae), its compatibility with common fungicides, insecticides, and an acaricide was evaluated for incorporation in IPM programs. The choice of pesticides (see Table 1) for the compatibility tests was based on their availability to farmers in the village shops.

A technique known for its use in the assessment of food poisons was followed to assess the compatibility of pesticides with the *F. semitectum*. For each pesticide, 50, 100, 500, and 1,000 ppm solutions were prepared. One ml solution of each pesticide and 15 ml of Saboraud maltose agar supplemented with yeast extract (0.1%) was poured into a sterile Petri dish under a laminar flow hood. The control experiment (0 ppm) was prepared using 1 ml of distilled water instead of the pesticide. Discs of 6 mm diameter were cut from the pure culture of *F.semitectum* and one disc was transferred aseptically to the center of each Petri dish. The treatments were replicated four times, using a completely randomized design. Petri dishes were then incubated in the laboratory at 26±2 °C and 78±4% r.h. Radial growth of the colony was measured at 24-h intervals for 5 days.

RESULTS AND DISCUSSION

Overall, the fungicides and insecticides appeared moderately inhibitory and equally toxic (41 and 37%, respectively) to the mycopathogen, followed by the acaricide (27%). Average inhibition of radial growth of *F. semitectum* by the fungicides ranged from 21.5 to 54.5% (Table 1). Among them, carbendazim (54.5% inhibition) and benomyl (53.3%) were significantly more detrimental. Mikunthan (1995) reported that benomyl is most toxic to *Metarhizium* sp. at the lowest concentration (100 ppm), whereas edifenphos inhibited mycelial growth to a lesser degree. Gupta et al. (2002) found that *M. anisopliae* and *B. bassiana* are sensitive (70-100% growth inhibition) to carbendazim, a fungicide similar to benomyl, at 10 and 100 ppm. Benomyl is also known to be detrimen-

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Table 1 Pesticide-induced inhibition of radial growth of *Fusarium semitectum* compared to water control (%).

Pesticides			Inhibition of radial growth (%)				
			50 ppm	100 ppm	500 ppm	1,000 ppm	Mean
Fungicides	Copper oxychloride	50% WP	13.16	15.84	26.77	29.80	21.4g
	Captafol	80% WP	8.20	20.22	60.09	88.69	41.8d
	Thiophanate methyl	70% WP	28.42	46.99	53.55	62.84	48.0b
	Sulphur	80% WP	10.39	16.94	22.40	40.98	22.7g
	Thiram	75% WP	30.60	37.16	48.63	81.42	49.5b
	Chlorothalonil	75% WP	22.95	40.98	51.91	63.38	44.8c
	Dithane M-45	75% WP	8.75	19.12	32.24	68.30	32.1e
	Carbendazim	50% WP	12.57	38.25	67.21	100	54.5a
	Benomyl	50% WP	15.84	32.79	64.65	100	53.3a
Insecticides	Chlorpyriphos	20% EC	15.30	32.79	47.54	62.29	39.5d
	Imidacloprid	17.8% SL	9.29	25.14	45.35	49.73	32.4e
	Monocrotophos	50% EC	18.03	26.78	33.34	38.79	39.2e
Acaricide	Dicofol	18.5% EC	8.75	25.68	31.70	41.53	26.9f

EC, emulsifiable concentrate; SL, soluable liquid; WP, wettable powder.

*Means followed by the same letter are not significantly different (Duncan's Multiple Range Test, P>0.05).

tal to three *Entomophthora* species (e.g., Olmert & Kenneth, 1974). Conversely, copper oxychloride and sulphur were significantly safer to *F. semitectum* (21.5-23% inhibition) followed by dithane M-45 (32% inhibition). Gupta et al. (2002) reported that copper oxychloride and azadirachtin (0.3%) are tolerated by *M. anisopliae* at concentrations of 1,000 and 2,000 ppm.

The three insecticides were moderately toxic to the fungus (32.4-39.5% inhibition). Monocrotophos was the least inhibitory insecticide, chlorpyriphos was most detrimental (Table 1). A similar effect of chlorpyriphos was also reported against *Verticillium* spp., but it did not inhibit *B. bassiana* (Olmert & Kenneth, 1974). Dicofol, the only acaricide tested, was less fungicidal (26.9% inhibition). That fungicides are more toxic to the mycopathogen than other agrochemicals, is in agreement with earlier findings (Soper et al., 1974).

Chemicals can hamper entomopathogenic fungi, but it has also been reported that they may enhance the activity of fungi. This occurs when fungi are combined with normally sub-lethal doses of insecticides, e.g., *Metarhizium* radial growth is stimulated in presence of copper oxychloride (Mikunthan, 1995).

Among the fungicides tested, copper oxychloride and sulphur caused the least inhibition and were compatible with *F. semitectum* (Table 1). This is supported by Olmert & Kenneth (1974) who found that copper oxychloride has no effect on *B. bassiana* and was moderately toxic to *V. lecanii*. Copper oxychloride was also reported to be safe to *Metarhizium* spp. (Mikunthan, 1995). Therefore, selecting compatible fungicides or insecticides should be given priority along with selecting compatible isolates of entomopathogenic fungi.

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Pesticide-induced mortality and prey-dependent life history of the predatory mite *Neoseiulus longispinosus* (Acari: Phytoseiidae)

N. Nusartlert, P. Vichitbandha[1], G.T. Baker[2] & A. Chandrapatya

Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
E-mail: chandrapatya@yahoo.com; [1]Biology Division, Science Section, Faculty of Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom Province 73140, Thailand; and [2]Department of Entomology and Plant Pathology, PO Box 9775, Mississippi State University, Mississippi State, MS 39762, USA

The life history of *Neoseiulus longispinosus* (Evans), a common predaceous mite on various plants in central, eastern, and southern Thailand, was studied under laboratory conditions at 30±2 °C, 50±5% r.h., and continuous light. Eggs or larvae of *Tetranychus kanzawai* Kishida, *T. truncatus* Ehara, *T. urticae* Koch, *Eutetranychus africanus* (Tucker), *Oligonychus mangiferus* (Rahman et Sapra), or *O. simus* (Pritchard et Baker) were provided as prey on excised mung bean, cassava, strawberry, tangerine, mango, and sugarcane leaf arenas, respectively. The predator completed its development on each of these prey, except for the eggs of *O. mangiferus.* Larvae were observed to feed after hatching. To develop from egg to adult, this predator required minimally 3.2±0.3 days when fed *T. truncatus* eggs or *E. africanus* larvae, and slightly more when fed eggs of *E. africanus*. Females reared on various stages of *T. truncatus* lived 22.0±2.8 days, during which 47.1±8.8 eggs/female were laid (= 2.6±1.1 eggs/female/day). Ten commonly used pesticides were sprayed on *N. longispinosus* and mortality was assessed after 24 h for larvae, nymphs, unmated females, and gravid females. Egg mortality was checked after 3 days. Almost all pesticides tested were toxic to all stages of *N. longispinosus*. The only exception was Nissourun®, which caused 12.4 and 56.5% mortality of eggs and gravid females, respectively.

Key words: *Neoseiulus longispinosus*, spider mite, life history, fecundity, pesticide

Neoseiulus longispinosus (Evans) was first described from Indonesia as *Amblyseius longispinosus* (Evans, 1952). Since then, this predator has been found in many countries especially in subtropical and tropical regions, including Taiwan, Philippines, Thailand, India, Australia, New Zealand, China, Malaysia, Pakistan, Papua New Guinea, and Hawaii (USA) (Ehara, 2002a,b; Ho et al., 1995). This species was first recorded in Thailand by Charanasri et al. (1977) and Ehara & Bhandhufalck (1977). At present, it is considered as one of the most abundant predatory mite species in Thailand. It is commonly found to be associated with various phytophagous mites, on a variety of host plants. Kongchuensin et al. (2005) reported the collection of *N. longispinosus* from 33 plant species, ranging from perennial plants (e.g., fruit trees) to annual crops (e.g., vegetables), distributed all over Thailand throughout the year. This mite was also abundant on plants infested with spider mites producing heavy webbing on the lower surface of leaves.

Neoseiulus longispinosus is reported as a potential control agent against several spider mites, such as *Aponychus corpuzae* Rimando and *Schizotetranychus nanjingensis* Ma & Yuan in China (Zhang et al. 1998, 1999), *Tetranychus urticae* Koch in Thailand and southeast Queensland (Kongchuensin, 2001; Singh & Singh, 2005; Waite, 1988), and *Eutetranychus africanus* (Tucker) and *Eotetranychus cendanai* Rimando in Thailand (Kongchuensin, 2001; Thongtab et al., 2001). This predatory mite is easy to rear in the laboratory. However, more information is needed about their prey in order to mass-produce this mite for the purpose of biological control.

This paper presents the results of experiments to assess the egg-to-adult development of *N. longispinosus* feeding on different spider mite species. Longevity and egg production of the predator were also assessed, but only when fed on *T. truncatus*. In addition, the effects of several commonly used pesticides on mortality of *N. longispinosus* was also determined.

MATERIALS AND METHODS

Stock cultures

Five species of spider mite were collected from mung bean (*T. kanzawai*), cassava (*T. truncatus*), tangerine (*E. africanus*), mango (*O. mangiferus*), and sugarcane (*O. simus*) at Kasetsart University, Bangkhen Campus; *T. urticae* was obtained from Manita Kongchuensin's laboratory (Dept. of Agriculture, Ministry of Agriculture and Co-operatives, Thailand). Adult female mites of each species were kept on detached leaves of their original host. The leaves were kept on moistened cotton pads on top of sponges in a plastic box (15 × 2 × 5 cm). Each box was kept in an incubator set at 30±2 °C and 50±5% r.h. Water was added when necessary and if leaves deteriorated, they were cut into small pieces and placed on the top of new fresh leaves.

Neoseiulus longispinosus was collected from cassava and reared on mulberry leaves resting on moistened cotton pads with *T. truncatus* as prey. All rearing units were kept at 30±2 °C and 50±5% r.h. Gravid females were transferred to leaf arenas with the same prey and the next generation was used to start the experiment.

Life history of *Neoseiulus longispinosus*

The life history studies were performed at 30±2 °C and 50±5% r.h., under continuous light. Leaf arenas (2 × 2 cm) of mung bean, cassava, tangerine, mango, sugarcane, and strawberry were placed on moist cotton pads (one arena per rearing cell). Five gravid females of *N. longispinosus* were transferred onto each arena and a known number of eggs or larvae of a particular spider mite species was added as prey. After 24 h gravid females and predator eggs were removed from each leaf arena, except for 1 predator egg that was left to start the experiment. Observations were made every 6 h until the mites reached adulthood and the duration of each

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Table 1 Development of *Neoseiulus longispinosus* fed with eggs or larvae of various spider mites at 30±2 °C and 50±5% r.h.

Spider mite species	Stages	n	Mean duration of predator stages ± SD (days)				
			Egg	Larva	Protonymph	Deutonymph	Egg-Adult
Tetranychus truncatus	eggs	23	1.34±0.14b	0.36±0.06b	0.73±0.15bc	0.75±0.18ab	3.18±0.29b
Tetranychus kanzawai	eggs	20	1.34±0.22b	0.44±0.11ab	0.88±0.19ab	0.80±0.13ab	3.46±0.34a
Tetranychus urticae	eggs	14	1.52±0.32a	0.39±0.13ab	0.84±0.23b	0.76±0.27ab	3.52±0.46a
Eutetranychus africanus	eggs	23	1.53±0.09a	0.50±0.23a	0.62±0.30c	0.88±0.42a	3.55±0.29a
Oligonychus simus	eggs	8	1.47±0.21ab	0.50±0.19a	1.03±0.39a	0.66±0.30b	3.66±0.33a
Tetranychus truncatus	larvae	29	1.44±0.38bc	0.41±0.17b	0.78±0.15a	0.83±0.20a	3.46±0.5ab
Tetranychus kanzawai	larvae	25	1.70±0.13a	0.53±0.19ab	0.74±0.32a	0.66±0.23a	3.63±0.32a
Tetranychus urticae	larvae	23	1.50±0.30abc	0.52±0.17ab	0.78±0.34a	0.76±0.24a	3.57±0.40a
Eutetranychus africanus	larvae	7	1.36±0.13c	0.45±0.09ab	0.68±0.19a	0.75±0.32a	3.24±0.26b
Oligonychus mangiferus	larvae	9	1.45±0.30bc	0.57±0.22a	0.63±0.47a	0.76±0.36a	3.36±0.53ab
Oligonychus simus	larvae	13	1.62±0.29ab	0.46±0.09ab	0.81±0.11a	0.79±0.14a	3.68±0.34a

Means within a column and within a spider mite stage followed by the same letter are not significantly different at 0.05 level as determined by LSD.

developmental stage was recorded. Numbers of eggs or larvae consumed by each predator were also recorded and prey was added onto each leaf arena at each observation until the predatory mite (unsexed) completed its development.

Longevity and fecundity of mated *Neoseiulus longispinosus*

A study on longevity and fecundity of *N. longispinosus* was conducted on a mulberry-leaf arena, (2 × 2 cm) with various stages of *T. truncatus* as prey. Per rearing cell a female teleiochrysalis and two active males were transferred onto a leaf arena on a moist cotton pad. Data on female longevity, egg production, and pre-oviposition, oviposition, and post-oviposition periods were recorded daily. Males of the predatory mite were added to the rearing cell when necessary.

Effect of pesticides on mortality of *Neoseiulus longispinosus*

The effect of 10 pesticides commonly used in Thai agriculture – Omite®, Kelthane®, Mitac®, Nissourun®, Azodrin 60®, Poss®, Malathion®, Vidate L®, Actelic®, and Anthio® – was tested against all stages of *N. longispinosus*, using tap water as control. Mulberry leaf arenas (1 × 1 cm) with various stages of *T. urticae* were placed on a 9-cm Petri dish lined with moist cotton wool. Either 15 eggs, 15 larvae, 15 unmated females, or 15 gravid females of *N. longispinosus* were transferred to the leaf arenas. Recommended doses of each pesticide were applied onto each leaf arena with a handheld sprayer (9 replications/treatment). Dead mites were counted 24 h after application. Eggs were observed daily for three consecutive days to determine egg hatch.

RESULTS AND DISCUSSION

Development of *Neoseiulus longispinosus*

Neoseiulus longispinosus was able to complete its development on all prey species except for the eggs of *O. mangiferus* (Table 1). When 24 eggs produced by females fed with *T. truncatus* were transferred onto a mango-leaf arena with eggs of *O. mangiferus* as food, all eggs hatched and 91.6% of the larvae developed into protonymph but no feeding was observed in both stages. Only two protonymphs (4.1%) molted into deutonymphs and wandered around the leaf arena without feeding and finally they either died or escaped from the leaf arenas.

Neoseiulus longispinosus fed with spider mite eggs required 3.2±0.3 to 3.7±0.3 days to complete its development. The fastest egg-to-adult development was recorded when *T. truncatus* eggs served as prey (Table 1), development rates on eggs of the other spider mites were not significantly different. Approximately 39-42% of the total developmental time was required for embryonic development.

Neoseiulus longispinosus fed larvae developed slightly slower than those provided with eggs of the same species, except for *E. africanus* (Table 1). However, *N. longispinosus* offered *O. mangiferus* larvae did not take any prey. On larvae of the other prey species *N. longispinosus* required 3.2±0.3 to 3.7±0.3 days to complete its development, and 42-47% of the developmental period was spent in the egg stage. Fastest development was recorded when *E. africanus* larvae were provided as prey. An additional preliminary study indicated that *N. longispinosus* could successfully be reared on *T. truncatus* or *O. simus*, offered on blotted paper coated with paraffin.

The larval stage of *N. longispinosus* was seen feeding as all other mobile stages. This contrasts with results of Kongchuensin et al. (1989) and Ibrahim & Palacio (1994) who reported that larvae of *N. longispinosus* were not observed to feed, when supplied with all stages of *T. urticae*. Surayothi & Siri (2003) also stated that *N. longispinosus*, when offered *T. truncatus*, *Polyphagotarsonemus latus* Banks, or *Scirtothrips dorsalis* Hood, did not require any food during the larval stage.

Kongchuensin et al. (1989) found that *N. longispinosus* fed mixed stages of *T. urticae* completed its development in 3.8 days, similar to the data reported here (3.5-3.6 days). Surayothi & Siri (2003) reported 1.9 days to complete egg-to-adult development, when fed all stages of *T. truncatus*, which agrees with our results. In contrast, Ibrahim & Palacio (1994) reported that *N. longispinosus* needed 4.3 days when fed *T. urticae* at 25-28 °C and 65-85% r.h. in the laboratory, and Urisakul (1982) reported an egg-to-adult period of 5.5-6.0 days on cassava plants infested with *T. truncatus*. Thongtab et al. (2001) reared *N. longispinosus* on *Eotetranychus cendanai* Rimando at 28±1°C, 58±5% r.h. and found that the total developmental time was 4.8±0.6 days, which exceeds the values reported here.

The number of eggs or larvae required by *N. longispinosus* to complete its development depends on the food source. Table 2 shows that 7-9 eggs of *T. kanzawai*, *E. africanus*, or *T. urticae* were enough for this predator to complete development. However, 13 eggs were required when *T. truncatus* or *O. simus* were provided as prey. When larvae were offered, *N. longispinosus* required 4-6 larvae of either *O. mangiferus*, *T. urticae*, *T. truncatus*, or *T. kanzawai* to com-

Table 2 Average number of spider mite eggs or larvae required by *Neoseiulus longispinosus* stages to complete development at 30±2 °C and 50±5% r.h.

Spider mite species	Stages	n	Larva	Protonymph	Deutonymph	Total
Tetranychus truncatus	eggs	23	0.13	6.35	7.26	13.74
Tetranychus kanzawai	eggs	20	1.00	3.50	3.05	7.55
Tetranychus urticae	eggs	14	1.28	4.86	3.57	9.71
Eutetranychus africanus	eggs	23	3.00	2.17	2.87	8.04
Oligonychus simus	eggs	8	1.65	4.28	6.38	13.00
Tetranychus truncatus	larvae	29	0.14	2.48	2.62	5.24
Tetranychus kanzawai	larvae	25	1.07	2.89	2.68	6.64
Tetranychus urticae	larvae	23	0.17	2.26	2.52	4.95
Eutetranychus africanus	larvae	7	1.29	3.86	5.29	10.44
Oligonychus mangiferus	larvae	9	0.00	2.00	2.11	4.11
Oligonychus simus	larvae	13	0.31	3.46	3.46	7.23

Table 3 Effectiveness of pesticides (% mortality) on various stages of *Neoseiulus longispinosus* under laboratory condition (30±2 °C, 50±5% r.h.). Sample size is 9 in all cases.

Pesticides	Egg	Larvae	Unmated female	Gravid female
Omite® 0.05%	100aA	97.5aAB	65.1bC	70.7bBC
Kelthane® 0.05%	100aA	100aA	97.8aAB	95.0aB
Mitac® 0.06%	100aA	100aA	100aA	100aA
Nissourun® 0.005%	12.4cD	90.6bA	72.1bB	56.5cC
Azodrin 60® 0.1%	100aA	100aA	100aA	100aA
Poss® 0.25%	100aA	100aA	100aA	100aA
Malathion® 0.07%	100aA	100aA	98.8aA	100aA
Vidate L® 0.09%	95.5bA	100aA	100aA	98.1aA
Actelic® 0.08%	99.0abA	100aA	100aA	100aA
Anthio® 0.05%	100aA	100aA	100aA	100aA
Control (Water)	0dA	5.5cA	4.1cA	2.1dA

Means followed by the same letter are not significantly different at 0.05 level as determined by LSD. Lower case letters refer to comparison within column, capital letters refer to comparison within row.

plete its development, but it required up to 10 larvae when fed *E. africanus* (Table 2).

Longevity and fecundity of mated *Neoseiulus longispinosus*

The average longevity of predator females was 22.0±2.8 days (range 17-29). Females laid their first eggs approximately within 3-5 days after adult emergence (average 4.0±0.63 days). The oviposition period lasted 17.8±2.1 days (range 14-22). Each female laid on average 47.1±8.8 eggs (range 35-67) during her life span, or 2.6±1.1 eggs/day (range 1-6). Females died, on average, 1.2±1.1 days (range 0-4) after the last egg was laid. Kongchuensin et al. (1989) found that female longevity and oviposition period of *N. longispinosus* fed *T. urticae* averaged 25.1 and 15.4 days, respectively – similar to our results.

Zhang et al. (1999) found that *N. longispinosus* fed on *S. nanjingensis* females laid 3.6 eggs/day on average with a maximum of 7 eggs/day, which is slightly higher than our results. Urisakul (1982) found that the predator laid 62.1 eggs/female (1.82 eggs/female/day) when fed on *T. truncatus*, where 54.4 eggs/female (3.6 eggs/female/day) was reported for a diet of *T. urticae* (Kongchuensin et al., 1989) – also higher than reported here. A similar result was obtained by Ibrahim & Palacio (1994), who found that egg laying of *N. longispinosus* fed with *T. urticae* started on day 2 after emergence and the oviposition period lasted at most 28 days during which 43.3 eggs/female were laid.

Because *N. longispinosus* completed development fastest on a diet of *T. truncatus*, and because *T. truncatus* is very easy to rear on mulberry leaves, this species is recommended as prey for mass production of *N. longispinosus*.

Effect of pesticides on mortality of *Neoseiulus longispinosus*

All pesticides tested are highly toxic to all stages of *N. longispinosus*, except Nissourun®, where 12.4 and 56.5% mortality was recorded for eggs and gravid females, respectively (Table 3). Adult mites treated with Omite® showed 65.1% mortality, not significantly different from those sprayed with Nissourun® (72.1%). Singh & Singh (2005) studied the toxicity of some pesticides against *N. longispinosus* and found maximum mortality (99.5%) for dicofol under laboratory conditions, which agrees with our results. Also ethion, monocrotophos, and endosulfan have been found to be equally harmful to *N. longispinosus*. Hence, care must be taken when pesticides are applied in the field, because all except Nissourun® were highly toxic to all stages of *N. longispinosus.*

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Agricultural Acarology: Host Plant Effects and Damage

Effect of nitrogen, phosphorus, and potash levels on population fluctuation of European red mite, *Panonychus ulmi*, on apple

Sangita Sharma & S.P. Bhardwaj
Regional Horticultural Research Station, Dr Y.S. Parmar University of Horticulture & Forestry, Mashobra, Shimla-171 007 (Himachal Pradesh), India. E-mail: bhardwajspdr@yahoo.co.in

The European red mite, *Panonychus ulmi* (Koch), has assumed the status of major pest in recent years in Himachal Pradesh, India. It is essential that a fresh approach to mite control be undertaken especially by studying its population fluctuation in relation to host plant nutrition. The effect of three levels of N, P, and K, each at the recommended dose (N_1, P_1, K_1), 25% above (N_2, P_2, K_2) and 25% below the recommended dose (N_3, P_3, K_3), plus the untreated control ($N_0P_0K_0$) was studied on the build-up of *P. ulmi* populations on potted apple cultivars 'Delicious' at RHRS, Mashobra, Shimla (2,286 m above sea level). Among ten combinations of $N_xP_xK_x$, the lowest mite population was recorded at $N_3P_2K_2$ (i.e., 25% less than recommended N and 25% more than recommended P and K), with 21.9 adults, 29.2 immatures, and 103.2 eggs/leaf. In the control treatment ($N_0P_0K_0$), 40.0 adults, 44.3 immatures, and 166.3 eggs were obtained. Treatment combination $N_2P_3K_3$ supported the largest population of 74.8 adults, 91.8 immatures, and 320.9 eggs/leaf, significantly different from the other treatments. These results suggested that slight manipulation in the recommended doses of NPK fertilizers can contribute substantially to controlling the abundance of *P. ulmi* population on apple trees.

Key words: Population development, Delicious, plant nutrition, mite control, orchard pest

The European red mite, *Panonychus ulmi* (Koch), earlier referred to as *Paratetranychus pilosus* (C&F), has established as a key pest of apple in Himachal Pradesh (HP). The mite is believed to have been introduced in the USA in 1911 from Italy where it was first seen in 1878 (Ewing, 1912). During subsequent years it became a common pest in the Pacific North-West, Central California, Indiana and Utah (Newcomer & Yothers, 1929). Later it spread to many apple growing countries of the world. In India, it was reported for the first time in 1974 from the north-western Himalayan region of Jammu and Kashmir, HP, and Uttaranchal on apple, plum, peach, apricot, quince, jack-fruit, *Hibiscus*, rose, tomato seedlings, and ivy (Prasad, 1974).

In HP, the outbreak of this pest was noticed in 1991 in Thanedhar (Shimla district) and Dalash (Kullu district). The pest appeared in an epidemic form in all apple growing districts of the state (Bhardwaj & Bhardwaj, 2000). *Panonychus ulmi* passes through five principal life stages: egg, larva, protonymph, deutonymph, and adult, with each motile stage preceeded by a quiescent, non-feeding stage. Both young and adult mites cause injury to foliage by sucking the cell sap. Acaricidal treatments manage mite populations for a limited period and do not provide complete control. In view of the importance of this pest, studies were carried out to determine the effect of host plant nutrition on the population build up of the mites, which plays a vital role in their population dynamics in the apple orchard.

MATERIALS AND METHODS

Studies on the effect of host plant nutrition on the mite population were carried out during April-June 2002 on 2-year-old apple seedlings placed individually in plastic buckets under laboratory conditions at the Regional Horticultural Research Station (RHRS), Mashobra, Shimla (31°1'N, 77°1'E, 2,286 m asl). N, P, and K were applied in the ratio of 2:1:2 to these seedlings. Each treatment was applied to five plants, which were considered as five independent replications.

Table 1 Levels and sources of N, P and K used during the experiment.

Notation	Dose (g/20 kg soil)	Level
N_1	80	Recommended dose
N_2	100	25% above recommended dose
N_3	60	25% below recommended dose
P_1	62.5	Recommended dose
P_2	78.1	25% above recommended dose
P_3	46.9	25% below recommended dose
K_1	33.3	Recommended dose
K_2	41.6	25% above recommended dose
K_3	25.0	25% below recommended dose
$N_0P_0K_0$	Control	no fertilizer application

N: applied as calcium ammonium nitrate (CAN) containing 25% N; P: applied as P_2O_5 through single super phosphate (SSP) containing 16% P; K: applied as K_2O through muriate of potash (MOP) containing 60% K.

To study the effect of various levels of nutrients on mite density, the treatments followed a 3^3 Factorial Completely Randomized Design (Gomez & Gomez, 1984), resulting in nine combinations of $N_xP_xK_x$, with x being 1 = recommended level, 2 = 25 % above recommended level, or 3 = 25% below recommended level (Table 1). The effect of plant nutrients on mite density was recorded by releasing 10 adult mites (5 males and 5 females) gently on individual plants with a fine camel hair brush (no. 0), 20 days after the application of the respective nutrient dose. The mites were allowed to settle, feed, and oviposit on the seedlings for 10 days at room temperature. The data on the mite population (numbers of adults, immature stages, and eggs) were collected at weekly intervals spread over 9 weeks on leaves selected one each from the top, middle, and bottom layer of the five seedlings.

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RESULTS

Adults

Fluctuation of the numbers of adults varied significantly among the various combinations of N, P, and K (Table 2). At the first count, 30 days after fertilizer application, the mean mite population was low on plants receiving a combined treatment of P_2 and K_2 with different levels of N. At the last count, 86 days after fertilizer application, the lowest no. adults/leaf was observed in the treatment $N_3P_2K_2$ (66.3), whereas the no. adults/leaf was highest in $N_2P_3K_3$ (255.0). In the control treatment (no fertilizer applied), the mean mite population rose from 0.3 to 103.7 adults/leaf, from 30 to 86 days after start of the trial.

Comparison of means of the population of adults over nine subsequent weeks after fertilizer application showed that the lowest overall mean was observed in $N_3P_2K_2$ combination (21.85 adults/leaf), followed by 23.81/leaf in $N_1P_2K_2$. In $N_1P_1K_1$ and $N_1P_3K_3$ treatments the mean adult population was 44.96 and 45.19 adults/leaf, respectively, compared to

Table 2 Impact of different N, P, and K levels on the population build up of *Panonychus ulmi* adults on apple during April-June 2002 at Mashobra, Shimla, HP, India.

	Mean mite population per leaf (days after treatment)									
Treatment	30	37	44	51	58	65	72	79	86	Overall mean
$N_1P_1K_1$	1.00	2.00	3.66	9.33	23.00	47.00	65.67	93.00	160.0	44.96cd
$N_1P_2K_2$	0.33	1.33	2.66	7.00	12.33	26.00	42.67	54.67	67.33	23.81g
$N_1P_3K_3$	1.00	2.66	5.33	10.67	23.67	49.00	64.00	90.33	160.00	45.19c
$N_2P_1K_1$	1.33	3.33	10.67	21.00	41.67	68.00	85.00	134.30	213.30	64.30b
$N_2P_2K_2$	0.66	1.33	4.66	8.33	14.67	30.67	49.67	62.67	80.0	28.07f
$N_2P_3K_3$	2.00	6.33	14.00	27.33	54.00	70.00	94.33	150.00	255.0	74.78a
$N_3P_1K_1$	0.66	1.66	3.33	9.00	15.33	29.00	50.33	72.00	115.0	32.93ef
$N_3P_2K_2$	0.00	0.33	1.66	6.00	11.33	21.00	36.00	54.00	66.33	21.85h
$N_3P_3K_3$	1.00	2.00	3.66	9.33	16.33	31.67	62.00	79.00	127.30	36.93de
Control	0.33	1.33	7.33	13.33	25.33	45.33	71.33	92.00	103.70	40.00cd
Overall mean	0.83t	2.23s	5.70r	12.13q	23.77p	41.77o	62.10n	88.20m	134.80l	

Figures indicate log transformed values. Analysis of variance based on log-transformed data yielded: Treatment: P = 0.06; Time: P = 0.06; Treatment*Time: P = 0.19. Means between columns or between lines followed by different letters differ significantly (comparisons based on t tests).

Table 3 Impact of different N, P, and K levels on the population build up of *Panonychus ulmi* immature stages on apple during April-June 2002 at Mashobra, Shimla, HP, India.

	Mean mite population per leaf (days after treatment)									
Treatment	30	37	44	51	58	65	72	79	86	Overall mean
$N_1P_1K_1$	1.33	3.33	7.00	24.00	26.00	50.33	70.00	103.70	170.70	50.71cd
$N_1P_2K_2$	1.66	3.66	7.33	10.67	15.67	31.33	48.33	66.67	85.00	30.04e
$N_1P_3K_3$	1.66	3.66	11.33	13.67	28.33	53.00	73.33	108.30	180.70	52.66c
$N_2P_1K_1$	1.00	4.33	12.33	25.33	49.00	72.67	95.33	175.00	260.00	77.22b
$N_2P_2K_2$	1.00	2.66	6.66	11.67	19.67	38.33	57.00	82.67	101.30	35.66e
$N_2P_3K_3$	2.66	6.66	14.67	29.67	55.33	76.00	111.30	220.00	310.00	91.81a
$N_3P_1K_1$	2.00	3.62	4.33	10.00	17.33	34.00	65.33	82.67	160.00	42.14de
$N_3P_2K_2$	1.33	2.66	6.66	11.33	21.33	36.67	42.33	58.67	82.00	29.22e
$N_3P_3K_3$	2.00	3.00	7.00	12.67	19.00	34.33	78.67	88.33	166.70	45.74d
Control	1.33	3.00	6.66	11.00	37.67	59.33	78.33	94.00	107.70	44.33d
Overall mean	1.60t	3.66s	8.40r	16.00q	28.93p	48.60o	72.00n	108.00m	161.41l	

Figures indicate log transformed values. Analysis of variance based on log-transformed data yielded: Treatment: P = 0.05; Time: P = 0.04; Treatment*Time: P = 0.14. Means between columns or between lines followed by different letters differ significantly (comparisons based on t tests).

Table 4 Impact of different N, P, and K levels on the population build up of *Panonychus ulmi* egg counts on apple during April-June 2002 at Mashobra, Shimla, HP, India.

	Mean mite population per leaf (days after treatment)									
Treatment	30	37	44	51	58	65	72	79	86	Overall mean
$N_1P_1K_1$	7.00	19.67	38.33	67.67	91.33	98.67	226.70	340.00	542.70	159.10d
$N_1P_2K_2$	2.00	6.33	13.67	24.33	39.67	77.33	132.30	245.00	391.70	103.60h
$N_1P_3K_3$	9.66	24.67	47.67	73.00	96.00	146.70	220.00	376.70	526.70	169.00c
$N_2P_1K_1$	10.33	31.67	59.67	84.00	105.00	193.30	333.30	610.00	840.00	251.90b
$N_2P_2K_2$	3.00	8.33	14.33	28.00	43.33	74.33	158.30	270.00	416.70	112.90g
$N_2P_3K_3$	11.00	32.67	75.33	119.00	166.70	293.30	470.00	746.70	973.30	320.90a
$N_3P_1K_1$	3.33	7.33	20.67	46.00	66.67	95.67	176.70	283.30	460.00	128.90f
$N_3P_2K_2$	2.00	3.33	8.33	21.33	44.67	85.67	166.70	256.70	340.00	103.20h
$N_3P_3K_3$	6.33	12.33	31.33	53.67	75.33	100.00	186.77	336.70	490.00	143.60e
Control	6.33	15.33	21.33	55.33	93.33	178.30	283.00	333.30	510.00	166.30cd
Overall mean	6.10t	16.17s	33.07r	57.23q	82.20p	134.300	235.40n	379.80m	549.10l	

Figures indicate log transformed values. Analysis of variance based on log-transformed data yielded: Treatment: P = 0.05; Time: P = 0.05; Treatment*Time: P = 0.15. Means between columns or between lines followed by different letters differ significantly (comparisons based on t tests).

the untreated control with 40.00 adults, all being not significantly different from one another. The maximum overall mean population of 74.78 adults/leaf was recorded in $N_2P_3K_3$ and this was significantly different from all other remaining treatment combinations.

Immatures

A combination of P_2K_2 with $N_{1\text{-}3}$ exhibited significant reduction in numbers of immature mites over the 9 weeks period (Table 3). At 86 days after fertilizer application the highest immature mite population per leaf was found for the $N_2P_3K_3$ combination (310.00). In the control treatment (no fertilizer) the immature count went up from 1.33 to 107.70 mites/leaf over the 9 weeks period.

The comparison of overall means, based on all weekly intervals, clearly established the superior effect of $N_3P_2K_2$ over the remaining treatments as the lowest population of 29.22 immatures/leaf was recorded. The untreated check was at par with $N_3P_3K_3$ and $N_1P_1K_1$ and recorded 44.33, 45.74, and 50.71 immatures per leaf, respectively. Treatment $N_2P_3K_3$ supported the highest mite population (91.81 immatures/leaf) (Table 3).

Eggs

Feeding of *P. ulmi* on apple plants treated with different combinations of N, P, and K revealed that host plant nutrition played a vital role in fecundity of the mites (Table 4). At 86 days after fertilizer application, plants treated with $N_3P_2K_2$ supported the fewest eggs/leaf (340.00), whereas many more eggs were recorded in treatments $N_2P_2K_2$ (416.70), $N_3P_1K_1$ (460.00), $N_3P_3K_3$ (490.00), and the untreated control (510.00), and the most (973.30/leaf) were found in the plants given $N_2P_3K_3$ treatment, followed by $N_2P_1K_1$ (840.00), and $N_1P_1K_1$ (542.70) and $N_1P_3K_3$ (526.70).

The comparison of overall means, based on all weekly intervals, indicated that the fewest *P. ulmi* eggs were recorded on plants treated with $N_3P_2K_2$ (103.20/leaf), immediately followed by $N_1P_2K_2$ (103.60/leaf), both significantly higher than the other treatments. A considerably higher count was recorded in treatment $N_3P_3K_3$ (143.60 eggs/leaf) which differed significantly from $N_1P_1K_1$ (159.10) and the control (166.30). The highest egg numbers were 251.90 and 320.90/leaf, recorded in $N_2P_1K_1$ and $N_2P_3K_3$, respectively, significantly different from all other treatments.

DISCUSSION

Studies on the effect of different N, P, and K levels on the build-up of the adult, immature, and egg populations clearly indicated that the combination of P and K at 25% above the recommended dose plus N at 25% below the recommended dose ($N_3P_2K_2$) significantly reduced the abundance of these stages on apple plants. On the contrary, $N_2P_3K_3$ (i.e., N at 25% above plus P and K at 25% below the recommended dose) supported the highest population of all three stages of *P. ulmi*.

Some information on the effect of NPK combinations on the population build-up of *P. ulmi* and other mite species is available. The present findings agree with Rodriguez (1958) who reported rapid increase in mite populations [*P. ulmi* and *T. telarius* (L.) (= *T. urticae*)] on apple with increased N, and population decline with increase in P and K. In Rumania, Lefter (1967) also reported a positive correlation between NPK content of leaves and population dynamics of *P. ulmi* on plum, being more evident for N than for P and K. Morris (1961) reported a six-fold increase in populations of the clover mite, *Bryobia praetiosa* (Koch) on plants with medium N supply, and about 11-fold with high N supply, but a lower mite population with higher levels of P and K.

On the other hand in Nova Scotia, Canada, Walde (1995) used higher N and lower P and K in apple orchards, and reported a non-linear relationship between N and *P. ulmi* density. Similarly, Archer et al. (1988) indicated that combinations of N and P fertilizers favoured abundance of *Oligonychus pratensis* (Banks) on sorghum more than those fertilized with N or P alone. They also found a weak positive effect on mite density on corn but no effect on abundance on sorghum. Phosphorus fertilizers had little effect on mite densities on sorghum and corn. Interpretation of experimental data pertaining to the population build up of mites on a treated plant is thus complicated by host plant response, nutritional needs by the mite species, and variable experimental conditions.

In the present study a significant effect of NPK nutrition on *P. ulmi* density on apple trees was recorded. $N_2P_3K_3$ allowed *P. ulmi* population to build up faster than $N_3P_2K_2$. The respective increase in population of adults, immature, and eggs was 3.42, 3.14, and 3.11 times higher in the former than the latter. In comparison, in the control the population of adults, immature, and eggs was 1.87, 2.07, and 1.93 times higher than with $N_2P_3K_3$, but 1.83, 1.51, and 1.61 times lower than with $N_3P_2K_2$.

These results clearly suggested that slight manipulation in recommended doses of NPK fertilizers can contribute substantially in controlling the development and abundance of *P. ulmi* population on apple trees. This approach being eco-friendly would help minimize pesticide application against *P. ulmi* on apple, but the degree to which plant growth and apple quality parameters are affected needs further investigation.

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Resistance of strawberry plants against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)

Abd-Allah M. Afifi, Ahamed Y.M. El-Laithy[1], Saied A. Shehata[2] & El-Sayed M.A. El-Saiedy[1]

Agricultural Zoology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. E-mail: amafifi51@gmail.com; [1]Plant Protection Department, National Research Center, 12622 Dokki, Cairo, Egypt; and [2]Vegetable Crop Department, Faculty of Agriculture, Cairo University, Giza, Egypt

Strawberry plant resistance to infestation with the two-spotted spider mite, *Tetranychus urticae* Koch was investigated, using two cultivars: Sweet Charlie (Sw-cv) and Camarosa (Ca-cv). Ca-cv is more susceptible to infestation with *T. urticae* than Sw-cv. Chemical analysis of leaves revealed that total phenols and amino acids were higher in Sw-cv than in Ca-cv in, whereas the opposite was recorded for total sugars. An increase in total phenol content likely suppressed mite infestation, whereas increase of total sugars may have a stimulatory effect on spider mites. Leaf trichomes of Sw-cv have a higher density and are longer and sharper pointed than those of Ca-cv. Such dense and long-hairy leaves of Sw-cv were not conducive to mite infestation. Also when the two cultivars were fertilized with $CaSO_4$ and K_2SO_4, mite infestation was lower than on unfertilized plants. These treatments lead to an increase in total phenols and amino acids in both cultivars. Increased potassium levels in strawberry plants lead to an increase in plant resistance to *T. urticae* infestation. Moreover, fertilization with $CaSO_4$ and K_2SO_4 gave an increased strawberry yield, of 7-17% for Sw-cv and 6-22% for Ca-cv.

Key words: Strawberry, resistance, *Tetranychus urticae*, fertilization, trichomes

Strawberry (*Fragaria ananassa* Duch) is a high-value crop. It has become one of the popular and favorite fruits to the Egyptian consumers due to its high nutritional value, reasonable price, availability in the markets, and its wide utility either fresh or processed. Moreover, it has become a strong export commodity to the Arab and European markets, particularly from November to April when production in European countries is diminished or limited to greenhouses.

The two-spotted spider mite, *Tetranychus urticae* Koch (red form), is an economically important pest of commercial strawberry production, as it decreases plant vigor, consequently resulting in the decrease of fruit size and yield (Lolaluzd, 2003). It is considered the most important pest of strawberry plants, as it is near invisible, yet has a nasty effect on the condition and productivity of plants (Wysoki, 1985; van Lenteren, 2000).

Strawberry plant resistance to *T. urticae* has been attributed to the chemical content of plant tissue (Rodriguez et al., 1960; Kielkiewicz & van de Vrie, 1983; Tomczyk et al., 1987) and to physical factors, leaf trichomes, or morphological structure (Kielkiewicz & van de Vrie 1983). Induced resistance can result from an environmental change and may temporarily protect the host plant. The application of fertilizers can change the chemical constituents of plant tissue and consequently their nutritional value for pests (Herbert & Butler, 1973; Ibrahim, 1988). Our objective was to study possible causes of strawberry plant resistance to mite infestation in terms of chemical and physical factors and the effect of fertilization by K_2SO_4 and $CaSO_4$ on the induced plant resistance to mite infestation.

MATERIALS AND METHODS

To estimate the impact of host plant resistance on the infestation rate of *T. urticae*, physical and chemical factors of strawberry leaves from two cultivars were studied, Sweet Charlie (Sw-cv) and Camarosa (Ca-cv). The following chemical constituents were determined: (1) total phenols, by the colorimetric method (Swain & Hillis, 1959), (2) total free amino acids, estimated according to Rosen (1956), and (3) total carbohydrates, as total soluble and total non-soluble sugars according to Smith et al. (1956).

Chemical analyses were carried out during the growing seasons of 2001 and 2002, at the start and at the peak of spider mite infestation, and at the end of the growing season. Leaflets were transferred to the laboratory and cut into small pieces for analysis. Five grams of these pieces were kept in a small glass with 50 ml ethyl-alcohol (80%) at 10°C in the refrigerator. Samples were homogenized using a mixer. Homogenized samples were filtrated through G 3 silica filter paper. Ethyl-alcohol was added to the filtrate up to 100 ml volume. Chemical analysis of the filtrate was done following the above methods using a spectrophotometer.

To study the effect of leaf trichomes on mite infestation, the length and thickness was measured of 10 trichomes per cultivar. Also trichome density (number/cm^2) was calculated for both cultivars.

To assess a possible effect of fertilization with calcium and potassium on the chemical composition of leaves and on strawberry susceptibility to mite infestation, calcium sulfate ($CaSO_4$) and potassium sulfate (K_2SO_4) were applied as foliar fertilizer at a concentration of 0.15 and 0.3%, respectively. Plots for this experiment were selected randomly within the experimental area at Giza Governorate. Three treatments (two fertilizers and a water-only control) were conducted, each replicated three times. No additional pesticides were applied throughout the experimental period. Fertilizers were sprayed 3 weeks after transplantation of strawberry seedlings and were repeated three times with 10-day intervals.

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Table 1 Chemical constituents of strawberry leaves of the two cultivars Sweet Charlie (Sw-cv) and Camarosa (Ca-cv), at Giza Governorate and mean two-spotted spider mite density during various phases of infestation.

			Chemical constituents (mg/g fw)		
Infestation period	Strawberry cultivar	No. mites/leaf	Total phenoles	Total amino acids	Total sugars
Start	Sw-cv	9.31	10.93	30.25	8.28
	Ca-cv	13.40	8.99	21.33	9.96
Peak	Sw-cv	117.73	11.04	14.03	6.32
	Ca-cv	148.47	9.78	10.02	18.68
Late season	Sw-cv	16.72	5.14	2.03	14.16
	Ca-cv	22.65	5.14	6.13	11.02

Table 2 Density, length, and thickness of leaf trichomes for the two strawberry cultivars Sweet Charlie (Sw-cv) and Camarosa (Ca-cv).

	Sw-cv			Ca-cv		
Replicates	No. trichomes /cm²	Length (µm)	Thickness (µm)	No. trichomes /cm²	Length (µm)	Thickness (µm)
1	187	4.8	1	124	4.8	1
2	214	4.9	1	118	3.7	1
3	188	3.8	0.9	133	4.2	1.3
4	216	3.4	1	165	4	1.5
5	272	4.2	1	173	3.8	1.4
6	252	4.6	0.8	123	3.9	1
7	268	5.2	0.9	156	4.1	1.3
8	210	3.9	1.2	146	4.2	1.4
9	272	4.8	1	108	3.5	1.5
10	315	4.9	1	126	3.7	1
Mean	239.4	4.45	1.03	137.2	3.99	1.24
SD	42.50	0.58	0.08	21.66	0.36	0.48

RESULTS AND DISCUSSION

Generally, Sw-cv was higher in total phenols and amino acids than Ca-cv during the first and second of the three periods of mite infestation, whereas total sugars was higher in Ca-cv (Table 1). In both cultivars amino acids was higher at the initial period of infestation, and then started to decrease in association with the developing mite infestation. The increase in phenolic compounds was associated with a decrease in mite infestation, while the increase in total sugar is associated with an increase in mite infestation. These trends were in agreement with those reported by Kielkewicz & van deVrie (1983) and Luczynski et al. (1990).

The density of leaf trichomes was higher on Sc-cv than on Ca-cv (Table 2). Moreover, the trichomes are longer and have a more pointed end than those of Ca-cv. This may have caused the decrease of *T. urticae* population density in Sc-cv, relative to Ca-cv. Length, thickness, and density of leaf trichomes are considered to be factors that affect strawberry plant resistance to infestation with *T. urticae.* Moreover, plant structure affects the functional response, particularly in relation to the ability of the predator to locate prey at low densities (Skirvin & Williams, 1999). High density and glandular trichomes are important resistance factors of tomato plants against the eriophyid mite *Aculops lycopersici* (Massee) (Leite et al, 1999).

Foliar fertilization with $CaSO_4$ (0.15%) and K_2SO_4 (0.3%) decreases mite infestation on the two cultivars when compared to water-treated control plants; $CaSO_4$ had less effect than K_2SO_4 (Fig. 1). Both fertilizers lead to an increase of total phenols and amino acids during the peak of mite infestation and late in the season in both cultivars, compared to the control during the peak of mite infestation (Table 3). Total sugars decreased in Ca-cv treatments relative to the control. The increased K-level in leaves lead to an increase in plant resistance to *T. urticae* infestation as also reported by Rodriguez et al. (1960) and Ibrahim (1988).

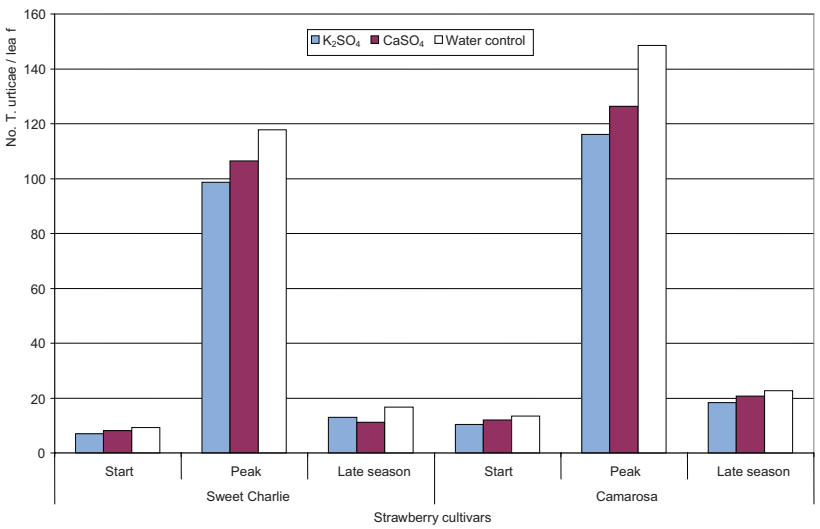

Figure 1 Density of *Tetranychus urticae* on strawberry cultivars Sweet Charlie and Camarosa affected by fertilization with $CaSO_4$ (0.15%) or K_2SO_4 (0.3%) at Giza Governorate during the three phases of mite infestation development.

Table 3 Effect of fertilization by $CaSO_4$ and K_2SO_4 on the chemical constituents of strawberry leaves of the two cultivars Sweet Charlie (Sw-cv) and Camarosa (Ca-cv), at Giza Governorate during during various phases of mite infestation.

			Chemical constituents (mg/g fw)		
Infestation period	Strawberry cultivar	Treatment	Total phenoles	Total amino acids	Total sugars
Peak	Sw-cv	$CaSO_4$	13.21	15.20	10.00
		K_2SO_4	14.28	16.67	7.80
		Control	11.04	14.03	6.32
	Ca-cv	$CaSO_4$	10.83	13.42	16.08
		K_2SO_4	11.60	12.12	16.72
		Control	9.78	10.02	18.68
Late season	Sw-cv	$CaSO_4$	6.36	3.33	11.92
		K_2SO_4	6.58	4.02	12.68
		Control	5.14	2.03	14.16
	Ca-cv	$CaSO_4$	6.36	7.92	10.13
		K_2SO_4	6.58	8.43	10.92
		Control	5.14	6.13	11.02

Strawberry cultivar Camarosa is more susceptible to *T. urticae* infestation than Sweet Charlie. This is due to chemical and physical factors. Increase in phenolic compounds lead to decrease in mite infestation, whereas mite infestation increased with an increase in total sugars. Leaf trichomes of Sweet Charlie are longer and more pointed, and the density on leaves of Sweet Charlie was higher than on Camarosa leaves. Fertilization by $CaSO_4$ and K_2SO_4 increased total phenols and amino acids in both Sweet Charlie and Camarosa leaves. It is argued that the increase of potassium in leaves leads to an increase in plant resistance to *T. urticae* infestation.

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Weight loss of copra due to infestation by *Aceria guerreronis*

M.A. Haq & T.R. Sobha[1]
Division of Acarology, Department of Zoology, University of Calicut, Malappuram, India-673635.
E-mail: haqzas@yahoo.co.in; and [1]School of Biosciences, Safi Institute of Advanced Study, Vazhayoor, Malappuram, India-673633

The commonly known coconut mite, *Aceria guerreronis*, inhabits the meristematic tissue of the coconut and has a high reproductive potential, hence it is important to assess its impact on coconut yield loss. This article focuses on yield loss in terms of copra. Infestation by *A. guerreronis* reduced the weight of copra of highly infested coconuts by 32%. When mite infestation was low, weight loss was only 7%.

Key words: *Aceria guerreronis*, weight loss, copra

The commonly known coconut, *Cocos nucifera*, represents one of the most important plantation crops of South India, particularly in Kerala. It constitutes one third of agricultural income of the state and hence any problem connected with loss in yield of coconut may be considered as a serious economic problem. Fortunately, coconut plantation of the state was free of major pests and parasites for a long time, except for a few instances of damage due to the Rhinoceros beetle, Red palm weevil and Black headed caterpillar. Hence, crop yield and coconut-based industries processing coconuts to produce copra, oil, soap, coir, handicraft, soft drinks, etc., in certain parts of India did not face much problems. This situation changed when the coconut mite, *Aceria guerreronis* (Eriophyidae) invaded India.

The incidence of *A. guerreronis* on coconut was first reported from Mexico by Keifer in 1965. Later, injurious effects of mite attack were also recorded from South America and neighbouring islands and in West Africa, for instance in Benin (Doreste, 1968; Mariau, 1969, 1986; Hall & Espinosa, 1981; Griffiths, 1984). The first report of its incidence from South India was made by Sathiamma et al. (1998). Subsequently the incidence of the mite, its invasion and injurious effects from the peninsular region including India, Sri Lanka, and Lakshadweep were demonstrated (Haq, 1999). Considering the high reproductive potential of the mite, a study was made to assess its damage potential. This article focuses on yield loss in terms of copra.

MATERIALS AND METHODS

In order to assess the loss of copra weight due to mite infestation, naturally infested nuts were categorised into four groups: uninfested (control) and slightly, moderately, and highly infested (50 nuts per category). Nuts were processed at an ambient temperature of 31°C for 2 weeks, and then the copra was weighed per category. The effect of mite infestation level (i.e., copra weight loss) was analysed by one-way ANOVA.

RESULTS

When the mite infestation level was higher, the weight of the copra was lower. Low, moderate and high infestation coincided with 7, 11, and 32% weight loss (Table 1). This effect of infestation level was highly significant (ANOVA: $F_{3,196}$ = 355.23, P<0.001).

DISCUSSION

Outbreaks of A. *guerreronis* cause various types of impact on the agricultural economy in the state of Kerala, India. Loss in weight of copra is one of the serious hazards. Feeding by mites under the bracts of the perianth of growing nuts results in uneven growth, measurable as a reduction in copra yield.

Yield loss in copra due to *A. guerreronis* infestation was estimated in different parts of the world: 10% in Benin (Mariau & Julia, 1970), 16% in Ivory cost (Mariau & Julia, 1979), 30% in Mexico (Hernandez, 1977), and 20-30% in St. Lucia (Moore et al., 1989).

After the outbreak of *A. guerreronis* in India, especially in Kerala, various aspects of the mite were studied. Preliminary studies of copra yield loss in Kerala indicated

Table 1 Weight (g) and weight loss (% relative to the uninfested control) of copra in different categories of coconut mite infestation.

Infestation level	Average weight (g)	% weight loss
Control	268.32	0
Low	249.98	6.8
Medium	223.80	10.5
High	153.12	31.6

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that there is a production decrease of 30-40% in seriously affected districts of the state. The annual loss due to mite infestation alone is estimated to be 100-150 crores (units of 10 million) in Indian Rupees. Muralidharan et al. (2000) studied the incidence and yield loss due to eriophyid mites on coconut in the Alappuzha district and estimated this to be 1,952 tonnes (31%) for copra alone. In the present study, weight loss in copra in the Thrissur district, one of the two states with highest infestation, was shown to be 32% in groups of highly infested nuts. Although copra content varies according to season, nutrient availability to the plant, and coconut variety, a 30% drop in production is likely to be a hard hit in the market of copra. Apart from the impact on copra, mite infestation will also affect other coconut-related products and industry, such as oil, food processing and soap manufacturing. Proper measures to control this pest are urgently needed for safe-guarding our coconut plantation.

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Veterinary Acarology

Dermanyssus gallinae in Dutch poultry farms: Results of a questionnaire on severity, control treatments, cleaning, and biosecurity

Monique F. Mul, Thea G.C.M. van Niekerk, Berry F.J. Reuvekamp & Rick A. van Emous
Wageningen University and Research Center, Livestock Research, PO Box 65, 8200 AB Lelystad, The Netherlands.
E-mail: Monique.Mul@wur.nl

In 2005 a questionnaire has been sent to 1,390 Dutch poultry farmers to investigate the severity of the poultry red mite (PRM) as a pest problem in The Netherlands. The response rate amounted to 31%. As the questions were not independent of each other, four clusters of questions were formed, based on two observed, discrete variables: (1) directly visible PRM infestation, and (2) indirect signs of the presence of PRM. Respondents were distributed over the most common housing systems in The Netherlands and reflected the Dutch situation with regards to housing of laying hens quite well. More than 80% of the poultry farmers reported infestations of PRM. Chicken flocks without PRM problems were significantly younger than flocks where (signs of) PRM infestation were observed. Where poultry was housed in battery cages, more farmers reported problems with PRM infestations, they tend to apply the first control treatment earlier, and repeat it more often than farmers with poultry in other housing systems. When PRM or signs of PRM were reported, farmers more often applied a combination of control treatments. The mean costs paid for control treatments and the costs incurred in terms of production losses were estimated to be € 0.43 per hen in an average flock. Given that there are 300 million layer hens in Dutch poultry houses and that ca. 85% of the flocks are PRM-infested, the overall annual cost to the national poultry industry is estimated at € 11 million. Since the answers to the questionnaire revealed a significant underuse of several measures that could prevent PRM infestation, there is room for improvement by more stringent management.

Key words: *Dermanyssus gallinae*, poultry red mite, laying hens, poultry farms, The Netherlands

The blood-feeding poultry red mite (PRM), *Dermanyssus gallinae*, is a frequent pest of chickens in Dutch poultry houses. The negative effects of PRM are thought to be transmission of poultry diseases, reduced egg production, reduced hen immunity, hen agitation, possibly inducing feather pecking, but also (skin) irritation in humans, usually workers in poultry houses. Due to more and more strict regulations regarding the use of chemicals in poultry houses, hardly any measures are left for effective control of PRM. Although farmers and extension services often mention PRM as the major problem in commercial farms with laying hens (further referred to as layers), there were no systematic attempts to quantify this. Therefore, the Dutch Product Board for Poultry and Eggs gave Wageningen UR Livestock Research the task to get more insight in the severity of this pest by means of a questionnaire to be sent to Dutch poultry farmers. In this way it was expected to assess the influence of housing system, control measures, and hygiene on the severity of PRM infections at a national level, and to quantify the costs due to PRM infections.

MATERIAL AND METHODS

A questionnaire was sent to 1,390 poultry farmers. The response rate was 31%. Some of the respondents (2% of all recipients of the questionnaire) did not complete the survey either for a reason (e.g., they left poultry industry) or not (12 cases). The respondents were asked to consider only the oldest flocks still on the farm. The answers had the form of discrete numbers (1-3) and could therefore be stored in an Excel database for quantitative statistical analysis.

A biplot analysis (Genstat 6.0; Rothamsted Research, Harpenden, UK) showed that the answers to several questions on the severity of PRM infestation were correlated and could therefore be grouped. Bi-plots (Gabriel, 1971) were made as a graphical representation of the relationships between farms and answers to questions. Questions about the presence of PRM, agitated hens during night, and farmer complaints on itches and other irritation of the skin were correlated. Also answers to questions concerning agitation of hens, quality of the feathering, feed consumption, egg production, extra mortality, and comb colour were positively correlated.

Cluster analyses showed that the farms could be divided into four clusters, based on two criteria: (1) PRM present or absent: direct signs of mites, e.g., visible in seams and cracks and/or unprotected surface, blood spots on eggs, irritation on human skin; and (2) Signs present or absent: indirect signs of mites, e.g., agitated hens in the dark, feather pecking, higher feed consumption, lower egg production, higher mortality, more mortality due to *E. coli*, and pale colour of the comb (signs suspected but not proven to be related to the presence of PRM). The four clusters are: (1) PRM no, signs no; (2) PRM no, signs yes; (3) PRM yes, signs no; and (4) PRM yes, signs yes.

For determining the differences in mean age of the flocks between the four clusters, the continuous data was analysed by RPAIR (Genstat 6.0) which gives t-tests for all pairwise differences of means from a regression. The significance level was set at 0.05.

Because several farmers did not answer all (sub)questions adequately, only 270 questionnaires were left for the analysis for correlations between the clusters and factors.

RESULTS

The respondents had farms with a range of the most common housing systems in The Netherlands and they provided a representative sample if it concerns the Dutch situation of housing layers (see Table 1A). There was a slight tendency

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Table 1A Number of respondents per housing system on questions concerning severity of PRM infestation and pest control treatments (n = 398).

	Cages	Deep litter	Aviary	Organic	Total
No. respondents (%)	128 (32.2)	202 (50.7)	39 (9.8)	23 (5.8)	392 (98.5)
Free range (no. respondents)	0	63	23	23	109
Covered veranda (no. respondents)	0	63	23	3	89

Table 1B Mean scores for farmers perception of severity of PRM infestation and pest control treatments (n = 392).

	Cages	Deep litter	aviary	Organic
Age of flocks (weeks)	50	49	46	50
PRM in cracks and crevices[2]	2.4	2.0	2.2	1.8
Bundles of PRM at unprotected surfaces[1]	1.9	1.4	1.5	1.3
spots with blood on eggs[1]	1.8	1.3	1.3	1.2
Agitated hens in darkness[1]	1.6	1.3	1.5	1.3
Feather pecking[1]	1.7	1.9	1.7	1.8
Extra mortality by *E. coli*[2]	1.3	1.4	1.3	1.3
Egg production[3]	1.2	1.3	1.2	1.4
Average age when first treatment was applied (weeks)	29	34	35	36
No. treatments during laying period	6.1	3.2	2.6	4.0
Most frequently used treatment	Heat	Not one in particular	Spirit and green soap	Biodiesel or no answer

[1]1, none; 2, some; 3, many.
[2]1, <2% extra; 2, 2-5% extra; 3, >5% extra.
[3]1, good; 2, moderate; 3, bad.

($P \leq 0.1$) for mean age of the flocks to depend on the housing system. Flocks with no PRM problems were significantly younger (45 weeks) than flocks where PRM was observed (52 weeks) or only indirect signs of it (53 weeks) ($P = 0.005$; Table 2).

Severity of the PRM infestation

In farms equipped with battery cages, the problems seemed more serious than in organic farms with outdoor access for the hens. In houses with battery cages farmers tended to observe more PRM in cracks and crevices, more aggregations of PRM at unprotected surfaces, and more chicken eggs with blood spots on the shell. This is typical of what we consider a PRM infestation. Of the farms with battery cages, 87% were infested with PRM. For farms with aviary systems, farms with deep litter, and organic farms, these percentages were 82, 83, and 78, respectively.

Housing system had little relation to indirect signs the hens may show under PRM infestation, such as agitation in darkness, intensified feather pecking, reduction in egg production, and increased mortality (Table 1B). There was no correlation between these putative signs of PRM and the extent of PRM infestation in farms.

Significant correlations were found between PRM clusters and housing system ($P<0.001$), yielding the following trends: (i) PRM was seen more often on farms with battery cages (35.2%) when compared with farms with deep litter (19.4%); (ii) indirect signs of PRM infestation were seen more often on farms with deep litter (25.4%) than on farms with battery cages (3.3%); and (iii) both direct evidence and indirect signs of PRM were seen more on farms with battery cages (24.2%) than on farms with deep litter (11.2%).

Treatments

There was a significant correlation between housing system and mean age of the hens at first pest treatment ($P<0.001$). Farmers with battery cages tend to treat hens significantly earlier (mean age = 29 weeks) than farmers with deep litter systems (34 weeks), or aviary systems (38 weeks), but not significantly earlier than organic farmers (34 weeks). There was no significant correlation between PRM problems and the timing of first treatment. But when there were no PRM problems observed, farmers with an aviary system tended to apply the first treatment later (70 weeks) than farmers with battery cages (25 weeks; $P<0.001$).

PRM problems were significantly correlated with treatment during the laying period ($P<0.001$): when PRM was observed, farmers answered more often that treatment was carried out during the laying period (Table 2). On farms with battery cages, hen houses were treated on average 6.1 times during the laying period, on organic farms 4 times, on farms with a deep litter housing system 3.2 times, and on farms with an aviary system 2.6 times.

There was a significant correlation between housing system and the type of treatment chosen ($P<0.001$). Farmers within the same category of housing system seemed to choose the same type of treatment against PRM. Organic farmers preferred biodiesel (or did not answer the question), farmers with battery cages chose mostly for a heat treatment in between two flocks, farmers with a deep litter system did not choose for a particular treatment, whereas farmers with an aviary system chose mainly for spirit and green soap.

There was a significant correlation between the PRM problem and the application of single or multiple treatment methods ($P<0.001$). At farms that had no PRM problems, farmers answered more frequently that only one method was applied (45.7%) rather than a combination of methods (13.2%). At farms where PRM was observed as well as indirect signs of PRM in the chicken flock, the farmers answered more often that a combination of treatment methods was applied (45%) instead of a single method (12.5%).

The most frequently used methods – sometimes applied more than once – were treatments with silica dust, biodiesel, spirit and green soap, and chemical treatments. Among the methods requiring continued treatment, the most frequent applications were with garlic, lesser mealworm beetle (*Alphitobius diaperinus*), and modified light regimes (to reduce foraging time of PRM).

Table 2 Mean age of flocks and number of farms with preventive or control treatment in relation to the four clusters of answers to poultry red mite (PRM)-severity estimates (n = 270).

Type of problem			Mean age[1]	Treatment during	
	PRM	Signs	(weeks)	empty-house period[2]	laying period[3]
1	–	–	45.3a	50 (25)	60 (42)
2	–	+	53.5bc	23 (4)	28 (11)
3	+	–	51.7c	40 (11)	58 (9)
4	+	+	48.1ab	33 (4)	41 (4)

[1]Means followed by different letters are significantly different (P<0.05).

Between parentheses: number of farms where no information was obtained regarding [2]treatment in between flocks, or [3]treatment during laying period.

Table 3 Preventive measures and the percentage of farmers that did (not) apply them.

	Yes	No
Trees within 10 m of hen houses	64	36
Bird nests in or within 10 m of hen houses	44	56
Farm clothes for visitors	96	4
Clean egg trays, containers or pallets	73	27
Cleaning with water	57	43

Prevention

Measures to prevent a PRM infestation are, amongst others, removal of all trees and/or bird nests within a range of 10 m from the hen house, strict rules for visitors to wear farm clothes, usage of clean materials (egg trays, containers, and pallets), and the use of water for cleaning the hen houses between subsequent flocks (Table 3). These measures may prevent PRM from invading poultry houses. Table 3 shows that 36% of the farms did not have trees within 10 m of the houses, 56% of the farms had no bird nests in or in the neighbourhood of the houses, 96% of the farms had rules for visitors to wear farm clothes when entering the hen houses, 73% of the farms had their egg trays, containers, or pallets cleaned, and 57% of the farms used water to clean the hen houses.

After depopulation of a flock of hens, there is a good opportunity for cleaning and reducing the numbers of PRM. During this period the houses were treated either chemically, with heat, with water, or with biodiesel. Yet, still a total of 110 respondents answered that during the empty-house period between subsequent flocks no treatment was applied, other than the usual, more superficial dry cleaning of the house.

Costs

Poultry farmers estimated the cost for treating PRM to be about € 0.14 per hen. Costs due to PRM infestation were thought to arise from higher feed conversion, reduced egg quality, and extra hen mortality. The farmers estimated these costs at € 0.29 per hen per laying cycle. Total costs per hen were therefore estimated to be € 0.43.

DISCUSSION

The data acquired from the response to our questionnaire can be used to estimate the total costs for the Dutch egg production industry. Assuming that the distribution of housing systems covered by the questionnaire is representative, these costs can be calculated as the product of the costs per hen per flock (€ 0.43), the flock infection fraction (ca. 0.85), and the number of flocks × the number of hens per flock (= the total number of hens, which is approximately 30 million in The Netherlands). This amounts to ca. € 11 million as an estimate of the total annual cost for the egg production industry in The Netherlands. However, experts in the field of poultry science estimate these costs to be even higher (MF Mul, unpubl.).

The correlations reported in this article should be interpreted with great caution. For example, the mean age of hens in a flock may differ per housing system and the older the flock the more likely it is for a PRM infestation to arise. Thus, a correlation between PRM severity and housing system may actually reflect a difference in the mean age of the flocks. Since population growth of PRM and foraging activity strongly depends on temperature (Kilpinen, 2001; Maurer & Hertzberg, 2001; Nordenfors, 2000), the correlation may also reflect a difference in the sum of the daily mean temperatures experienced by the PRM population.

It may also be questioned whether the observations on PRM infestations depend on how easy it is to notice PRM in each of the housing systems. For example, cages may provide more possibilities for PRM to hide than for instance a housing system with deep litter. Moreover, the answers to the questionnaire obviously are of a subjective nature. Farmers may be more aware of the problems, notice an infestation more quickly in one or the other housing system (in deep litter systems and organic systems it is not as easy to see PRM as it is in cage systems). Finally, farmers may rate the pest status differently, depending on the housing system they are using. Also, some of the indirect signs of PRM infestation (e.g., agitated hens in darkness, increased feather pecking) may be judged subjectively by the farmers and their judgment may depend on the housing system they are using.

Given the associations between poultry housing systems and PRM infestations it is interesting to see whether these trends also translate into different control methods and/or preventive measures. We found that farmers using battery cage systems tend to apply the first pest control treatment earlier than farmers using the other housing systems. They also apply pest control treatments more frequently. One may wonder whether this is so because (1) these farmers are more easily alarmed, (2) they can apply these control measures more easily in their type of housing system, or (3) they apply less effective treatments, thereby necessitating more frequent applications.

Preventive measures in between subsequent flocks are known to differ between housing systems. For example, farmers with battery cages mostly applied a heat treatment, which in other systems is hard to apply for various reasons. Research is needed to develop good, safe, cheap, and easily applicable preventive methods, as well as better control treatments for all housing systems. These methods should be designed so as to reduce the costs to the farmers and to increase the profitability of poultry farming.

In conclusion, more than 80% of the poultry farmers responding to our questionnaire reported PRM infestations on their farm. Those employing a housing system with battery cages tend to observe PRM infestations earlier, to apply the first treatment earlier, and to apply the treatments more frequently than farmers employing other housing systems. Various methods to prevent PRM infestation are available, but our questionnaire revealed that they were applied less frequently than needed. The overall costs due to pest con-

trol treatments and due to the damage incurred from PRM infestation are estimated to be € 0.43 per hen (averaged over all hens in a flock of average size under average conditions).

Acknowledgement

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A bioassay to assess the activity of repellent substances on *Ixodes ricinus* nymphs

Francesco Nazzi, Norberto Milani & Igor Saccon
Dipartimento di Biologia e Protezione delle Piante, Università di Udine, via delle Scienze 208, 33100 Udine, Italy.
E-mail: francesco.nazzi@uniud.it

A simple laboratory assay was developed to evaluate substances and extracts for their repellent effect on the tick *Ixodes ricinus* L. The bioassay involved testing the locomotory activity of *I. ricinus* nymphs in a circular glass arena. The stimulus to be tested was applied onto the arena outside a circle line (4 cm diameter). One field-collected *I. ricinus* nymph was placed in the centre of the arena and the time spent before entering the treated area was compared with that recorded in suitable control experiments where no stimulus or the solvent alone was used. Apart from a DEET-based product that was used as a positive control, extracts of basil leaves (*Ocimum basilicum* L.) and leaves of various grasses were tested. The commercial DEET-product proved to be active and so was an acetone extract of basil leaves; the hexane extract of basil leaves and the acetone extract from leaves of mixed grasses had very little or no activity. The bioassay appeared to be suitable to assess the repellent activity of natural products. Basil seems to contain substances that are repellent to *I. ricinus* nymphs.

Key words: Bioassay, *Ixodes ricinus*, *Ocimum basilicum*, repellents, sheep tick, sweet basil

Tick-transmitted diseases (e.g., Lyme borreliosis, tick-borne encephalitis) are causing increasing concern in northeastern Italy. In particular 22 cases of TBE have been reported between 2001 and 2005 in the region of Friuli; Lyme borreliosis is regarded as an endemic disease in the area (Ruscio & Iob, 1999). This situation prompted research into tick distribution and ecology as well as possible control methods for the main vector of such diseases: the hard tick *Ixodes ricinus* L.

Protection against tick bites by means of tick repellents is an effective method to prevent human infection. Both in vivo and in vitro assays have been developed to test candidate substances for their repellency on ticks. Normally in vitro tests involve study of the displacement of one or more ticks in an observation arena that is treated with the stimulus to be tested; a moving-object assay was also described (Dautel et al., 1999). In vitro tests suffer several limitations, in particular the interaction of the stimuli under study with possible attractants from the host have to be considered (McMahon et al., 2003). However, simple and effective lab bioassays still represent a very useful tool for the screening of candidate substances to select those that are worth of further and more reliable studies.

Like other aromatic plants from the Lamiaceae family, sweet basil (*Ocimum basilicum* L.) produces secondary metabolites that could confer arthropod-repellent properties to the plant (Hasegawa et al., 1997). Other *Ocimum* species have already been tested for their potential repellency against mosquitoes (Bindra et al., 2000; Gbolade et al., 2000; Seyoum et al., 2002). Here we describe a simple bioassay for testing the repellent activity of compounds extracted from the plant *O. basilicum* against *I. ricinus* nymphs.

MATERIALS AND METHODS

Bioassay

A circular glass arena – a 5-cm diameter Petri dish placed upside down on wet filter paper inside a larger Petri dish – was used to observe the behaviour of ticks towards a stimulus. Two concentric circles were drawn on the lower surface of the arena, one with radius 1 cm (start line), the other with radius 2 cm (finish line). The treatment stimulus was applied outside the outer circle (Fig. 1). A single nymph was placed with a fine paintbrush in the centre of the arena. The time between the crossing of start and finish line was assessed. If the start line was not crossed before 30 s the nymph was discarded; if the tick had not yet crossed the finish line after 3.5 min, 210 s was recorded as the finish time. Bioassays were run at room temperature under daytime light conditions; temperature was never lower than 22 °C, humidity was not controlled. Four replicates were run with each stimulus. Each replicate consisted of 7-14 bioassays with a single tick against the test stimulus and 7-14 bioassays against a control (Table 1).

Ticks used in the bioassay

The ticks under test were field-collected nymphs. They were collected in the field by dragging a fleece blanket over the vegetation and subsequently kept inside sealed vials with a

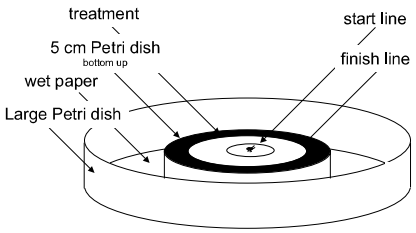

Figure 1 The observation arena used for the bioassay.

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Table 1 Stimuli tested in the bioassay: doses and details (n = 4 for each stimulus).

Stimulus	Dose	Nymphs used	Active nymphs
DEET based commercial product	2,6 mg of DEET	85	71
Hexane basil extract	100 mg equivalents	105	83
Acetone basil extract	100 mg equivalents	84	72
Mixed grasses acetone extract	100 mg equivalents	98	78

strip of wet filter paper as a water source, until they were used in the test.

Stimuli tested in the bioassay

A DEET-based commercial product was used as a positive control; in this case 2.6 mg of DEET, corresponding to 4 µg/mm², was sprayed outside the finish line using the roll-on dispenser provided with the product. Control arenas were untreated.

Both an acetone and a hexane extract of leaves and stems from fresh organic basil were tested to assess their possible bioactivity on the sheep tick. One-hundred-mg equivalents of basil leaves (i.e., the amount of extract obtained from 100 mg of leaves) in 100 µl solvent were used to treat the arenas (corresponding to 0.3 mg material in the hexane extract, and 14.4 mg in the acetone extract). Control arenas received 100 µl of the solvent used for the extraction. An acetone extract of leaves from mixed grasses (100 mg equivalents of mixed grasses leaves corresponding to 18.1 mg of material) was also tested as a negative control.

Extraction and chromatographic analysis

Extraction of natural sources was carried out by immersion into the solvent (100 g/750 ml) for 1 h. Extracts were kept at -20 °C until use. The desired concentration was obtained by evaporating the solvent under a gentle stream of nitrogen.

The volatile compounds in the extracts were collected by means of solid phase micro-extraction (SPME) using a polyacrilate fibre (85 µm) that was exposed for 5 min to the volatiles emitted by 100 mg equivalents of an acetone extract of basil, contained in an air-tight vial, sealed after evaporation of the solvent and maintained at room temperature. Identification was based on gas chromatography-mass spectrometry (GC-MS) using a Varian 3400 GC coupled to a Varian Saturn 2000 MS. The column (CP-SIL 8, 30 m × 0.25 mm ID, film thickness: 0.25 µm) was maintained at 40 °C for 1 min, then programmed to 250 °C at 10 °C/min. The carrier gas was helium (flow: 1 ml/min).

Statistical analysis

The median of the time spent by a tick between start and finish line was considered. The non parametric Mann-Whitney U test was used to check for possible significant differences between treatment and control.

RESULTS AND DISCUSSION

Only a few nymphs crossed the finish line when the DEET-based commercial product was used as a treatment, whereas most nymphs did so in about 20 s when no treatment was applied (Fig. 2A). The difference between treatment and control was highly significant ($P<0.0001$), suggesting that the bioassay is suitable for showing the repellent effect of active products.

No significant differences between treatment and control were found when a hexane extract of basil was used as a treatment (Fig. 2B) although the difference approached significance ($P = 0.076$); this suggests that this apolar solvent does not effectively extract possible active compounds from basil.

When an acetone extract of basil was used in the bioassay, ticks often turned back when approaching the treated area; a few of them did not cross the finish line before the end of the experiment (Fig. 2C). As a result, the difference between treated and control was significant ($P = 0.0017$). Thus, acetone seems to extract repellent compounds from basil suggesting that polar compounds may be involved in the bioactivity.

When an acetone extract of mixed grasses was used, the difference between treatment and control was not significant (Fig. 2D), suggesting that repellency observed when basil was used is related to specific compounds extracted from basil.

SPME-GC-MS analysis of the active extract revealed several compounds including α-terpineol, linalool, β-caryophyllene, and eucalyptol. Further research will include tests with some pure compounds identified in the active extract.

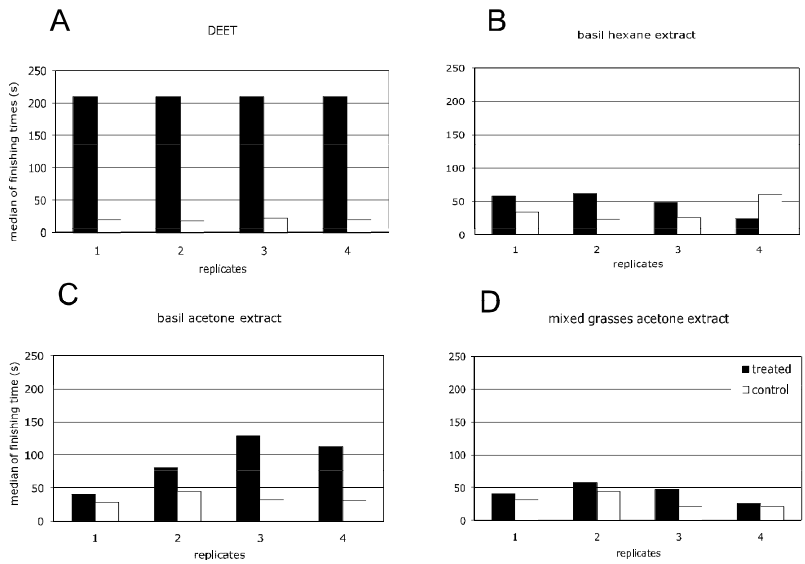

Figure 2 Results of the bioassay of (A) the DEET based product, (B) the hexane extract of basil leaves and stems, (C) the acetone extract of basil leaves and stems, and (D) the acetone extract of mixed grasses. The median time spent by the ticks for crossing the finishing line is given.

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Experimental studies on the potential role of the poultry red mite, *Dermanyssus gallinae*, as a vector of *Salmonella* serotype Enteritidis

Claire Valiente Moro, Philippe Fravalo[1], Michel Amelot[2], Claude Chauve, Gilles Salvat[1] & Lionel Zenner
UMR 958 ENVL/INRA Protozoaires Entéricoles et Parasites des Volailles, Ecole Nationale Vétérinaire de Lyon, 1, Avenue Bourgelat, 69280 Marcy l'Etoile, France. E-mail: valienteclaire@yahoo.fr; [1]Unité Hygiène et Qualité des Produits Avicoles et Porcins, Agence Française pour la Sécurité Sanitaire des Aliments, Zoopole, BP 53, 22440 Ploufragan, France; and [2]Service d'Elevage et d'Expérimentation en Pathologie Aviaire, AFSSA, Zoopole, BP 53, 22440 Ploufragan, France

Dermanyssus gallinae is the most important and common ectoparasite of laying hens in Europe and it is suspected of being a vector of pathogens. Salmonellosis is a major sanitary problem in poultry farms. We performed an in vitro experiment to evaluate the role of *D. gallinae* as a vector of *Salmonella enterica* serotype Enteritidis (SE). Two ways of infection of the mites were tested: through the blood meal by artificially engorging mites on contaminated blood, and after cuticular contact by leaving mites on a bacterial coating. The results showed that the mites could be infected via both infection routes. Bacterial multiplication within the mites has also been demonstrated, as well as the transstadial passage of *Salmonella* from the protonymph to the deutonymph stage, and the transovarial passage associated with a negative effect of SE on *Dermanyssus* oviposition. Mites have been shown to retransmit bacteria through a blood meal. In order to check whether the ingestion of previously contaminated mites by chicks led to an infection dose sufficient to contaminate birds, sets of 10 mites were orally administered to 1-day-old chicks. Each bird was found to be positive 12 days post-inoculation. *Salmonella* colonized the intestinal tracts and invaded liver, spleen, and caeca. Therefore, this study demonstrated experimentally that *D. gallinae* is a vector of SE. The mite may represent a suitable environment for the development of *Salmonella* and could be an additional factor for the persistence of *Salmonella* infection between two flocks. This underscores the importance of effective red mite control during the down time following a batch of poultry infected by *Salmonella*.

Key words: *Dermanyssus gallinae*, *Salmonella* transmission, vector, chicks, organ colonization, poultry

The poultry red mite, *Dermanyssus gallinae*, is suspected to be a vector and/or a reservoir for many pathogenic agents (Valiente Moro et al., 2005). This mite is frequently present in laying-hen facilities and it is responsible for anaemia, dermatitis, weight loss, and a decrease in egg production (Kirkwood, 1967; Chauve, 1998). It has become resistant to many available pesticides and, consequently, it is an emerging problem of real importance which must be taken into account if poultry producers wish to maintain good health in their commercial egg production facilities (Beugnet et al., 1997). Another sanitary problem in poultry farms is *Salmonella* infection. Since poultry is one of the most important reservoirs of *Salmonella enterica* serotype Enteritidis (SE), the prevention of the food-borne transmission of these bacteria is of major public health concern (Lacey, 1993). These Enterobacteriaceae can persist for long periods in the environment (2.5 years in avian faeces) probably due to their capacity to survive desiccation better than other coliforms (Morse & Duncan, 1974). More and more studies have emphasized the role of invertebrates, such as litter beetles, nematodes, and houseflies, in the transmission or persistence of SE in poultry farms (Fischer et al., 2003; Skov et al., 2004; Olsen & Hammack, 2000). Zeman et al. (1982) observed that these bacteria could survive inside *D. gallinae* for 4 months but did not study their vectorial capacity. However, observations in breeding hen facilities have demonstrated both the presence of mites in avian faeces, a surface frequently contaminated by *Salmonella*, and their capacity to feed regularly on birds which can show transient bacteraemia.

The objective of this study was to assess whether *D. gallinae* could play a role in the transmission of the pathogen in poultry farms. Using an in vitro experimental model to test the potential of the mite as a vector of *Salmonella*, we studied whether the mite could be infected, whether *Salmonella* could survive and multiply inside the arthropod, and/or be transmitted from one mite generation or stage to another, and finally whether the mite could contaminate normal vertebrate blood in an artificial feeding device. Since birds commonly peck and even ingest red mites in breeding hen facilities, the ingestion of contaminated mites by chickens as a potential route for poultry infection is of particular interest. We therefore fed chicks orally with mites previously contaminated by *Salmonella* and analysed the ability of SE to colonize their intestinal tract and invade their internal organs after inoculation to poultry in this way.

MATERIALS AND METHODS

Infection of mites

The populations of *D. gallinae* were collected from laying-hen breeding facilities in the Rhone-Alpes region (France). The absence of *Salmonella* in mites was previously checked by culture on the selective medium SM ID (BioMérieux, Craponne, France), as previously described (Valiente Moro et al., 2007). *Salmonella enterica* subsp. *enterica* serotype Enteritidis (SE) was obtained from the French Food Safety Agency (Ploufragan, France). Two methods of infecting the mites were tested: infection through a blood meal, or by cuticular contact. To ensure that any bacteria subsequently detected were those located inside the mites, they were cleaned following the protocol described by Zeman et al. (1982). This protocol was previously validated on groups of mites which were washed or not and then cultured on SM ID. Cuticular infection was achieved by leaving them on a dry *Salmonella* coating. Briefly, 500 µl of a solution containing a high SE concentration in peptone water (BioMérieux, Craponne, France) was applied in streaks onto SM ID agar and incubated at 37 °C. Sterile Wattman paper was placed in contact with the bacterial coating and then transferred to a

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sterile Petri dish. This allowed the mites to walk on the paper without suffering from the humidity produced by the agar. They remained in contact with *Salmonella* for 48 h at room temperature and were finally stored in sterile plastic tubes until inoculation of chicks. The contamination of mites through the blood meal was performed by using an in vitro feeding device (Bruneau et al., 2001). Using a pipette the mites were then placed in contact with the chicken skin overlying the infected blood reservoir and allowed to feed for 4 h in an incubator under standardized conditions (27±1 °C, 75% r.h., and total darkness). Blood was drawn from the occipital sinus of live chickens and no specific treatment was applied. It was directly contaminated with a bacterial level of 10^8 CFU/ml. To test the presence of *Salmonella* within the mites immediately after contamination, 100 and 40 mites infected by both infection routes, respectively, were analyzed (D0). To detect *Salmonella*, a culture of crushed mites in 20 µl of buffered peptone water (BPW) made up to 100 µl was plated onto SM ID and incubated at 37 °C for 18-24 h. For all experiments of the study, a SM ID Petri dish was added in the incubation chamber to check that there was no environmental contamination between the cultural media.

Survival and multiplication of *Salmonella* in mites

The presence of *Salmonella* was then investigated over time by analyzing mites at 1, 3, 7, and 14 days after their infection. To test whether bacteria multiplied, the colonies inside the mites were counted over time (D1, D7, and D14). Mites were crushed individually in 20 µl of PBW made up to 120 µl. Five 10-fold serial dilutions were made and 100 µl of each dilution were plated onto SM ID and incubated for 18-24 h at 37 °C. Concerning the contamination through the blood meal, we assumed that all mites with a *Salmonella* count 5× higher (statistically determined) than the theoretical value of 20,000 bacteria provided evidence of bacterial multiplication. This latter value was determined by considering that the number of Enterobacteriaceae inside freshly engorged mites was about 2×10^4 CFU, since a mite absorbs about 0.2 µl of blood, containing in our experiment a bacterial level of 10^8 CFU/ml. Similarly, to have an idea of the threshold value in mites infected after cuticular contact, the average of *Salmonella* count inside the mites 1 day after contamination was estimated to be 7.6×10^3 CFU/ml. All mites with a bacterial count 5× higher than this threshold value, i.e, 3.8×10^4 CFU/ml, proved bacterial multiplication.

Effect of *Salmonella* on mite oviposition and on transovarial and transstadial passages

To test whether the presence of the pathogen inside engorged females decreased the number of laying females as well as their fertility rate, comparisons of the number of eggs laid were made using 165 females engorged on either infected or uninfected blood. Each engorged female was then isolated and stored until its eggs had developed to the protonymph stage (N1). The protonymphs from each female were then washed and crushed with a sterile P10 micropipette tip in 10 µl of BPW. Samples were made up to 50 µl and incubated for 18-24 h at 37 °C to pre-enrich them and improve the detection of *Salmonella*. The following day, these pre-enriched 50-µl samples were plated onto SM ID and incubated for 18-24 h at 37 °C. In the same way, N1 offspring from a female fed uninfected blood were engorged with SE-infected blood to obtain deutonymphs (N2) which were analyzed for the presence of *Salmonella* as previously described.

Retransmission of *Salmonella*

One or 2 weeks after the mites had been contaminated regardless of the infection route, they were allowed to take an uninfected blood meal. After engorgement, the blood was removed and 2 x 100 µl was pre-enriched for 18-24 h at 37 °C in 900 µl of PBW, plated the following day onto SM ID, and incubated for 18-24 h at 37 °C. After their blood meal, all mites were washed individually and crushed in BPW to estimate the number of infected mites by culture on SM ID. Each experiment was performed 18 (engorged mites) and 12 times (cuticle-contaminated mites). A negative control was added to each experiment in which uninfected mites were allowed to take an uninfected blood meal. The blood was analysed as described above.

Oral inoculation of chicks with contaminated mites

One hundred 1-day-old chicks were inoculated orally: 34 received mites contaminated during the blood meal, 34 were infected with mites contaminated by the cuticular route, and 30 chicks, the negative control, were inoculated with uncontaminated mites. Using a 200-µl pipette, each chick received orally 10 mites suspended in 100 µl of TS. Each group of birds was housed in separate isolator units and kept 12 days after inoculation. The final washing solutions of mites were analysed for the presence of *Salmonella* in order to check the effectiveness of the washing solution. Six pools of 10 mites were kept for each route of infection to assess the titre of the feeding suspension. All *Salmonella* detections were performed according to the ISO 6579 procedure which requires a pre-enrichment step in BPW followed by an enrichment step on a semi-solid medium MSRV (Modified Semi-solid Rappaport Vassiliadis agar). Characteristic colonies are transferred on two selective media XLD (Xylose Lysine Desoxycholate) and Rambach, and finally confirmed biochemically and serotyped. Fecal samples and organs were analyzed at 6 and 12 days post-inoculation, respectively. Spleens and livers were pooled in groups of five before analysis, caeca were analyzed individually. *Salmonella* counting was performed using an MPN approach based on miniaturisation of MSRV enrichment (Fravalo et al., 2003).

Statistical analysis

Differences between numbers of infected mites over time, as well as the role of *Salmonella* on mite oviposition were analyzed by means of χ^2 tests. The difference obtained between females engorged on contaminated blood or uncontaminated blood was analyzed by a two-sided Student's t-test.

RESULTS

Success of experimental infection of mites

Mites could be infected via both infection routes. After an infectious blood meal, 29% of mites became carrier of *Salmonella*, and after cuticular contact, 55% of mites were infected.

Survival and multiplication of SE within mites

By either infection route, a significant increase in the number of mites carrying *Salmonella* was observed in comparison to day 0 (D0) (Fig. 1A). For mites infected by cuticular contact, the increase was noted on D7, and as soon as D3 for mites contaminated by the oral route. The fact that the number of infected mites is higher 3 or 7 days after infection, than 1 day after infection, suggests that the *Salmonella* may multiply

Table 1 Oviposition comparison between mites engorged with contaminated or uncontaminated blood.

	No. engorged females	No. egg-laying females	Total no. offspring/female	Average no. eggs/female
Uncontaminated blood	72	49	94	1.92
Contaminated blood	93	29	38	1.31

Table 2 Isolation and enumeration of *S.* Enteritidis in spleen, liver, and caecum on day 12 post-inoculation.

	Spleen		Liver		Caecum	
	Positive birds (%)	CFU/g tissue [×5]	Positive birds (%)	CFU/g tissue [×5]	Positive birds (%)	CFU/g tissue [×1]*
Engorged mites	100	2.7±1.8 ($\times10^3$)	100	1.9±1.9 ($\times10^2$)	100	8.6×10^4
Cuticular mites	100	3.6±3.5 ($\times10^3$)	100	1.8±2.9 ($\times10^3$)	100	8.6×10^4

*Caecal walls and contents estimated using MPN method (MPN *Salmonella*/g).

inside the mites. After counting the number of colonies inside the mites, bacterial multiplication was shown for 21 and 24% of mites on D7 and D14, respectively (Fig. 1B). For the other mites, we observed either a stable state or even a decrease in the number of bacteria. Similarly, multiplication was demonstrated for mites after cuticular contact for 42 and 25% of mites on D7 and D14, respectively (Fig. 1C).

Effect on mite oviposition and transovarial and transstadial passages

The number of ovipositing females is significantly lower when mites were engorged on contaminated blood than when they were fed uncontaminated blood (t-test, $P<0.05$; Table 1). Also the fecundity rate is slightly lower (1.31 vs. 1.92), but this difference is not significant. Of the 74 ovipositing females, 37 showed transovarial passage: in vitro females engorged on contaminated blood produced infected protonymphs (N1). Out of a total of 22 N1 obtained from uncontaminated females and subsequently fed contaminated blood, three deutonymphs (N2) were detected as positive for *Salmonella*, demonstrating transstadial passage.

Contamination of blood by infected mites

In cases of oral acquisition, only one *Salmonella* transmission was observed among the 18 separate assays performed. In cases of cuticular contact, blood was infected in five cases out of a total of 12 separate experiments. The blood on which uninfected mites were feeding remains negative in each experiment.

Infection of chicks after oral inoculation with contaminated mites

The mean infectious titres contained in the suspension given to the chicks were 3.0×10^4 and 2.7×10^6 CFU/chick for birds inoculated with cuticular contaminated mites and blood-engorged mites, respectively. The residual contamination measured for the 4-ml pools of the final washing solutions from the 340 cuticle-contaminated mites was 10 CFU/100 µl and 7.5×10^4 CFU/100 µl for the blood-contaminated mites. At 6 days after inoculation, faecal samples from both sets of infected chicks were positive for *Salmonella*, while the control corresponding to chicks inoculated with uncontaminated mites remained negative. On day 12 post-inoculation, SE was isolated from the caeca of all birds which had received contaminated mites with an average SE number $>8.5\times10^4$ MPN *Salmonella* g^{-1} (Table 2). Using direct plating, SE was counted from the spleen and the liver of all birds. No significant difference was found between the infection routes (χ^2 test, $P>0.05$).

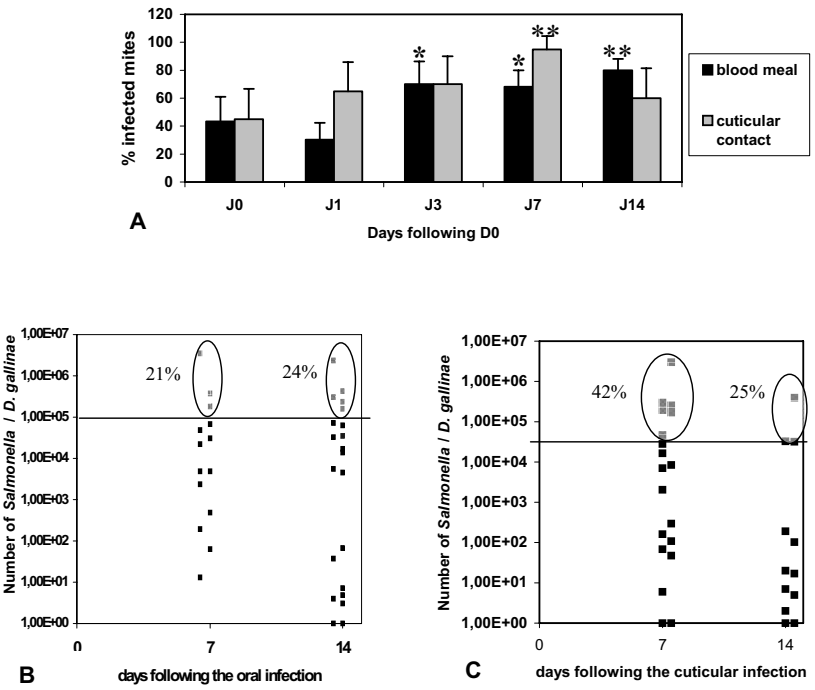

Figure 1 (A) Detection of *S.* Enteritidis inside *Dermanyssus gallinae* infected through a blood meal or cuticular contact. Vertical bars are standard errors. Significance of difference between D0 and others dates is indicated (** $P<0.01$, * $P<0.05$). (B) *S.* Enteritidis multiplication in infected *D. gallinae* after a blood meal (theoretical value = 2×10^4 bacteria in freshly engorged mites and multiplication if the number of bacteria on SM ID = $5\times2\times10^4 = 1\times10^5$). (C) *S.* Enteritidis multiplication in infected *D. gallinae* after cuticular contact (theoretical value = average at J1 = 7.6×10^3 and multiplication if the number of bacteria on SM ID = $5\times7.6\times10^3 = 3.8\times10^4$).

DISCUSSION

Diseases transmitted by arthropods play a major part in human and animal health. The superfamily Dermanyssoidea and in particular *D. gallinae* have already been suspected in the transmission of pathogens (Valiente Moro et al., 2005). Nowadays, the large number of red mites in breeding facilities with recurrent *Salmonella* infections raises the question of the potential role of *D. gallinae* in the transmission of this pathogen.

In poultry farms, mites can be infected with *Salmonella* in two ways: by taking a blood meal from birds with transient bacteraemia, or through contact with *Salmonella* found on various surfaces such as droppings. Most studies on the transmission of pathogens by *D. gallinae* have used in vivo assays allowing mites to feed on vertebrate hosts (Zemskaya & Pchelkina, 1967; Durden et al., 1993). Under our experimental conditions, the mites acquired *Salmonella* immediately after infection through the blood meal or after cuticular contact. Given the effectiveness of paraformaldehyde in eliminating all external contaminations, two possibilities remain: either a transcuticular passage of the bacteria and/or entry of the microorganisms through the stigmata. *Salmonella* multiplication in mites infected by both routes has also been demonstrated. However, in some cases, there was a decrease in the number of bacteria in the mites. This may result from a similar antibacterial response by the mites to that demonstrated for ticks, or even from the destruction of bacteria by the digestive system of the mite (Weyer, 1975; Nakajima et al., 2003). The transovarial passage of *Salmonella* has been observed when mites acquired bacteria during the blood meal and is often reported for ticks (Macaluso et al., 2001; Rennie et al., 2001), although it is less frequent in other mites. In our experiments, we observed that *Salmonella* may have a negative effect on mite oviposition. As we demonstrated transovarial passage, the decrease in fertility could be explained by the presence of Enterobacteriaceae in the reproductive organs. We also showed transstadial passage of *Salmonella* from protonymph to deutonymph. Generally, transstadial passage of bacteria is largely facilitated by arthropods, such as *D. Gallinae*, which require a blood meal to moult (Rodhain & Perez, 1985).

Two means of infecting the birds were relevant to explore: either by the mite biting, or after the ingestion of contaminated mites by birds. *Dermanyssus gallinae* was able to recontaminate the blood after acquiring the bacteria during a previous blood meal or contact with *Salmonella*. Even if a few cases were observed, it may be noteworthy in infested poultry facilities due to the large number of red mites (Nordenfors & Chirico, 2001). Similarly, when chicks were orally inoculated with contaminated mites, the excretion of *Salmonella* was observed from 6 days post-inoculation following both challenge tests and *Salmonella* colonized, multiplied, and persisted for 12 days in liver, spleen, and caeca ($>8.5\times10^4$ MPN *Salmonella* g^{-1} tissue) in all birds which received contaminated mites. The mean infectious titre of the inoculum given to chicks was equal to 3×10^4 and 2.7×10^6 CFU, for chicks inoculated with cuticle-contaminated and engorged mites, respectively. Other experimental studies performed with 1-day-old or 1-week-old chicks showed that low dosages of SE ranging from less than 10 organisms to 5×10^4 CFU could induce infection in chicks (Cooper et al., 1994; Duchet-Suchaux et al., 1995; Van Immerseel et al., 2004). It is important to note that this dose is dependent on the age of the host or on the number of bacteria in the organs. For example, it is about 10^{12} CFU for older chickens, considering the fact that they can set off their host defence mechanisms after the colonization of the gastrointestinal tract (Muir et al., 1998). In our experiment, mites used as the infecting inoculum contained sufficient infectious levels to cause infection in 1-day-old chicks.

The results of our tests on the final washing solutions were 10 and 7.5×10^4 CFU/100 µl for cuticle-infected and engorged mites, respectively. This suggests that the washing procedure was not performed adequately. However, it would be expected that the mites that were infected by walking on a *Salmonella* coating would have more *Salmonella* on the outside than the mites that have been infected by a blood meal. But the results show that the washing solution of the engorged infected mites is more contaminated than the other group. So, another explanation for this contamination could be the accidental crushing of a mite during the washing process. This hypothesis is supported by the fact that the level of contamination in the solution could be compatible with the concentration found in a single infected mite 7 or 14 days after engorgement, considering the fact that bacterial multiplication has been observed in some mites. In a test using 1-week-old chicks, CFU levels were highest during the first week(s) after oral inoculation with 5×10^4 SE and then decreased progressively (Duchet-Suchaux et al., 1995). Hinton et al. (1989) further demonstrated that birds may readily become infected by eating food contaminated with *Salmonella.* This latter experiment is closer to our study because *Salmonella* transmitted via feed given to birds, is similar to *Salmonella* transmitted via mites ingested by birds. The invasion of organs such as liver and spleen is an indication of systemic infection that might also reach reproductive organs and can be correlated with the frequency of deposition of the bacteria inside eggs which, ultimately, can contaminate consumers.

It would be interesting to further investigate whether such infection takes place in older birds. To estimate the possibility of this type of contamination in the field, it would also be necessary to study the doses found in naturally contaminated and infested flocks, before and after each batch. Carriage of *Salmonella* by arthropods has already been reported in previous studies. Most recorded examples refer to litter beetles (*Alphitobius diaperinus*) or cockroaches, but their role in the transmission of infection remains unproven (Davies & Wray, 1955; McAllister et al., 1994; Gray et al., 1999). In view of our results, it appears that *D. gallinae* is an experimental vector of SE. Although *D. gallinae* can both acquire SE and contaminate the blood under experimental conditions, this does not mean that the mite is a natural vector. In order to better understand the role of the mite in the epidemiology of *Salmonella* in poultry farms, much remains to be explored, such as mite population density, dispersion in poultry facilities, frequency of bloodmeals, and resistance to acaricides. In an epidemiological context, it is possible that infected mites crushed or eaten by chicks may be the main source of infection rather than the mite blood meal, but there is as yet no evidence to prove that.

In conclusion, this study has demonstrated an experimental test of *S.* Enteritidis infection that mimics a natural infection which could take place in poultry. By demonstrating that *D. gallinae* is an experimental vector of SE, we have new information that may guide future field studies.

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'*Candidatus* Midichloria mitochondrii', formerly IricES1, a symbiont of the tick *Ixodes ricinus* that resides in the host mitochondria

D. Sassera, T. Beninati[1], S. Epis, C. Bandi, L. Beati[2], M. Montagna, M. Alba, C. Genchi, L. Sacchi[3] & N. Lo[1]
DIPAV, Sezione di Patologia Generale e Parassitologia, Università di Milano, Italy. E-mail: davide.sassera@unimi.it; [1]School of Biological Sciences, University of Sydney, Australia; [2]Institute of Arthropodology and Parasitology, Georgia Southern University, Statesboro, GA, USA; and [3]Dipartimento di Biologia Animale, Università degli Studi di Pavia

Intracellular bacteria are widespread in nature and may adopt a wide array of life styles. They can be found free in the cytoplasm of their host cells, within host-derived vacuoles, or even in the nucleus. Here, we review current knowledge about '*Candidatus* Midichloria mitochondrii', an intracellular bacterium that invades the host mitochondria in the ixodid tick *Ixodes ricinus*. This bacterium was first detected by electron microscopy in two independent studies, published in, 1979 and, 1992, that showed it in the cells of the ovary in adult ticks and also in the cells of the ovarian primordia in larvae and nymphs. This symbiont resides not only in the cytoplasm, but also inside the mitochondria of the ovarian cells, where it appears to penetrate the outer mitochondrial membrane and colonize the intermembrane space. Molecular studies have recently been performed, addressing the phylogenetic position, transmission, and prevalence of this novel bacterium. The functional significance of this symbiotic association has yet to be revealed. Even though '*Candidatus* M. mitochondrii' seems to behave as a 'predator' towards the host mitochondria, this does not appear to interfere with egg development, thus ensuring the vertical transmission of the bacteria to the progeny. The 100% prevalence in the ovaries of females of *I. ricinus* may indicate a mutualistic association, whereas the peculiar intramitochondrial localization suggests that '*Candidatus* M. mitochondrii' might be exploiting the energy available in the mitochondrial environment. The possibility that the bacterium is a reproductive parasite should also be considered.

Key words: Intracellular bacteria, vertical transmission, mutualism, reproductive parasite, Rickettsiales

According to the broad definition of symbiosis proposed by De Bary in 1879 (the living together of dissimilar organisms), the epithet 'intracellular symbionts' encompasses a wide range of bacteria, belonging to many different taxa. The ubiquity of symbiotic relationships, established with a wide number of hosts, indicates the possible evolutionary advantages of symbiosis. These associations can be very diverse. Most intracellular symbionts colonize the cytoplasm, either free or within various kinds of host-derived membranes. Some bacteria (e.g., *Rickettsia* spp.) have been found within the nucleus, but even more exclusive is the mitochondrion: very few symbionts have been found within this organelle. There is evidence for the presence of bacteria within the mitochondria of three ciliate species (Yamataka & Hayashi, 1970; de Puytorac & Grain, 1972; Fokin et al., 2003), but there is only one intramitochondrial bacterium observed in any other kind of eukaryote, in the sheep tick *Ixodes ricinus* (Lewis, 1979; Zhu et al., 1992). This tick of the family Ixodidae (hard ticks), common in Europe and in parts of Northern Africa and the Middle East, is the main vector of Lyme disease in Europe, as well as an important vector of other pathogens of humans and animals (e.g., TBE virus, *Rickettsia* spp., *Anaplasma* spp.; for a review see Parola & Raoult, 2001).

The first description of the presence of intramitochondrial bacteria in the ovaries of *I. ricinus* dates back to 1979, when Lewis published an electron microscopy (EM) study on adult ticks. A second study reporting the presence of similar bacteria also in larvae and nymphs of *I. ricinus* was published by Zhu et al. (1992). In 2003 Beninati and co-workers, using PCR with universal bacterial primers, cloning and sequencing of the libraries, obtained a novel bacterial 16S rRNA sequence from the ovaries of *I. ricinus* adult females (Gene Bank accession AJ566640). This preliminary evidence for a novel bacterium led to a series of molecular (Beninati et al., 2004) and EM studies (Sacchi et al., 2004), which 're-discovered' the presence of the symbiont within *I. ricinus* mitochondria and determined its phylogenetic position. These investigations resulted in the temporary naming of the bacterium as IricES1 (*Ixodes ricinus* endosymbiont 1). After further molecular information was collected on the distribution, transmission, and phylogeny of this bacterium (Lo et al., 2006a), the official naming '*Candidatus* Midichloria mitochondrii' was proposed for this organism (Sassera et al., 2006). Here we present and discuss all the current knowledge regarding this fascinating symbiont.

ELECTRON MICROSCOPY STUDIES

EM has thus far been performed on ovaries of engorged (Lewis, 1979; Sacchi et al., 2004) and unengorged (quoted by Zhu et al., 1992 as unpublished) adult females, and on larvae and nymphs (Zhu et al., 1992). '*Candidatus* M. mitochondrii' appears as a typical Gram-negative bacterium, oval or coccoid shaped, measuring 0.6-1.3 µm in length (up to 2.5 µm) and 0.3-0.45 µm in diameter. In adult ticks the symbiont is present in the cytoplasm of all types of ovarian cells (i.e., oocytes, funicular, and luminal cells) surrounded by a host-derived membrane. In oocytes and lumenal cells '*Candidatus* M. mitochondrii' is also present within the mitochondria, in the intermembrane space between the outer and inner membranes (Fig. 1). Some mitochondria contain a single bacterium, whereas others may include more than 20. The more bacteria there are, the more degraded the mitochondrial matrix appears, as if the bacteria consumed the organelle and multiplied within it.

Intramitochondrial bacteria have been observed also in the primordial ovarial tissues of female larvae and nymphs, whereas no bacteria were seen in the primordial testicular tissues of males, nor in the malpighian tubules. Nymphs at

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different developmental stages (i.e., 15 and 21 days after repletion) have been observed and the number of 'parasitized' mitochondria seems to increase with the development of the female gonads. In nymphs several vesicles and long fine tubules are present within the mitochondria harbouring symbionts. Based on the EM information available we could hypothesize that the life cycle of '*Candidatus* M. mitochondrii' consists of: 1) invasion of the mitochondrion by one or a few bacteria; 2) assimilation of the contents of the mitochondrial matrix, with deformation and/or enlargement of the mitochondrion and parallel multiplication within the mitochondrion; and 3) burst of the vacuolated mitochondrion and release of the bacteria.

Mitochondrial symbionts have thus far been found in three species of ciliates as well. Studies on *Urotricha ovata* (de Puytorac & Grain, 1972) and on *Spirostomum minus* (Fokin et al., 2003) found the presence of single bacteria within mitochondria, with no signs of multiplication or degradation of the mitochondrion. The symbionts found in *Halteria geleiana* instead show remarkable similarities with '*Candidatus* M. mitochondrii' (Yamataka & Hayashi, 1970). They seem to have a similar life cycle, ending with swollen, degraded mitochondria containing up to >20 bacteria. Tubules and vesicles similar to the ones seen in *I. ricinus* nymphs were also observed. Such similarities suggest a possible phylogenetic proximity, which is yet to be investigated. Another interesting analogy with '*Candidatus* M. mitochondrii' is the one shown by the group of predatory bacteria called Bdellovibrio-and-like-organisms (BALOs). These are small, highly motile Gram-negative bacteria that prey on other Gram-negative bacteria, by entering the prey periplasm and multiplying therein. EM photographs of BALOs preying on other bacteria are remarkably similar to the ones of '*Candidatus* M. mitochondrii' within mitochondria. BALOs to date include three subgroups, two highly diverse families of the δ-proteobacteria class, but also the novel group *Micavibrio* spp., which forms a deep branch within the α-proteobacteria (Davidov et al., 2006). Considering the phylogenetic proximity of *Micavibrio* spp. to '*Candidatus* M. mitochondrii', the analogies in their life cycles may derive from the use of similar molecular mechanisms.

MOLECULAR IDENTIFICATION

The first molecular studies on '*Candidatus* M. mitochondrii' were performed by Beninati et al. (2004), who obtained a 1,427 nt sequence of the 16S rRNA. The *gyrB* gene, coding for DNA gyrase B and widely used for phylogenetic analyses, was then sequenced as well, obtaining a 1,432 nt partial sequence (AM159536) (Sassera et al., 2006). Both gene sequences where used for phylogenetic analysis and gave congruent results, placing '*Candidatus* M. mitochondrii' within the Rickettsiales, an order of the α-proteobacteria. This order comprises bacteria that share the characteristic of being intracellular symbionts, but that differ in many other aspects. Members of this order can be found in a variety of hosts, from arthropods to nematodes, from cattle to humans, adopting a wide range of life styles, from obligate mutualism in which the bacteria are necessary for the host reproduction (e.g., *Wolbachia* in nematodes, see Bandi et al., 2001a) to extremely virulent parasitism that often causes death of the host (e.g., *R. prowazekii* etiologic agent of louse-borne typhus; Andersson & Andersson, 2000). The bacteria of the order Rickettsiales are also considered to be the closest relatives of the mitochondria (Andersson et al., 1998); it is interesting that '*Candidatus* M. mitochondrii' is phylogenetically related to the organelle it invades.

Phylogenetic trees based on the *gyrB* gene do not permit clear resolution of '*Candidatus* M. mitochondrii' within the order Rickettsiales, probably due to the small number of available sequences, but 16S rRNA phylogeny does. In 16S rRNA analyses (Fig. 2) '*Candidatus* M. mitochondrii' clearly differs from all other Rickettsiales species, clustering with a group of uncharacterized/unnamed sequences obtained from four additional tick species (*Hyalomma anatolicum*, *Ixodes brunneus*, *I. persulcatus*, *Haemaphysalis wellingtoni*), as well as bacteria from a free-living amoeba, a marine sponge, and from a microbial mat. This is a monophyletic cluster of highly similar sequences (>90% nucleotide identity), placed in most analyses as the sister clade of the Anaplasmataceae, one of the two families of the order Rickettsiales (Fig. 2). The deep branching observed indicates the importance of this group of sequences, which, based on percentage of 16S rRNA identity, could be elevated at the rank of a novel family (Sassera et al., 2006). Of the other bacteria of this clade only the ones from *Acanthoamoeba* have been characterized by EM (Fritsche et al., 1999) and these

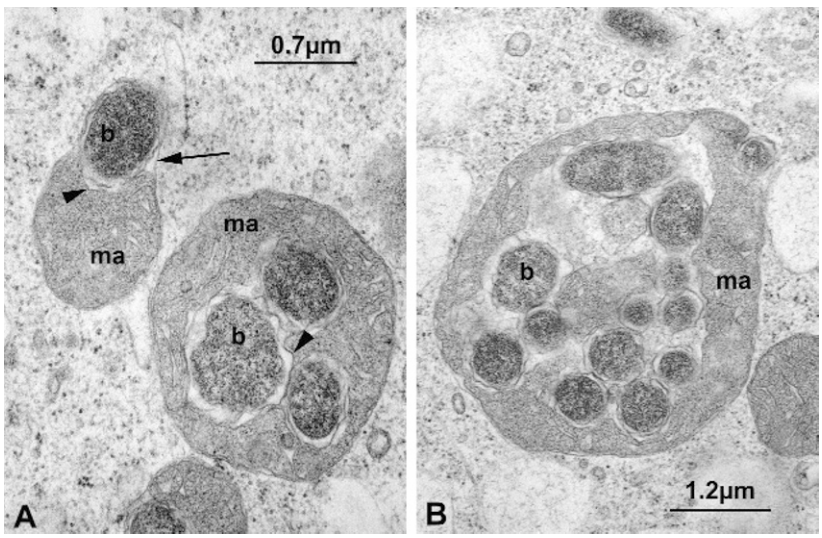

Figure 1 Electron micrographs of ovarian cell of *Ixodes ricinus* containing '*Candidatus* Midichloria mitochondrii'. (A) Initial steps of mitochondrial invasion: note a bacterium enclosed between the outer (arrow) and inner (arrow head) membranes of the mitochondrion, and three bacteria encircled by the inner mitochondrial membrane (arrow head). (B) Multiple infection showing clusters of bacteria embedded into the degraded mitochondrial matrix. b. bacterium; ma, matrix.

bacteria are only found in the cytoplasm of the amoebas, with no relationship with the mitochondria. There is currently no information on whether bacteria of the *Midichloria* clade invade mitochondria in other tick species.

A recent study by Epis et al. (2008) investigated the presence of bacteria phylogenetically related to '*Candidatus* M. mitochondrii' in 197 tick specimens, belonging to 21 species encompassing the five subfamilies of the family Ixodidae. Individuals from seven species of five genera were found to be PCR positive for bacteria closely related to '*Candidatus* M. mitochondrii'. A 1100 bp fragment of the 16S rRNA gene obtained from all positive specimens was sequenced in order to infer phylogenetic relationship between these bacteria. The resulting tree indicates the presence of a strongly supported family of tick associated bacteria, belonging to the Rickettisiales. The lack of correspondence between the phylogenies of these bacteria and their hosts makes the authors believe that the *Midichloria* symbionts have not co-evolved with the tick hosts, thus suggesting that the symbiotic relationship between them is not a long lasting obligate mutualistic one. Finally, EM pictures obtained from ovaries of *Rhipicephalus bursa* adult females show the presence of bacteria inside the mitochondria. This finding indicates the possibility that the intra-mitochondrial niche is exploited not only by '*Candidatus* M. mitochondrii'of *I. ricinus*, but by similar bacteria in other tick species.

TRANSMISSION AND PREVALENCE

Some aspects of the biology of '*Candidatus* M. mitochondrii' have been elucidated by molecular investigations. The transmission of the symbionts from mother to offspring, hypothesized based on EM observations showing their presence in both oocytes and larvae, has been confirmed with a PCR screening of newly laid eggs, which exhibited a positivity of 100% for the bacterium (Lo et al., 2006a). Transovarial transmission is the only mode of transmission so far demonstrated, but horizontal transmission cannot be excluded. An indication for horizontal transmission comes from the similarity of bacterial 16s rRNA sequences obtained from ticks of different genera. Moreover, we should consider that horizontal transfer occurs in *Wolbachia* (another member of the order Rickettsiales), a bacterium that is mainly vertically transmitted (Huigens et al., 2004).

Information regarding the prevalence of the symbiont in *I. ricinus* populations was also obtained by means of a PCR screening performed on both the 16S rRNA and the *gyrB* genes. Adult ticks were collected from 11 countries throughout the distribution of *I. ricinus* and tested for the presence of '*Candidatus* M. mitochondrii'. The 16S rRNA screening found 95% of the females to be positive, while all males were negative. PCR performed with primers specific for *gyrB* was more sensitive, as 100% of the females and 44% of the males were positive (Lo et al., 2006a). These results clearly show that the symbiont has a strong preference for females, which could perhaps be expected for a transovarially transmitted microorganism (see Bandi et al., 2001b). Forty-four percent of the males are positive, only to the more sensitive *gyrB* assay (16S rRNA protocol estimated detection limit was 20,000 copies, *gyrB* limit was 400 copies; Lo et al., 2006a), which probably indicates that the bacteria inherited from the mother slowly diminish in number during the male development, possibly because they do not find the right habitat for multiplication. Sequence fragments of the 16S rRNA and gyrB genes were obtained from samples from each of the 11 populations, and showed low genetic variability, as only one polymorphic site was found in the 16S rRNA fragment (380bp) and only two synonymous substitutions were detected in the *gyrB* fragment (519bp). The low variability reported could indicate that the invasion of the *I. ricinus* population by '*Candidatus* M. mitochondrii' is relatively recent, possibly due to a fast sweep. The two gene fragments analysed are considered fairly conserved, so these data need to be confirmed with the sequencing of faster evolving genes. Laboratory maintained *I. ricinus* were also screened for the presence of '*Candidatus* M. mitochondrii' (Lo et al., 2006a). Tick lines maintained in the lab for a few generations exhibited 100% prevalence of the symbiont, whereas two older lines, which had been maintained in the lab for 5 and 7 generations, showed a remarkably lower prevalence values (18 and 43%, respectively).

RELATIONSHIP WITH THE HOST

The evidence obtained so far does not provide a clear classification of the relationship between *I. ricinus* and its mitochondrial symbiont. Here, we discuss the different possible symbiotic relationships, indicating the evidence for and against each scenario (Lo et al., 2006b).

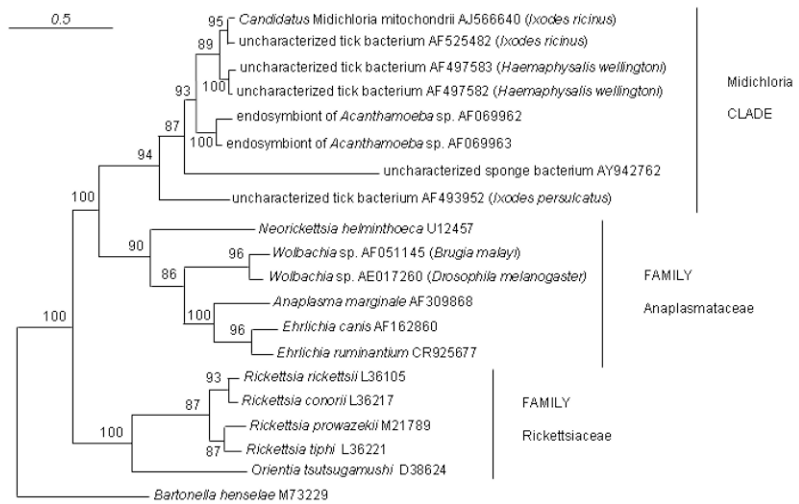

Figure 2 Phylogenetic tree based of near full length 16S rDNA sequences (>1200 bp) of '*Candidatus* Midichloria mitochondrii' and other members of the order Rickettsiales. The neighbour-joining analysis was performed with the Kimura correction. *Bartonella henselae* was used as outgroup.

Parasitism

The EM study of the host mitochondria seems to indicate consumption of the mitochondrial matrix by the bacterium, which could utilize this material for its multiplication. On the other hand the 100% prevalence of '*Candidatus* M. mitochondrii' in females is not easily reconciled with the hypothesis of parasitic behaviour. Indeed, if the symbiont reduces the host's fitness we would expect some individuals to develop some form of resistance.

Reproductive parasitism

The presence in the ovary, the 100% prevalence in females, and the transovarial transmission are clues that indicate a form of reproductive parasitism, which could be parthenogenesis, male-killing, feminization, or cytoplasmic incompatibility (CI) (for a review see O'Neill et al., 1998). The first three types of reproductive parasitism result in a distortion of the sex ratio in the host towards a higher percentage of females. This distortion has never been reported in *I. ricinus*, neither in nature nor in lab populations. CI is an incompatibility that causes the death of the progeny in crosses between infected males and uninfected females, which, due to the fact that the symbiont causing it is maternally transmitted, may result in rapid spread of the symbionts in the host population. For CI to be effective, it is expected that the bacterium will reside in the reproductive tissues of both females and males. The low prevalence in males and the low number of bacteria in infected males do not support the hypothesis of CI activity by '*Candidatus* M. mitochondrii'. However, it could be a former CI inducer. When a CI causing bacterium reaches fixation in a population, a novel phenotype is advantageous, one that rescues the CI effect, but that does not cause it (Hurst & McVean, 1996).

Commensalism

With commensalism the symbiont does not harm nor help the host. The loss of '*Candidatus* M. mitochondrii' in laboratory populations of *I. ricinus* indicates that the ticks can live without the symbionts, but it is not clear evidence of commensalism. The 100% prevalence in females suggests the relationship is not of this form: if there is no benefit to the host, it is expected that resistant individuals will evolve that do not bear the cost of harbouring the symbiont.

Mutualism

'*Candidatus* M. mitochondrii' could provide some benefit to *I. ricinus*. All female ticks do have this symbiont in nature and they develop normally. The bacteria are indeed lost after a few generations in the lab. Thus, the relationship cannot be an obligate mutualism, because the ticks develop anyway. A question to be addressed is why the laboratory-raised colonies lose the bacteria: is it because of possible antibiotic treatments on the tick hosts, or is it because the symbionts are not as important in the controlled conditions of the laboratory? The selective advantage of the symbiosis in nature could be lost in the laboratory, where lower or different selective pressures are present. Another possibility is that, since the life cycle is strongly accelerated in laboratory populations, i.e., from the 1-3 years it takes in the wild to as low as 3 months in the laboratory (Soneshine, 1991), the bacteria cannot keep up with this faster development of the host.

What is the reason behind '*Candidatus* M. mitochondrii' entering the host mitochondria? It could perhaps enhance the energetic metabolism of the females, e.g., during the important steps of engorgement and egg deposition, when the host metabolism is at its peak. Otherwise, it could act on the membranes of mitochondria and uncouple the mitochondrial respiration, in the same way as thermogenin (UCP1) does in the brown adipose tissue present in newborn or hibernating mammals.

This protein causes an influx of H+ into the mitochondrial matrix. Bypassing the ATP synthetase channel and dissipating as heat, the energy from the proton motive force results in thermogenesis instead of ATP production (Leninger & Cox, 2001). If '*Candidatus* M. mitochondrii' could provide an analogous system it would grant the host a higher temperature during the cold European winters the females must often endure between engorgement and egg-deposition. Such a hypothesis would explain the loss of the symbionts in laboratory populations, as this activity would be useless in a habitat where temperature is constant. Mitochondria are also important in the process of apoptosis, as they are involved in the 'mitochondrial pathway' to programmed cell death (Lawen, 2003). In response to cell damage the mitochondria release two proteins, cytochrome c and Apaf-1, which start a cascade of caspases, enzymes with proteolytic activity that ultimately leads to the phagocytosis of the cell. '*Candidatus* M. mitochondrii' must somehow inhibit this pathway, that would otherwise lead to cell death whenever the bacteria cause the degradation of the mitochondria.

'*Candidatus* M. mitochondrii' is a fascinating bacterium, with a peculiar lifestyle. It is a symbiont of an arthropod of major medical and veterinary interest. Advances in the knowledge of this symbiosis could be important in the control of ticks, but could also give novel information on symbiosis in general and on the biology of mitochondria.

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The tick *Ixodes persulcatus* (Acari: Ixodidae) is a vector of various disease agents in the Cisural region, Russia

Edward I. Korenberg, Valentina V. Nefedova, Yurii V. Kovalevskii & Nataliya B. Gorelova
Gamaleya Research Institute for Epidemiology and Microbiology, Russian Academy of Medical Sciences, 18 Gamaleya Street, Moscow, 123098 Russia. E-mail: ekorenberg@tochka.ru

Four hundred adult unfed taiga ticks (*Ixodes persulcatus*) collected in the Cisural region, Russia, were analyzed by means of PCR with primers specific for the DNA sequences of disease agents pathogenic for humans: *Borrelia afzelii*, *B. garinii*, *Ehrlichia muris*, *Anaplasma pagocytophilum*, *Babesia microti*, and *Rickettsia* spp. Only the DNA of *B. microti* was never found, DNA of at least one of the other agents was detected in 326 ticks (81.5%), 166 ticks (41.5%) contained DNA of two or more agents (17 variants of mixed infections were revealed), and five ticks (1.3%) proved to contain DNA of four or five agents. These results confirm that, as a rule, antagonistic relationships between disease agents do not take place in the tick body.

Key words: Taiga tick, vector, *Borrelia*, *Ehrlichia*, *Anaplasma*, *Babesia*

The taiga tick, *Ixodes persulcatus* Schulze, whose range is mostly confined to the territory of Russia, is one of the main vectors of TBE virus and *Borrelia burgdorferi* sensu lato in natural foci and plays a leading role in the transmission of these disease agents to humans (Korenberg, 1994; Korenberg & Kovalevskii, 1999; Korenberg et al., 2002). The prevalence of these ticks and the risk of human tick-borne encephalitis (TBE) and *Borrelia* infections are very high in the Cisural region (Korenberg et al., 2001a, 2002b), which places it among Russia's most vulnerable regions with respect to morbidity caused by these agents (Frizen et al., 2004). According to recent data, *Ehrlichia muris*, *Anaplasma phagocytophilum*, and *Babesia microti* also circulate in the natural foci of this region (Ravyn et al., 1999; Telford et al., 2002). Cases of human ehrlichiosis and anaplasmosis acquired after a tick bite have been recorded, and clinical manifestations of these diseases have been described (Grigoryan et al., 2000; Vorobyeva et al., 2002; Afanasieva et al., 2006). Moreover, different variants of mixed infections transmitted by ticks are relatively frequent (Korenberg, 2003).

The purpose of this study was to analyze *I. persulcatus* ticks for the prevalence of mixed infection by several disease agents, including *Ehrlichia*, *Anaplasma*, and *Babesia*, which were first detected in Russian natural foci only in recent years.

MATERIALS AND METHODS

Ticks

Studies were performed with 400 adult unfed *I. persulcatus* ticks collected by flagging in mountain taiga forests of the Western Urals (Chusovskoi District, Perm Region, Russia) in May and June 2004, at the seasonal peak of tick abundance. This region was described in detail previously (Korenberg et al., 2001, 2002a).

Sample processing for PCR analysis

Ticks were washed in 70% ethyl alcohol and distilled water and dissected under a binocular microscope. Their internal organs were removed and placed in 1.5-ml Eppendorf tubes with 70% ethyl alcohol, which were stored before analysis at 4 °C. Genomic DNA was isolated from tick organs using guanidine isothiocyanate and phenol-chloroform extraction as described (Gushchin & Khalilov, 1996).

DNA amplification and analysis

PCR was performed out in a Tertsik four-channel thermocycler (DNK Tekhnologiya, Russia). The reaction mixture (30 µl) contained 67 mM Tris-HCl (pH 8.6), 16.6 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.001% Triton X-100, 0.12 mg/ml BSA, the four dNTP (0.2 mM each), 20 pmol of each primer, 1.5 U of *Taq* polymerase, and 5 µl of the initial total DNA. Nested PCR in all cases was performed with 1 µl of the primary PCR product used as a template. To control the isolated genomic DNA, it was amplified with primers cytb1 and cytb2 for the mitochondrial cytochrome *b* gene of ixodid ticks. PCR screening of the tick material for the presence of *Borrelia* DNA was performed by amplifying it with group-specific primers LD1 and LD2. All positive amplicons were then analyzed in nested PCR with *B. afzelii*-specific primers (BAfz1 and BAfz2) and *B. garinii*-specific primers (BGar1 and BGar2) flanking a fragment of the 16S rRNA gene. The DNA of *Eh. muris* was amplified with primers HE3 and MuHE1 flanking a fragment of the 16S rRNA gene. The DNA of *A. phagocytophilum* was first amplified in nested PCR with primers ge3a and ge10 and then with primers ge9 and ge2 specific to a fragment of the 16S rRNA gene. To amplify the DNA of *B. microti*, the pairs of primers flanking a fragment of the 16S rRNA gene were used, Bab1–Bab4 in the first round of PCR and Bab2–Bab3 in nested PCR. Rickettsia-specific pairs of primers recognizing a fragment of the 16S rRNA gene (fD1 and Rc16S.452n) and

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Table 1 General results of PCR analysis of *Ixodes persulcatus* ticks.

	% ticks containing specific DNA sequences (n)*		
DNA source	Females	Males	Total
Cyt gene (tick)	100 (299)	100 (101)	100 (400)
Borrelia afzelii	28.8±5.2 (86)	33.7±9.4 (34)	30.0±4.6 (120)
Borrelia garinii	33.4±5.4 (100)	39.6±9.7 (40)	35.0±4.7 (140)
Ehrlichia muris	17.1±4.3 (51)	24.7±8.6 (25)	19.0±3.9 (76)
Anaplasma phagocytophilum	2.0±1.6 (6)	2.0±2.8 (2)	2.0±1.4 (8)
Babesia microti	0	0	0
Rickettsia spp.	54.2±5.8 (162)	38.6±9.7 (39)	50.3±5.0 (201)
Total no. ticks	299	101	400

*Figures in parentheses indicate sample sizes.

the gltA gene (RpCS.877p and RpCS.1258n) were used for amplification and sequencing of rickettsial DNA.

The results of PCR with DNA of *B. afzelii* Ip-21, *B. garinii* Ir-2200, and *Eh. muris*, *A. phagocytophilum*, *B. microti*, and *R. sibirica* were used as positive controls. PCR products were resolved by horizontal electrophoresis in a 1-2% agarose gel. Electrophoresis was performed in Tris-borate buffer with ethidium bromide at 165 V. Gels were analyzed using a DNA Analyzer system with the Gel Imager and Gel Analysis v. 1.0 programs (Russia).

RESULTS

The DNA of at least one disease agent recognized by the primers used in this study was detected in 326 out of 400 ticks (81.5%). Table 1 shows the frequencies of detection of the various agents by PCR with these primers. Approximately one-third of all ticks contained DNA of *B. garinii* or *B. afzelii*, with *B. garinii* being slightly more frequent. Nucleotide sequences characteristic of *Eh. muris* were detected in approximately one-fifth of ticks, but only a few percent of these vectors contained the specific sequences of *A. phagocytophilum* DNA. None of the ticks carried the agents of babesiosis, but half of them proved to contain rickettsial DNA. Preliminary results of selective sequencing and their comparison with GenBank data indicate that, at least in some cases, the amplicons of *Rickettsia tarasevichiae* DNA sequences were obtained in our experiments. DNA of all disease agents (except for rickettsial DNA) was detected more frequently in male than in female *I. persulcatus* ticks.

DNA of two or more disease agents was detected in 166 out of 400 ticks (41.5%), with such a mixed infection being represented in 17 variants (Table 2). *Borrelia–Rickettsia* combinations, which often included both *Borrelia* species circulating in the study region, occurred more frequently than other variants. On the whole, positive results of PCR with primers recognizing DNA of three disease agents were obtained for 41 ticks (10.3% of all ticks studied and 24.7% of mixed-infected ticks). Five ticks (1.3% of the total number) contained DNA of four or five disease agents. Of special interest is the fact that four ticks (1.0% of the total number) contained DNA of *Eh. muris* and *A. phagocytophilum*, in combination with DNA of *Borrelia* and *Rickettsia* (two cases) or with rickettsial DNA alone (two cases).

DISCUSSION

The above data on the prevalence of *B. afzelii*, *B. garinii*, or both these agents in adult unfed *I. persulcatus* ticks virtually coincides with those previously obtained in the study region by detecting the DNA of *B. burgdorferi* sensu lato with group-specific primers (Nefedova et al., 2001) and by inoculating the BSK medium with the material from the tick gut (Korenberg et al., 2002). The detection of *Eh. muris* and *A. phagocytophilum* DNA in *I. persulcatus* ticks and the cases of ehrlichiosis and anaplasmosis among patients bitten by them (Vorobyeva et al., 2002; Afanasieva et al., 2006) provide evidence for their possible role as vectors of these disease agents in the Cisural region of Russia. However, we failed to detect the DNA of *Babesia* in adult ticks, although *B. microti* was previously isolated in the study region from voles of the genus *Chletrionomys*, the main hosts of *I. persulcatus* nymphs and larvae (Telford et al., 2002). This may account for the fact that no cases of babesiosis have as yet been reliably identified either in the Cisural region, where a considerable number of patients with diseases acquired after a taiga tick bite have been examined by serological and PCR methods, or within the entire *I. persulcatus* range. The point is that, in most cases, people are attacked by adult *I. persulcatus* ticks, which appear to be ineffective as *B. microti* vectors. However, this assumption needs verification.

In recent years, the problem of diagnosis, treatment, and prevention of mixed infections transmitted by ixodid ticks has acquired special significance (Korenberg, 2001, 2003; Belongia, 2002; Alekseyev et al., 2004a). In this context, attention should be given to the above data on 17 combinations in which DNA of different disease agents was detected in individual taiga ticks. In fact, there must be more variants of mixed infection in *I. persulcatus* ticks, because they are the main vectors of TBE virus (Korenberg et al., 2001), whose indication was not performed in this study. Our previous data, obtained by means of TBE virus isolation and dark-field microscopy of *Borrelia*, show that about 25% of adult unfed taiga ticks in the study region are simultaneously infected by both these agents (Korenberg et al., 1999). Therefore, the population of these ticks actually includes a considerably larger proportion of individuals that may carry two to five or even six pathogenic microorganisms simultaneously. For example, one tick from our sample analyzed by the PCR method was found to contain DNAs of *B. garinii*, *Eh. muris*, *A. phagocytophilum*, and *Rickettsia* spp., but Dr. V. Popov (University of Texas, Medical Branch at Galveston, TX, USA), using electron-microscopic methods, additionally detected in it a typical flavivirus (Popov et al., 2007). Even without taking into account infection by TBE virus, the results of this study show that at least 3% of the total number of mixed-infected ticks may contain DNA of four or five disease agents. This fact contradicts the opinion that one tick cannot be infected by more than three agents of human diseases and that *Eh. muris* and *A. phagocytophilum* cannot coexist in the tick body (Alekseev et al., 2004a,b). New data confirm that antagonistic interaction between various disease agents need not take place in the tick body, because these agents are mainly localized in certain organs, tissues, or even cell structures that may be

Table 2 Combinations of DNA of two and more disease agents detected in the same *Ixodes persulcatus* tick and their occurrence frequencies.

Variant of mixed infection	No. ticks	% mixed-infected ticks	% total ticks
B. afzelii + B. garinii	21	12.6	5.3
B. afzelii + B. garinii + Eh. muris	6	3.6	1.6
B. afzelii + B. garinii + Eh. muris + Rickettsia spp.	2	1.2	0.6
B. afzelii + B. garinii + Eh. muris + A. pagocytophilum + Rickettsia spp.	1	0.6	0.2
B. afzelii + B. garinii + Rickettsia spp.	21	12.6	5.2
B. afzelii + B. garinii + A. pagocytophilum + Rickettsia spp.	1	0.6	0.2
B. afzelii + Eh. muris	2	1.2	0.5
B. afzelii + Eh. muris + Rickettsia spp.	3	1.8	0.8
B. afzelii + Rickettsia spp.	36	21.8	9.0
B. garinii + Eh. muris	7	4.2	1.8
B. garinii + Eh. muris + Rickettsia spp.	8	4.8	2.0
B. garinii + Eh. muris + A. pagocytophilum + Rickettsia spp.	1	0.6	0.2
B. garinii + A. pagocytophilum + Rickettsia spp.	1	0.6	0.2
B. garinii + Rickettsia spp.	33	19.9	8.2
Eh. muris + A. pagocytophilum + Rickettsia spp.	2	1.2	0.5
Eh. muris + Rickettsia spp.	20	12.1	5.0
A. pagocytophilum + Rickettsia spp.	1	0.6	0.2
Total mixed-infected ticks	166	100	41.5

regarded as their specific ecological niches (Balashov, 1987, 1998; Friedhof, 1990; Korenberg, 1999, 2001, 2003).

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Seasonality of *Megninia ginglymura*: a one-year study in a hen farm in Yucatan, Mexico

M.T. Quintero, M. Itza*, G. Juarez & A. Eleno

Laboratorio de Entomología, Departamento de Parasitología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, CP 04510, México, DF. E-mail: octq1010@yahoo.com.mx; and *Private farmer in Merida, Yucatan, Mexico

Mites of the genus *Megninia* are known to occur in Mexico since 1979. In 1987 three *Megninia* species were found in different states. In Yucatan, the presence of *M. ginglymura* was reported and recently hens were observed to suffer from feather loss and skin lesions in several hen farms. To determine the development of these mites during the year, samples were collected each month, from January until December 2005, in hen farms near Merida, Yucatan. Random samples were taken from five hens, taking feathers from various anatomical regions, such as head, dorsum, legs, wings, vent, anterior face of thigh, and pectoral zone. The samples were transported to the laboratory, where mites were mounted on slides employing Hoyer's liquid. All developmental phases – larvae, protonymph, tritonymph, and adult (female and male) – were identified and counted. A total of 2,461 mites were observed: 58.7% adult (41.4% females, 17.3% males), 12.3% tritonymh, 12.0% protonymph, and 16.7% larva. Two peaks of population development of *M. ginglymura* occurred, one in July and the other in November. These peaks suggest seasonality, with two biological cycles per year. *Megninia* is present all year long, although at a lower population density in March and October. Therefore, we propose to combat *M. ginglymura* in June and early November, i.e., 1 month before they become abundant.

Key words: Poultry pest, population dynamics, feather mites, Analgidae

Feather mites of the genus *Megninia* Berlese, belong to the Analgidae, subfamily Megniniinae (Gaud & Atyeo, 1996). They are found in various genera and species of poultry, in particular *Megninia ginglymura* (Megnin) that causes economic damage in commercial poultry farms. Presence of *M. ginglymura* has been reported in various countries in South-America. In 1939, Reis found it in the state Sao Paulo, Brazil, and observed that the adult and young phases live in association with the feathers. It was also found in Cuba by Cerny (1970, 1973) and more recently its presence in Cuba was reported by Sczypel et al. (2003). Tucci et al. (2005) reported *M. ginglymura* in 1997 in Sao Paulo, Brazil, and in 2004, they detected also a second *Megninia* species, *M. cubitalis*. In India, D'Souza et al. (2001) described problems caused by *M. ginglymura*. Several authors mention that these mites cause up to 20% lower egg production or even death in poultry (Monteiro 2005). Tucci et al. (2005) reported that saliva excretion of these mites cause lesions and an allergic reaction with pruritus and serous scabs, which in turn cause bird secretions. According to Monteiro (2005) this also causes a secondary infection.

In Mexico, mites in the genus *Megninia* are known to be present since 1979 (Quintero et al. 1979). Gaud et al. (1985) published a study on *Megninia* feather mites in chicken (*Gallus gallus domesticus*) to inform poultry farmers about the various species, since there was confusion in identifying *M. cubitalis* and *M. ginglymura*. Quintero & Acevedo (1987) reported the presence of three *Megninia* species in domestic fowl in Mexico, where *M. cubitalis* was found in layer hens in Nuevo Leon, *Megninia ortari* in Veracruz, and *M. ginglymura* in Merida, Yucatan, and the port of Veracruz. Quintero (1991) found this last species on turkeys (*Meleagridis gallipavo*) from Campeche. It has been supposed that this mite was originally a parasite of turkey and then passed on to chicken.

In recent years, severe loss of feathers as well as skin lesions have been observed in layer hens in Merida, Yucatan (Fig. 1). Considering the earlier presence of *M. ginglymura* in Yucatan and the lack of knowledge of genera and species of mites that harm various types of commercial poultry farms in this geographical region, a study was planned in a layer hen farm. Over the period of 1 year mites were systematically collected and identified, and their development phase was noted. This study was done to help choose an appropriate method of mite control.

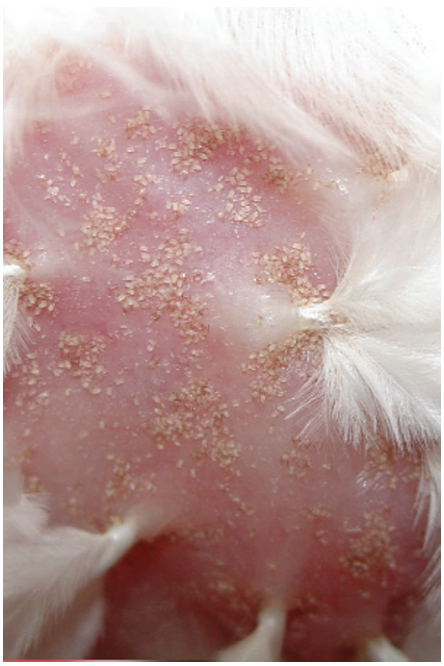

Figure 1 The skin of a hen, heavily infested by feather mites.

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MATERIALS AND METHODS

From January until December 2005, random samples were taken monthly from five hens, taking feathers from various anatomical regions: head, dorsum, legs, wings, vent, anterior face of thigh, and pectoral zone. The samples were transported to the Veterinary School's Entomology Laboratory from the Parasitology Department of the National Autonomous University of Mexico. The mites were mounted on slides in Hoyer's liquid. They were counted and the development phases (larva, protonymph, tritonymph, adult female/male) were determined. The mites collected were identified as *M. ginglymura* using the key of Gaud et al. (1985).

RESULTS

Of the total of 2,461 mites observed, 58.7% were adult (41.4% females, 17.3% males), 12.3% tritonymh, 12.0% protonymph, and 16.7% larva.Two population peaks of *M. ginglymura* were manifested, one in July, the other in November. These peaks suggest seasonality with two biological cycles completed during a single year, but *Megninia* is present all year long with low populations during March and October (see Fig. 2).

Loss of feathers of various anatomical regions of poultry was observed in commercial farms and it was determined that the damage was caused by *M. ginglymura.* In skin regions with a high concentration of mites feathers had disappeared altogether.

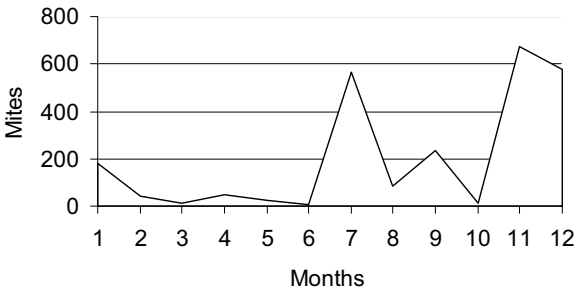

Figure 2 Annual dynamics of *Megninia ginglymura*.

DISCUSSION

Whereas *M. ginglymura* is known to be present for several decades in Yucatan, Mexico, and Sao Paulo, Brazil (Quintero et al., 1987; Tucci et al., 2005), massive mite infestations and lesions are found on layer hens in recent years. Despite these findings and their reports, the veterinarians in charge of the farms in Yucatan continue to call this a problem of 'corucos', meaning that it is a problem of *Ornithonyssus* sp., without substantiating their claim by taxonomic determination of the mites. This was the reason for carrying out the study reported here. The results suggest the mite's biseasonality, with peaks in July and November. Which factors determine this seasonality, is as yet unknown and this requires more research. However, if this seasonal pattern is fixed, control measures may be more effective when applied ca. 1 month before these peaks emerge.

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Acaricides

Acaricidal activity of some essential oils and their monoterpenoidal constituents against the house dust mite, *Dermatophagoides pteronyssinus* (Acari: Pyroglyphidae)

S.R. El-Zemity[1], H.A. Rezk[2], Saher Farok[2] & A.A. Zaitoon[2]

[1]Pesticide Chemistry Department, and [2]Economic Entomology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. E-mail: hussienrezk@yahoo.com

The acaricidal activity of 14 essential oils and 14 of their major monoterpenoids were tested against the house dust mite *Dermatophagoides pteronyssinus*. Mites were exposed for 24 and 48 h to five concentrations of each constituent, under laboratory conditions. In general, the acaricidal effect of both essential oils and monoterpenoids against the dust mites was time-dependent: LC_{50} values decreased with increasing exposure time. Essential oils from clove, matrecary, chenopodium, rosemary, eucalyptus, and caraway oils had high activity. The monoterpenoids cinnamaldehyde and chlorothymol were most effective, followed by citronellol. This study suggests the use of several essential oils and their major constituents as eco-friendly biodegradable agents for the control of the house dust mite, *D. pteronyssinus*.

Key words: Natural acaricides, essential oils, monoterpenoids, house dust mite, *Dermatophagoides pteronyssinus*

Among several species of the family Pyroglyphidae, *Dermatophagoides pteronyssinus* and *D. farinae* have been found to be predominant in household dust, accounting for about 80-90% of the total mite populations. They are also found in homes in various areas of Egypt (Rezk, 2004; Rezk et al., 1996). The two house dust mites are important sources of allergens that cause respiratory allergies including asthma worldwide inside homes in humid areas (Hallas, 1991; Arlian et al., 1992; Colloff et al., 1992; Solarz, 2001a,b). They are also identified as etiologic agent of sensitization and asthma-triggering in children (Lau-Schadendorf et al. 1989; Arshad & Hide 1992).

Control of house dust mites is usually done by three approaches: (1) physically, by applying high cleaning standards, thus removing the mites (Adilah et al., 1997; Nishioka et al., 1998), (2) biologically, by reducing humidity levels, thus making life miserable for the mites (Bischoff et al., 1998), and (3) chemically, by effective acaricides, thus killing the mites (Mitchell et al., 1985; Green et al., 1989). The latter option must only be employed after careful consideration.

Although good control has been obtained by currently used synthetic acaricides (benzylbenzoate, tannic acid), the risk to human health is a problem. This problem has led to research efforts to develop safer and efficient alternatives. Thus, the use of plant-derived acaricides has drawn attention and has been considered as a promising alternative to chemical acaricides (Miyazaki et al., 1989; McDonald & Tovey, 1993; Tovey & McDonald, 1997; Kim, 2001; Kim et al., 2003; Rezk & Gadelhak, 2004; Rembold, 2005). The present work explores the miticidal activity of some commonly available essential oils and their major constituents against *D. pteronyssinus*.

MATERIAL AND METHODS

Stock culture of house dust mite

The house dust mite, *D. pteronyssinus*, was isolated from mattress dust and reared on a finely-ground mixture of dust, dried yeast, and dried milk (1:1:0.5) in complete darkness (Saint Georges, 1987). Stock jars were kept in an incubator at an average temperature of 25±2 °C and 80±5% r.h. After 5 months considerable numbers of various stages were available for experimentation.

Plant essential oils

Fourteen essential oils were used: caraway, chenopodium, cinnamon, clove, eucalyptus, fennel, garlic, geranium, lemon grass, matrecary, peppermint, rose, rosemary and thyme. The oils were supplied by the Faculty of Pharmacy, University of Alexandria.

Essential oil constituents (Table 1) were analyzed by gas chromatography (Hewlett-Packard 58590 II Plus) coupled with a mass spectrometer detector (HP-5989 B), equipped with a HP-5 column (30 m × 0.25 mm × 0.025 μm). Temperature program: initial temperature 70 °C; hold 2 min; temp. rate 4 °C/min, final temperature 250 °C; hold 30 min; constant column flow rate 0.8 ml He/min. Injection temperature 250 °C; injection volume 1 μl/min. The mass spectrometer was accomplished with Wiley library ID 275 and settings were: electron impact ionization mode with 70 eV electron energy. Scan mass range m/z 50-45, detector temperature 250 °C.

Monoterpenoids

Fourteen monoterpenoidal constituents of the essential oils were tested (supplied by Sigma-Aldrich): eugenol, carvone (S), carvone (R), borneol, carveol, benzyl alcohol, thymol, camphor, menthol, chlorothymol, cinnamaldehyde, carvacrol, cineol, and citronellol.

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Table 1 Relative abundance of major constituents of the tested essential oils.

Essential oils	Major constituents	Abundance (%)
Caraway	l-Carvone	58.0
	Limonene	38.4
Chenopodium	p-Cymene	33.3
	Pinene-2-ol	11.7
	Ascaridole	10.0
Cinnamon	Cinnamaldehyde der.	36.1
	Cinnamic aldehyde	12.3
Clove	Eugenol	62.3
	ß-Caryophyllene	19.2
Eucalyptus	1,8-Cineole	45.1
	α-Pinene	14.9
	α-Terpineol	11.6
Fennel	Anthol	47.6
	Pinene	11.7
	1,8-Cineole	10.3
Garlic	Pentdecane	12.1
	Hexadecane	11.6
Geranium	Citronellol	31.0
	Citronellol formate	14.0
Lemon grass	Lemonene	91.1
	Terpinene	2.2
Matrecary	Camphor	51.4
	p-Cymene	20.1
	Thymol	9.5
Peppermint	Menthol	46.1
	p-Menthone	18.3
Rose	Citronellol	34.4
	Geranoil	20.4
Rosemary	1,8-Cineol	28.6
	Camphor	20.2
Thyme	p-Cymene	31.5
	Thymol	18.5

Experimental treatments

Following preliminary experiments, five concentrations for each essential oil or monoterpenoid were applied: 25, 50, 100, 250, and 500 ppm, plus a control. Each concentration was mixed with 0.5 g of house dust and placed on a microcell (3 × 1.5 cm). Ten mites were introduced on top of the microcell using a brush. Five replicates were maintained per tested concentration. All experiments were conducted under laboratory conditions at 25±2 °C and 80±5% r.h. Mortality counts were done after 24 and 48 h of exposure. The LC_{50} values and their 95% confidence limits were calculated according to Finney (1971).

RESULTS

The LC_{50}'s based on mortality data after 24 h exposure indicated that clove oil was the most effective (LC_{50} = 29.78) followed by matrecary (104.45), chenopodium (117.53), fennel (156.42) and caraway (158.05) (Table 2). According to the GC-MS analysis (Table 1), clove oil contains 62% of eugenol and 19% caryophyllene. Matrecary oil contains 51% camphor, 20% p-cymene and 9.5% thymol. After 48 h exposure to the tested oils, clove oil, matrecary, and chenopodium again ranked 1-3, followed by rosemary and eucalyptus (Table 2). No mortality was recorded in the controls.

Because of the strong acaricidal activity of clove oil and some others, the identification of the essential oil components by GC-MS was pursued, in an attempt to underpin the oil activity. Responses of *D. pteronyssinus* to the 14 monoterpenoids varied – most toxic after 24 h exposure was cinnamaldehyde (LC_{50} = 64.38), followed by chlororthymol (90.45), citronellol (159.48), and menthol (253.68) (Table 3). After 48 h, the LC_{50}'s for cinnamaldehyde and chlorothymol were alike.

Table 2 Acaricidal activity of essential oils against the house dust mite *Dermatophagoides pteronyssinus*.

Essential oils	Time (h)	LC_{50} (ppm)	95% Confidence limits
Caraway	24	158.05	179.66-192.89
	48	92.01	40.82-207.72
Chenopodium	24	117.53	97.23-142.19
	48	76.02	53.71-160.10
Cinnamon	24	390.51	260.52-586.83
	48	189.55	153.91-233.74
Clove	24	29.78	19.61-44.94
	48	21.17	15.97-27.97
Eucalyptus	24	275.42	183.93-414.46
	48	89.84	49.17-164.29
Fennel	24	156.42	122.40-185.09
	48	94.46	81.42-109.61
Garlic	24	736.30	94.01-6,054.90
	48	571.31	271.73-1,215.82
Geranium	24	181.53	146.07-225.93
	48	118.56	62.37-226.61
Lemon grass	24	300.66	213.32-425.03
	48	103.63	71.16-391.43
Matrecary	24	104.45	87.16-125.24
	48	71.64	14.52-349.55
Peppermint	24	274.27	175.57-431.16
	48	125.73	59.74-267.41
Rose	24	669.07	320.64-1,405.82
	48	409.28	160.34-1,064.27
Rosemary	24	375.71	238.48-594.9
	48	89.47	12.59-635.43
Thyme	24	488.65	259.86-919.04
	48	269.59	216.46-335.96

DISCUSSION

This study demonstrates the effectiveness of some essential oils and their monoterpenoidal constituents against *D. pteronyssinus*. Clove oil proved the most effective against the dust mites. Therefore, it would be interesting to also investigate its major constituent terpenoids, eugenol and caryophyllene. The most effective pure terpenoids tested, cinnamaldehyde and chlorothymol, certainly merit further study as potential dust mite control agents. Perhaps the acaricidal activity of these and other terpenoids can be improved by mixing several components. That mixtures may have different effects than separate compounds can, for example, be seen in the case of one of the most potent oils against dust mites, matrecary oil. Its major component was camphore (51%), and a less major component was thymol (9.5%), but when tested separately, thymol's LC_{50} appeared 3-4× lower than that of camphor. Clearly the relatively minor component here seems the more promising acaricide.

The public perception and acceptance of using natural essential oils may be more positive, because these chemicals also occur in washing products. The toxic effect of these oils may be due to their fumigant and/or contact action. The vapor of some essential oils has been shown to kill pyroglyphid mites (Watanabe et al., 1989). Further screening of essential oils in solution is required. Criteria for choosing the most suitable oil should include rate and persistence of acaricidal activity, in relation to temperature, volatility, human acceptability, and adverse effects on fabrics. Essential oils

Table 3 Acaricidal activity of monoterpenoids against the house dust mite *Dermatophagoides pteronyssinus.*

Essential oils	Time (h)	LC_{50} (ppm)	95% Confidence limits
Carvacrol	24	318.33	157.69-652.63
	48	115.48	86.49-154.5
Camphor	24	1,026.58	353.78- -
	48	420.66	187.83-953.23
Citronellol	24	159.48	123.13-170.07
	48	64.89	72.81-207.01
Carvone (R)	24	669.07	320.64-1,405.82
	48	253.13	118.33-547.46
Carvone (S)	24	643.89	72.81-6,170.07
	48	159.48	123.13- -
Carveol	24	309.36	272.15- -
	48	85.37	72.01-101.15
Cineol	24	808.8	328.02-2,023.69
	48	488.05	259.86-919.04
cinnamaldehyde	24	64.38	56.48-73.36
	48	48.05	2.98-738.35
Chlorothymol	24	90.45	76.63-106.79
	48	45.81	37.21-56.32
Borneol	24	638.65	272.14-1,506.23
	48	393	261.25-595.48
Benzyl alcohol	24	669.07	320.64-1,405.82
	48	181.79	114.12-270.20
Geraniol	24	431.96	58.03-3,329.67
	48	127.66	67.71-241.62
Menthol	24	253.68	73.06-907.36
	48	136.89	24.69-779.96
Thymol	24	345.93	127.45-957.04
	48	125.24	47.05-335.15

may also be particularly suitable for inclusion in carpet washing solutions to aid in mite control.

The use of plant essential oils as a source for the control of adult house dust mites is simple and inexpensive, and it seems safe and effective, because many of them are thought to affect pests only, with few if any harmful effects on non-target organisms and the environment (Susan & Ward, 1987; Kim et al., 2003; Kwon & Ahn, 2002).

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A gel formulation of formic acid for control of *Varroa destructor*

Fidel Avila-Ramos, Gabriel Otero-Colina*, Hussein Sánchez-Arroyo, María Teresa Santillán-Galicia & Alberto Tecante[1]

Colegio de Postgraduados Campus Montecillo; Km 36.5 Carr. Mexico - Texcoco, Montecillo, 56230 Edo. de México. *E-mail: gotero@colpos.mx; and [1]Departamento de Alimentos y Biotecnología, Facultad de Química 'E', UNAM, Cd. Universitaria, D.F., 04510, México

Two formulations of a starch-based gel containing 85% formic acid were evaluated to control *Varroa destructor* in bee hives. Gels were poured in polyethylene-wrapped plates provided with release areas from 10 to 300 cm^2 to regulate evaporation. In a laboratory test, at 32 °C and 40-60% r.h., evaporation rate of the plates, expressed as weight loss, was determined over 45 days. A selected formulation and release areas of 16, 32, and 64 cm^2, allowing evaporation of about 10 g per day, were used in field tests. In June and July 2004, plates were placed inside honey bee (*Apis mellifera*) hives in Tlalmanalco (State of Mexico) and Cordoba (State of Veracruz, Mexico). Weight loss of the plates was measured together with their effectiveness in *V. destructor* control and their possible effects on adult and brood population, presence and activity of the queen bee, and quantity of food supplies. Mean daily evaporation of formic acid ranged from 8 to 12 g in both locations. None of the treatments had negative effects on bee population and food supplies, but three queens were lost, apparently due to excessive handling. Mean effectiveness in reducing the *Varroa* population was about 73%. Treatment with formic acid in a gel formulation is considered to be economically sound and practically feasible.

Key words: Formic acid, methanoic acid, *Apis mellifera*, bees, honey

Formic acid has been extensively used for control of *Varroa destructor* (here simply called varroa), the most important pest of honey bees (*Apis mellifera*) around the world (Clarke, 1991). It inhibits varroa respiration and the mites are unable to degrade it. In contrast, in honey bees the effect on respiration is absent (Bolli et al., 1993). Formic acid acts in vapor form, in bee hives it is more effective when environmental temperatures are 18-25 °C (Imdorf et al., 1990). Under those conditions the vapors penetrate the capped cells and kill the mites inside them (Calis et al., 1998).

Several devices have been designed for short- and long-term release of formic acid vapors. Among the first group, the Illertisser plate is constructed in cardboard saturated with 22 g of 65% formic acid. It releases all formic acid in 4-8 h (Imdorf & Gerig, 1988). In many countries, beekeepers soak cardboard strips with 20 g of 60% formic acid and introduce them into bee hives to control varroa (Van et al., 1998).

The second group of devices, denominated closed, release-controlled or long-term releasers, has the advantage of reducing risks to the handler, by preventing exposure to vapors. Among them, the Kramer™ plate releases vapors through 12 holes of 1.5 cm diameter each, bored in a polyethylene bag that envelops a cellulose plate soaked with formic acid (Krämer, 1991; Vivas, 1997). The Nassenheider™ evaporator is a plastic container filled with 120 g of formic acid and connected to a wick that receives the formic acid by capillarity and gradually releases it (Ritter & Rutter, 1980). Other evaporation devices have been developed to maintain evaporation of formic acid for a longer time by physical means (Charrière et al., 1997).

The use of a gelled medium to prolong evaporation of formic acid and improve its effectiveness against varroa has been tested. Clark (1991), using formic acid in a gel, obtained 90% varroa mortality. Feldlaufer et al. (1997) developed the Beltsville formic acid gel packet, which regulates the release of formic acid, preventing its depletion in the first hours. Kochansky & Shimanuki (1999) developed an easy-to-handle gel formula from which formic acid evaporates gradually over 2 weeks. BeeVar™ is commercialized in Argentina. This gel is also easy to handle and contains 200 g of formic acid that causes more than 80% mortality after two applications (Eguaras et al., 2001). A second formula (Eguaras et al., 2003) contains 232 g of 70% formic acid and 8 g carboxypolymethylene.

The advantages of gels as systems to regulate evaporation motivated the design of a convenient device for control of varroa in bee hives using formic acid in a gelled medium.

MATERIALS AND METHODS

Preparation of the gel device

Gels were prepared from starch in two formulations and poured into 15 × 20 cm, 2 cm high polystyrene foam dishes. Each dish was wrapped in a 400 caliber polyethylene bag and sealed by heat. In a preliminary test, the polyethylene wrap proved to be impermeable to formic acid, both under laboratory conditions and at 5 °C. Each device (plate) contained 200 g of the gel vehicle and 200 g of 85% formic acid.

Evaporation of formic acid under laboratory conditions

A test was carried out to determine the release rate of formic acid vapors under controlled conditions simulating the interior of bee hives (32±2 °C, 40-60% r.h.). The variables were type of gel (A and B) and release area, which was controlled by cutting out rectangles of known areas from the polyethylene bags. The release areas were designated as treatments: 10, 16, 32, and 64 cm^2, plus plates with no polyethylene bag, where the release area was 300 cm^2. Controls containing distilled water or 85% formic acid without gel

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Table 1 Formic acid (FA) in a gelled medium applied to honey bee colonies.

Treatment	Active principle	Evaporation area (cm^2)	No. doses*	Day of application
A	FA in gel B	16	2	0
B	FA in gel B	32	2	0 and 15, 1 plate each
C	FA in gel B	64	2	0 and 15, 1 plate each
D	Fluvalinate	-	1	0
E	Control	-	-	-

*Each dose was equivalent to 200 g of 85% formic acid in gel.

were considered for each release area. Each treatment was replicated four times. All plates were weighed daily to estimate the formic acid lost by evaporation, until their weight stabilized around 200 g.

Evaporation of formic acid under field conditions

A field test was carried out in the municipality of Tlalmanalco (State of Mexico, Mexico), 2,500 m above sea level, during June and July 2004. Climate in this location is sub-humid temperate [Cb (wz)(w)(i') g'; García, 1988]. A second test was carried out in the municipality of Cordoba (Veracruz, Mexico), 924 m a.s.l., June to August 2004. Climate in this location is humid warm [(A) Cam (f)(i') gw'; García, 1988].

Bee colonies lodged in standard Jumbo (Dadant) hives were used. They were selected for the tests provided they satisfied the following conditions: (1) a mean daily natural fall of 25 or more varroa mites, (2) seven or more spaces between frames occupied by worker bees, (3) three or more combs completely filled with capped brood, and (4) queen with normal egg laying activity. These conditions were numerically evaluated to compare them before and after treatments. A screened bottom board with a metal sheet covered with oil (8 meshes per square inch with a wooden frame) was placed on the floor of each hive for 7 days to estimate natural varroa fall. Honey supers (i.e., boxes placed above the brood chambers to store surplus honey) were removed from the selected hives.

The inner cover of each hive was replaced by a 'California inner cover', which has a wooden frame 2 cm thick attached to its perimeter. The California inner cover opens a space above the frames where the plates fit in. Colonies that were to receive treatments were selected randomly. Only gel B was selected for use after the laboratory test. Treatments are shown in Table 1. Non-treated and fluvalinate-treated controls were included. For the fluvalinate control, two Apistan™ (Novartis) strips per colony were placed between frames 3-4 and 6-7. Each treatment included three replicates.

Plates were weighed weekly to estimate the quantity of formic acid evaporated. Data were recorded six times per plate for treatment A, and three times per plate for treatments B and C. A digital sensor (datalogger HOBO™) was placed in each hive, close to the plate, to record the temperature in the precise place where vapors were released. Temperature inside the hives was measured every hour.

Effectiveness of formic acid under field conditions

Mites fallen after treatments were collected using the metal sheets covered with oil, described for estimating natural mortality. They were replaced on days 1, 7, 15, 16, 22, and 30, starting on day 0, when treatments were applied.

A chemical shock was applied 30 days after formic acid was administered, to quantify the surviving varroa mites. It consisted of inserting two Apistan strips between combs 3-4 and 6-7, plus 5 ml of 20% coumaphos spray (Asuntol™, Bayer) distributed on all combs. Chemical shock was also applied to control colonies.

Once the plates were removed from the hives, the number of spaces between frames occupied by worker bees and the number of combs filled with brood were recorded. The presence of active queens was inferred by the observation of eggs or young larvae. By comparing estimates of bee population and food supplies, the effects of treatments on the general condition of the colonies were evaluated.

RESULTS

Evaporation of formic acid under laboratory hive conditions

Weight loss was gradual and continuous for all treatments (i.e., water and formic acid with and without gel), although the evaporation rate diminished when about 90% of the initial mass had been lost (Fig. 1). The number of days required to lose 50 and 75% of the original weight of the plates was subjected to a factorial ANOVA (Table 2). Significant effects of medium, release area, and their interaction were found for both 50 and 75% weight loss. As a general trend, for a given release area, loss of formic acid in gel formulation was 2-3 times slower than without. Water weight loss rate was intermediate.

In all cases a significant fit to linear regression was found between weight loss of plates with formic acid (gels A and B) and their respective release areas, explaining more than 96% of the variation (Table 3). It appears that for all the release areas, both gels led to almost constant release of vapors until the formic acid was depleted.

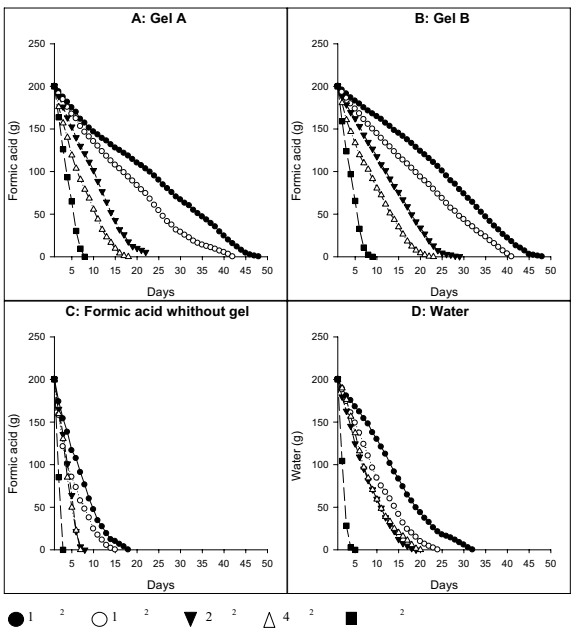

Figure 1 Evaporation of formic acid with and without gel and of water, as affected by the release area. (A) gel A, (B) gel B, (C) formic acid without gel, and (D) water.

Table 2 ANOVA on number of days required by formic acid plates to lose 50 and 75% of their original weight. Laboratory tests at 32±2 °C, 40-60% r.h.

		Time to 50% weight loss		Time to 75% weight loss	
Source of variation	d.f.	SS	F	SS	F
Medium	3	1093.00	38.72	2468.638	78.59
Release area	4	2289.325	60.83	4022.20	96.03
Medium*Release area	12	479.375	4.25	956.30	7.61

P<0.0001 in all six cases.

Table 3 Correlation between daily weight loss of formic acid from plates and time elapsed, for each type of gel and release area.

	Gel A		Gel B	
Release area (cm²)	Equation	r^2	Equation	r^2
10	y = -3.93x + 210.7	0.9620	y = -4.62x + 213.8	0.9927
16	y = -5.93x + 183.0	0.9747	y = -5.58x + 194.4	0.9882
32	y = -11.79x + 210.3	0.9977	y = -8.25x + 205.2	0.9992
64	y = -12.92x + 194.2	0.9851	y = -10.04x + 196.3	0.9906
300	y = -31.30x + 231.2	0.9981	y = -29.70x + 214.9	0.9721

Table 4 Comparison of number of days elapsed to 50 and 75% weight loss of formic acid from gel A and B as related to release area.

	Gel A		Gel B	
Release area (cm²)	50%	75%	50%	75%
10	22.75a	31.00a	24.25a	33.50a
16	16.00abc	25.75ab	18.00b	28.00b
32	8.75bcd	14.00bc	11.250c	17.25c
64	5.25cd	9.50c	7.50c	12.50c
300	2.50d	4.25c	2.75d	4.50d

Means followed by the same letter in a given column are not significantly different (Tukey, P>0.05).

Table 5 Comparison of weights of plates with formic acid in a gelled medium (formulation B), 8 and 15 days after placement inside bee hives in the field, in Tlalmanalco and Cordoba.

		Weight after 8 days		Weight after 15 days	
Release area (cm²)	n	Tlalmanalco	Cordoba	Tlalmanalco	Cordoba
16	6	146.92a	99.08abc	117.00a	55.67b
32	6	107.17ab	54.50c	65.83ab	12.33b
64	6	48.33c	62.67bc	11.83b	25b

Means followed by the same letter within a given period (i.e., 8 or 15 days) are not significantly different (Tukey, P>0.05).

The rates of weight loss of formic acid in gel formulations A and B, as a function of the release area, could be grouped in three blocks: (1) areas 10 and 16 cm², (2) 32 and 64 cm², and (3) 300 cm² (Table 4). According to Scott et al. (1999), Calderone & Nasr (1999), and Imdorf et al. (1990), the daily release of formic acid must average 10 g for effective varroa control. The plates with gel B and release area 32 cm² approached this value (and they looked okay), so it was selected for field tests.

Evaporation of formic acid under field conditions

Evaporation was gradual in all treatments, both in hives at Tlalmanalco and at Cordoba (Fig. 2). The gel retarded evaporation of formic acid: up to 95% of the formic acid had been lost 30 days later in treatment A (release area 16 cm²) and 15 days later in B and C (32 and 64 cm², respectively). Treatments at Tlalmanalco had a mean daily weight loss of 10 (treatment A; two plates placed simultaneously), 8.95 (B), and 12.5 g (C), whereas at Cordoba the daily weight loss was 11.26 (A), 12.5 (B), and 11.7 g (C). These values are equal to or higher than release rates proposed by Feldlaufer et al. (1997), Calderone & Nars (1999), Scott et al. (1999), and Eguaras et al. (2001, 2003), as the most adequate for varroa control.

Comparing mean weights of plates, 8 and 15 days after installation inside bee hives in Tlalmanalco and Cordoba, indicates that plates in Cordoba generally lost more formic acid (Table 5). The difference between locations is attributed to temperature inside hives. At Cordoba it was approximately 5 °C warmer than at Tlalmanalco (30.8±4.3 vs. 25.2±4.5 °C).

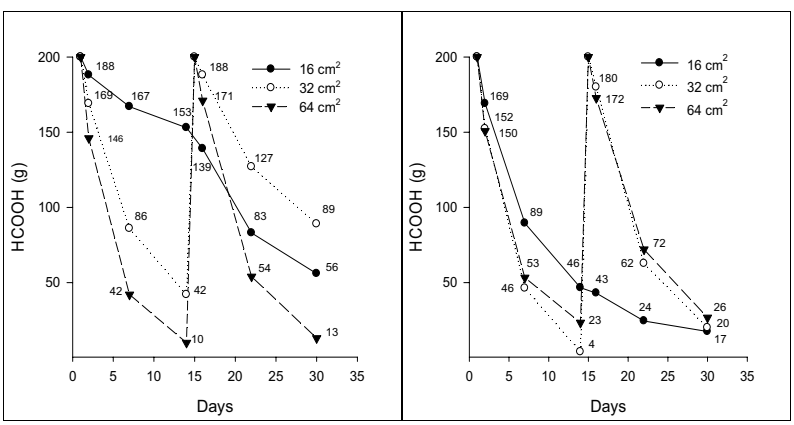

Figure 2 Weight loss of formic acid plates in field tests at Tlalmanalco (A), and Cordoba (B). On day 15 in treatments B and C, where weight increases abruptly, the plate was replaced by a new one.

Table 6 General condition of bee colonies after application of formic acid (gel B), in Tlalmanalco (T) and Cordoba (C). Values are estimates of population and food supplies after treatments minus the same estimates before treatments.

Treatments	Spaces between frames occupied by adult bees		Queen bee (0, absent; 1, present)		Spaces in combs occupied by brood		honey		pollen	
	T	C	T	C	T	C	T	C	T	C
Control	1.1	0.0	0.0	0.0	-1.5a	-0.0	1.0	0.5	0.0	-0.5
A	-1.0	-1.0	0.0	-0.6	-1.0a	-2.6	-0.3	-1.0	0.0	0.0
B	-1.0	-0.3	0.0	0.0	-0.6a	-0.3	0.1	-0.3	0.0	0.0
C	-2.3	-0.6	0.0	0.0	-1.3a	-0.0	0.0	-0.6	-0.1	0.0
Apistan®	-3.3	-1.3	-0.6	0.0	-5.6b	-0.6	1.3	0.4	-0.3	-0.3

No significant differences among means within columns were found (Tukey, P>0.05), except for 'Spaces occupied by brood in Tlalmanalco'.

Table 7 Effectiveness of treatments with formic acid (formulation B) in Tlalmanalco and Cordoba.

Treatment	Tlalmanalco	Cordoba
Control	34.50c	43.41b
A	72.04b	76.28ab
B	75.61b	67.52ab
C	78.86b	74.81ab
Apistan®	98.19a	91.64a

Means followed by the same letter, for a given column, are not significantly different (Tukey, P>0.05).

General condition of the colonies

No significant difference was found in any of the response variables, for any of the formic acid treatments, except one: in Tlalmanalco, brood was significantly reduced after treatment with fluvalinate (Apistan; Table 6). Two queens were lost from hives treated with fluvalinate at Tlalmanalco, and two queens were lost after formic acid treatment A (two plates, 16 cm² release area each) at Cordoba.

The loss of two queens after Apistan treatment is surprising because this pesticide is considered innocuous for bees after many tests (Borneck, 1988). Mexican beekeepers have used it since 1992. In the case of treatment A with formic acid, there is no way to determine whether the queens were lost as a consequence of the acid, excessive handling, or natural death. Treatment A did not have a higher evaporation rate, therefore it is assumed that formic acid was not responsible. However, the possibility that formic acid did kill the queens would pose a serious problem.

Previous experiences in testing negative effects of formic acid on bees have yielded variable results. Mutinelli et al. (1996) and Ciolino et al. (1999) registered queen loss, temporary lull in egg laying, swarming, mortality of worker bees, and increased aggressiveness. Eguaras et al. (2001) showed that regulated release of formic acid reduces negative effects, coinciding with the results of our work.

Efficacy of treatments in the field test

All treatments with formic acid caused varroa mortality of around 70% (Table 7). Apistan was over 90% effective; however, at Cordoba it was not significantly different from formic acid treatments.

DISCUSSION

A common feature of formic acid treatments is the irregularity of its effects. This is attributed to variations in the concentration of its vapors. Examples from the literature are 27-65% control (Hueso, 2000), 12-48% (Avila, 2001), and 34-65% (Vivas, 1997). Systems designed to regulate evaporation resulted in increased effectiveness. Using formic acid in gel, for instance, 56% (Calderone & Nars, 1999), 70.3% (Feldlaufer et al., 1997), and about 90% (Eguaras et al., 2001, 2003) varroa mortality was obtained.

Most treatments with natural products used to control varroa are short-term, which necessitates repeated applications. Illertissed plates, tymol, and oxalic acid are applied four times at weekly intervals (Imdorf et al., 1996). This is a disadvantage because costs increase. In our tests, the plates are applied once (treatment A) or twice (B and C), and costs and handling are reduced.

Starch-based gels were easy to prepare and handle, and raw material was cheap and easily available. Starch was gelled first and then mixed with formic acid. Thus, the user was not exposed to formic acid vapors during most of the process. Imbibition capacity of the gel allowed mixing with formic acid in a 1:1 ratio. A disadvantage was that the plate was too thick to fit into the space between frames and a normal inner hive cover, so the inner cover had to be replaced by the California inner cover, as described.

Evaporation of formic acid from our gel could be regulated within pre-established limits. Evaporation rate was affected by the gel medium, release area, and temperature. The mean effectiveness of treatments with formic acid included in the gel medium was about 70%. The devices (plates) are easy to handle and safe for the beekeeper; however, damage to queen bees is not totally excluded.

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Acarological Tools

Effect of eight storage modes on DNA preservation

Matthew Corriveau, Kaitlin Uppstrom & Hans Klompen[1]

Undergraduate Biology Program, and [1]Acarology Collection, Museum of Biological Diversity, Ohio State University, 1315 Kinnear Rd., Columbus, OH 43212, USA, E-mail: klompen.1@osu.edu

Proper storage procedures are essential for specimens that are to be used for future molecular studies. We tested the performance of eight storage modes for specimens of *Balaustium* sp. (Acariformes: Erythraeidae) collected on the same day and all fixed in 95% ethanol. In tests after 8 months of storage, all four treatments based on ethanol generated good quality DNA, irrespective of ethanol concentration (75 or 95%) or storage temperature (ambient or -20 °C). Fixation in ethanol and continued dry and cold storage performed adequately, but storage in Koenike's fluid, clearing in lactic acid prior to ethanol storage, and use of a lysis buffer, did not generate adequate DNA.

Key words: *Balaustium*, Parasitengona, DNA preservation, PCR

The increased use of molecular methods in acarology has refocused attention on mite storage modes. Clearly, the optimal resource for a wide range of applications, morphological and molecular, is fresh specimens. However, these are often not available. The focus of this study is on exploring the utility, in terms of DNA extraction, of a number of low-tech and cheap storage regimes already used in acarology collections. Dillon et al. (1996), in one of the first broad comparisons of killing and storage modes for arthropods, showed that small Hymenoptera killed and stored at -80 °C or in 100% ethanol (EtOH), immobilized with CO_2 and rapidly air dried, or EtOH-killed and air dried or critical point dried, all produced good DNA (as checked by successful amplification of part of the mitochondrial 16S rDNA gene). In contrast, material preserved in formalin, killed in ethyl acetate and subsequently dried, killed in ethylene glycol, or old museum specimens preserved in 70% EtOH did not produce quality DNA. Notably, these authors found no differences in success rate for short term (7 days) or medium- to long-term (16 months) storage. Unfortunately, these results may not be universally applicable, and some taxon-specific optimization for preservation techniques (and different extraction methods) may be required.

A number of recent studies have addressed this problem for Chelicerata. Vink et al. (2005) studied preservation of spiders and scorpions across four EtOH-based fixation and storage regimes, propylene glycol, and RNA*later*™. They did so using five temperature regimes: 40 °C (mimicking field conditions in the tropics), room temperature, 2-4 °C (refrigerator), -20 °C (standard freezer), and -80 °C (ultracold freezer). No storage method proved effective at 40 °C, but good quantities of long DNA segments were recovered using both RNA*later* and propylene glycol at various temperatures, as well as with storage using 95% EtOH in a freezer. However, the authors did not recommend long-term storage in RNA*later* (it may crystallize in the 95% EtOH used to dry the specimens prior to extraction) or propylene glycol (may cause soft tissue shrinkage). Instead they recommend storage of legs in RNA*later* or propylene glycol, with the remainder stored in 70% EtOH for morphological work. For acarologists this option is not optimal given the size of most mites. Mtambo et al. (2006) had good success using legs of properly stored specimens, but personal experience with extended storage of tick legs separated from the rest of the specimen has been poor.

Mtambo et al. (2006) noted that templates from ticks killed in 70% EtOH and stored in the same medium in a refrigerator over medium-long terms (1-2 years) amplified almost as well as templates from freshly killed specimens. Templates from specimens killed and stored in 70% EtOH at ambient temperature (~10 years), and cryopreserved ticks (stored 3-4 years) scored slightly less well. Ticks that were killed in a refrigerator, air dried, and stored at ambient temperatures did not yield adequate DNA at all. These results are more difficult to interpret than those of Vink et al. (2005), because specimens in the different treatments were not collected at the same time, introducing the added factor of storage time, and possible variation in initial processing modes. The same problem can be noted for a study by Rey et al. (2004) on water mites (Parasitengona, Hydracarina). These authors based their study on museum preserved material, the provenance of which is not always perfectly known. They did introduce investigations of some media not considered by any of the above studies, specifically Koenike's fluid [a mixture of acetic acid, glycerine, and water (Barr, 1973)], Angelier's fluid [a mixture of water, chromic acid and acetic acid (Valdecasas & Baltanás, 1989)], and direct freezing of mites in water (Rey et al., 2004). All are commonly used by researchers studying water mites. Success rates varied by taxon and storage time, but considerable success was

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achieved for all three of these media/procedures, as well as for specimens stored in 70% EtOH at room temperature (although generally not beyond 4 months storage time for the latter). Notably, these authors succeeded in amplifying small segments of DNA from 25-year-old specimens preserved in Angelier's fluid.

In short, much of the earlier research in this area for non-mite groups appears transferable to mites, but there are some differences, such as the relatively weak performance of cryopreservation for ticks (Mtambo et al., 2006). Moreover, the earlier studies did not consider various mite-specific storage regimes. On the other hand, the few mite-based studies (Rey et al., 2004; Mtambo et al., 2006) that were performed did not fully control for provenance or storage length. The goal of this study is to explore DNA preservation efficiency for eight storage regimes, based on specimens of one species, collected on the same date.

MATERIAL AND METHODS

Collection and storage

The species selected for this experiment was *Balaustium* sp. (Parasitengona: Erythraeidae). This species was selected because of its abundance, and therefore ease of collection. Using an aspirator, specimens were collected 31 May, 2005 from rocks in a suburban garden in Worthington, Ohio (40°05'56'N, 83°03'02'W). All mites were killed in 95% EtOH, after which approximately 30 mites were randomly assigned to each storage regime. Specimen vouchers are deposited in the Ohio State University Acarology Laboratory (OSAL) collection (OSAL0033618-33622).

The following treatments were explored:

(1) Storage in 70-75% EtOH at ambient temperature (18 °C) in a closed cabinet (R75). This is the standard storage regime for fluid-preserved specimens in most acarology collections.

(2) Storage in 70-75% EtOH in a freezer (-20 °C) (F75). This method explores the possible benefits of cold storage for material collected/processed in 75% EtOH.

(3) Storage in 95-100% EtOH in a freezer (F95). This approach combines the presumed benefits of cold storage and high-percentage EtOH (e.g., Vink et al., 2005).

(4) Storage in 95-100% EtOH at room temperature in a closed cabinet (R95). The main function of this approach was to isolate effects of cold storage or higher-percentage EtOH, in case of distinct differences between treatments (1) and (3).

A major consideration in deciding on storage in 70-75% or 95-100% EtOH is that specimens tend to harden even faster in higher-percentage EtOH than in 70-75% EtOH. This hardening makes specimens more difficult to clear and thus less suited for future morphological studies.

(5) Dry storage in a freezer (FDR). A vial with mites originally killed in 95% EtOH was placed in a freezer (-20 °C) uncapped, allowing the alcohol to evaporate. Once dry, the vial was then recapped and left in the freezer. This procedure intended to get the benefits of fixation in 95% EtOH while avoiding the problems of specimens hardening due to long-term storage in that medium. An added benefit would be that dry specimens do not pose safety issues, and can be shipped without problem. An obvious drawback is that recovery of small mites stored in this regime requires adding fluid, as dry recovery is typically quite problematic.

(6) Storage in Koenike's fluid at ambient temperature (RKO). This method is standard for fluid storage in water mite collections, but used infrequently for other mite groups. Koenike's fluid is recommended for long-term storage of all fluid-preserved mites because Koenike's avoids the hardening associated with long-term storage in EtOH (Krantz, 1978).

(7) Storage in EtOH after clearing in lactic acid, at ambient temperature (RLA). A common procedure for oribatid workers is to clear specimens, study them using temporary mounts in lactic acid in cavity slides, and subsequently return the cleared specimens to EtOH. This storage regime was tested because it would allow specific identification of individual mites (using cavity slides) before extraction. The standard clearing processes, using lactic acid or lactophenol, should not destroy DNA, but may inhibit or destroy nucleases. If so, the resulting material should be excellent for future molecular work. If successful, the logical next step would be to try and extract DNA from specimens already slide mounted, potentially bringing in a huge reservoir of available material.

(8) Storage in 'lysis buffer' at ambient temperature (Longmire et al., 1997) (RBU). This buffer (Tris-HCl, EDTA, NaCl, SDS) was developed for use with vertebrate tissues and blood samples, and is highly effective in preserving DNA in such samples under field conditions. We do not have experience with use of specimens preserved in this buffer for morphological studies. As with dry storage and Koenike's, this option would allow relatively unrestricted shipping.

Extraction

DNA was extracted in January and February 2006 and stored for 7-8 months. Extractions followed the 'Purification of Total DNA from Animal Tissues' protocol for the DNeasy™ (Qiagen) extraction kits, with minor modifications. Each extraction was based on two mites. Mites were removed from the storage vial using flame sterilized forceps, carefully dried on a clean tissue to remove excess fluid, and placed in a glass spot dish with 180 µl of ATL buffer. Mites were crushed with flame sterilized forceps, and the entire contents of the well was pipetted into a 1.5-ml Eppendorf tube. Addition of proteinase K (as per manufacturer's instructions) was followed by extended incubation at 55 ºC for no less than 12 h. The rest of the procedure followed the above protocol. Five replicates were extracted for each storage regime.

PCR

The three gene fragments amplified were: (1) Cytochrome oxydase subunit 1 (COI), a 600bp fragment of this multi-copy mitochondrial protein-coding gene. The primers used for this gene were P1 (5'-TTG ATT TTT TGG DCA YCC WGA AGT-3') (Simon et al., 1994) and R4 (5'-CCW VYT ARD CCT ARR AAR TGT TG-3') (Lekveishvili & Klompen, 2004), (2) 18S rRNA, a 800bp fragment of this multi-copy nuclear ribosomal gene. The primers used were 5F (5'-GCG AAA GCA TTT GCC AAG AA-3') (Giribet et al., 1996) and NS8 (5'-TCC GCA GGT TCA CCT ACG GA-3') (White et al., 1990), and (3) EF-1α, a 600bp fragment of this single copy nuclear protein-coding gene. A nested PCR was performed for EF-1α primer pair 40.6F (5'-ATY GAR AAR TTY GAR AAR GAR GC-3') and 41.21RC (5'-TGY CTC ATR TCD CGV ACR GCR AA-3'), followed by primer pair 40.71F (5'-TCN TTY AAR TAY GCN TGG GT-3') and M3RC (5'-GGY TCC ATV RCR TCN ARR GC-3') (Regier & Shultz, 1997; Lekveishvili & Klompen, 2004). The different fragments selected for amplification represent a variety of

Table 1 Amplification success for different genes based on templates from eight storage treatments for *Balaustium* sp.

Storage regime		COI	18S	EF1α
R75	75% ethanol, ambient	2-2-2-2-1[1]	2-2-2-2-2	2-2-2-1-0
F75	75% EtOH, frozen	2-2-2-2-2	2-2-2-2-2	2-2-2-2-2
F95	95% EtOH, frozen	2-2-2-2-2	2-2-2-2-2	2-2-2-2-1
R95	95% EtOH, ambient	2-2-2-2-1	2-2-2-2-2	2-2-2-2-2
FDR	dry, frozen	2-2-1-1-0	2-2-2-2-2	2-2-0-0-0
RKO	Koenike's fluid, ambient	2-0-0-0-0	1-1-1-0-0	*
RLA	lactic acid, ambient	2-1-0-0-0	2-1-0-0-0	*
RBU	lysis buffer	0-0[#]	0-0[#]	

Amplification success was measured by band intensity on an agarose gel for five trials each. Results for trials listed as best to worst.
[1]Scoring codes: 2, strong PCR product (band); 1, weak but distinct band; 0, no visible band. *not tested for this gene; [#]tests discontinued because of consistent lack of product.

organelles (mitochondria vs. nucleus) and gene types (protein coding vs. RNA). The COI and 18S fragments are multicopy and considered relatively easy to amplify. However, molecular studies increasingly involve larger DNA fragments and regions more difficult to amplify, so we added the fragment of EF-1α as a more severe test of template quality.

Examination of storage regime (8) lysis buffer, was discontinued early on, as templates from the first two extractions failed to generate any product for any PCR reaction. Storage regimes not performing well for COI or 18s rRNA, specifically regime (6) Koenike's fluid (RKO), and (7) lactic acid clearing and storing in EtOH (RLA), were not tested for EF-1α. All three gene fragments were sequenced [GenBank numbers: FJ515963 (COI), FJ515964 (18S), FJ515965 (EF-1α)] and compared with sequences for related taxa to exclude the possibility of contamination.

Comparison

PCR amplification of the genes of interest was used to correlate storage mode with quality of DNA isolated. Visualization of yield was based on products in 1.0% agarose gels stained with ethidium bromide. A successful amplification was expected to have a bright and distinct PCR amplification band (score 2 in Table 1; e.g., Fig. 1A, F75 lanes 1-5), whereas less successful or unsuccessful fixatives were expected to have a weak band (score 1 in Table 1; e.g., Fig. 1B, FDR lane 2) or no band at all (score 0 in Table 1). Results for all products tested were photographed using a Polaroid camera.

RESULTS AND DISCUSSION

Results for each test are summarized in Table 1, with examples of product yield shown in Figure 1. All EtOH-based treatments (1-4) generated good quality product for all genes tested. Storage in 70% at ambient temperature (R75) performed slightly less well than the other three, but the difference is inconsequential. This result is in contrast with results of Vink et al. (2005), who found relatively poor performance for EtOH-based treatments (e.g., fixed in 95% and stored in either 75 or 95% EtOH), with the possible exception of fixed and stored in 95% EtOH in a freezer. *Balaustium* sp. is much smaller than the spiders or scorpions tested by Vink et al. (2005), and the difference in outcomes may be related to body size. Ethanol is likely to penetrate tissues faster in smaller organisms, but this cannot be the full explanation. Our results are also better than those obtained by Rey et al. (2004) for water mites. Relative to that study, we hypothesize that the method of killing the mites may have influenced the results of the storage experiments.

Dry storage in a freezer (FDR) was clearly less successful than the EtOH treatments, but is still worth considering given the advantages of keeping specimens from hardening and the relative ease of shipping. Specimens preserved this way may yield less or lower-quality DNA, but with increasingly strict regulations for transport of containers with any EtOH, it is worthwhile to consider this simple alternative involving less paperwork and less expense. A notable difference between our results and those of Rey et al. (2004) was the relatively poor performance of specimens preserved in Koenike's (RKO). Rey et al. (2004) had good success rates with this medium (33-100% success after 4 months), but in our experiment Koenike's preserved specimens performed poorly, much worse than dry frozen ones (FDR). As with treatments 1-4 this may be related to the killing method, although in this case killing in 95% EtOH and transfer to Koenike's may have had a negative effect, an issue worth studying. Storage time may also be a factor, as Rey et al. (2004) had much lower success rates with specimens stored for 10 months or more. Finally, the clearing in lactic acid and storing in EtOH (RLA) treatment was generally unsuccessful.

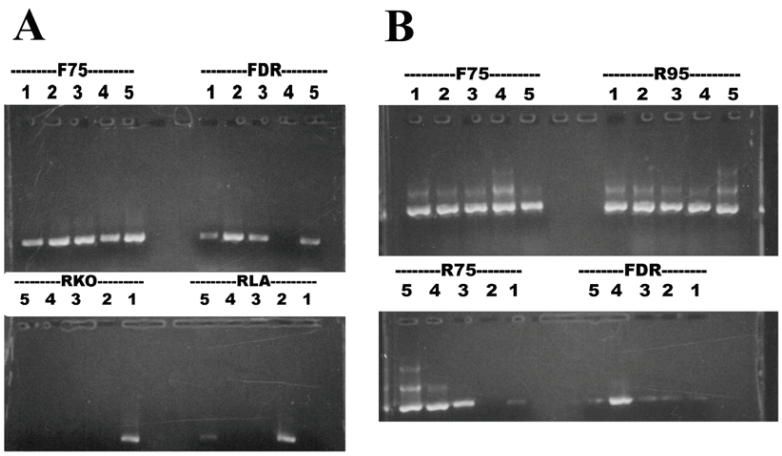

Figure 1 Agarose gels showing band intensity of different treatments, (A) based on COI, and (B) based on EF-1α. Treatment abbreviations as in Table 1.

If any success is to be obtained with previously cleared mites it will be by alternative extraction procedures. Thus the long-term hope of using already slide-mounted specimens for molecular studies remains unfulfilled.

In conclusion, our results support the common practice of using EtOH to store specimens to be used for basic sequencing. We found very limited influence of EtOH concentration or temperature on DNA preservation on the time scale tested (8-9 months). The only alternative with reasonable results was dry storage in a freezer. We stress that the current study addresses only storage, not differences due to fixation/killing or DNA extraction mode. The latter variables may well be more important than the storage method in obtaining quality genomic DNA (Dillon et al., 1996). We suspect that our procedure of killing all specimens in 95% EtOH improved our storage results with the various EtOH regimes, but this too needs further testing. We have planned follow-up experiments on specimens from the same series to examine effects of medium- to long-term storage (4-5 years).

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Spider Mites Web: A comprehensive database for the Tetranychidae

Alain Migeon, Elodie Nouguier & Franck Dorkeld
INRA, UMR CBGP (INRA / IRD / Cirad / Montpellier SupAgro), CS 30016, 34988 Montferrier-sur-Lez Cedex, France.
E-mail: migeon@supagro.inra.fr

Online databases are becoming a standard tool to study taxonomy, biodiversity, and ecology. We have developed a comprehensive database for the family Tetranychidae (spider mites). The Tetranychidae is one of the most important families of the Acari in terms of economic impact, because it comprises several agricultural pest species of major relevance, such as the cosmopolitan *Tetranychus urticae*. The aim of the web site is to gather information on all described spider mites in the world. The main goal is to provide a synthetic view of the biodiversity of this mite family. 1,280 literature references are included, from 1758 to present. 1,257 species, more than 11,745 host plants, and 5,380 geographical data are recorded in 17 tables. The database includes taxonomic data relating the history of nomenclature, geographical distribution, and host plants for all species examined. Three types of query are available to retrieve this information: (1) 'By Species' allows to search on species names and synonyms; (2) 'Advanced' performs a cross search including taxonomical, geographical, and host-plant information; and (3) 'Bibliography' allows searching references combining one, two, or three authors, and it displays all nomenclatural, host plant, and geographical data for the selected reference.

Key words: Database, geographical distribution, host-plant, internet, taxonomy

Online databases are more and more used in taxonomy and biology. They are useful to summarise and share information regarding both taxonomical or geographical information [see Magowski (2004) for a review on Acari databases]. They constitute a standard tool for taxonomical studies on biodiversity and ecology. Examples of specialized databases are given in ScaleNet (Ben-Dov et al., 2006) and The BioSystematic Database of World Diptera (Thompson, 2006). Fauna Europaea (Fauna Europaea Web Service, 2004) is another example dealing with a precise geographical region, Europe.

No database of the acarine family Tetranychidae was available, yet this family of phytophagous mites totals about 1,250 species and includes several ubiquitous pests. These major pests in the family have motivated a large number of studies. The availability of a database is of great value when the state of knowledge is sufficiently advanced (Shimano et al., 2004), as is the case for Tetranychidae.

A substantial amount of literature on spider mites has contributed to the general description of the family. The first of these studies was by McGregor (1950), followed by the work by Pritchard & Baker (1955), that is still being used, and the more recent catalogue of Bolland et al. (1998), giving a list of species of the family. These studies have been complemented by other more regional works such as Baker & Pritchard (1960) and Meyer (1974, 1987) for Africa, Tuttle et al. (1976) for Mexico, Baker & Tuttle (1994) for USA, Mitrofanov et al. (1987) for the former USSR, and Ehara (1999) for Japan. Bolland et al.'s (1998) book is a catalogue of the systematics of the family, which was reviewed and updated by Migeon & Flechtmann (2004). Although this catalogue remains extremely useful, it has limitations inherent to traditional publications: permanent update of the information is problematic, and storage and display of all available information remains difficult or even impossible.

The Spider Mites Web has been designed to reduce these major drawbacks. This site is aimed at providing comprehensive information for every species of the Tetranychidae, including their taxonomy, distribution, and host plants, with reference to the literature since 1758. In addition to the advantages of interactivity, Spider Mites Web, like other databases, provides cross-analyses, giving a synthetic view of the biodiversity of the Tetranychidae, ranging from host plant to continent.

DATA SOURCE AND DATABASE

Inputs are essentially based on bibliographical references (books and articles) on Tetranychidae, combining specialized, generalist, and agricultural publications. Heterogeneity of these sources requires validating every record.

The database (http://www1.montpellier.inra.fr/CBGP/spmweb/index.php) was originally created with Microsoft Access, then transferred to the Unix/Linux system. For site development we used PHP language, v. 5.04, in addition to HTML. PostgreSQL was used for the database engine and PHP-PostgreSQL for database queries. PHP-PostgreSQL is a universal, free, well-documented, and standard package for developing such applications.

Figure 1 is a schematic representation of the relational structure of the database. The main tables are Species-Nomenclature and References. These tables allow the description of nomenclatural history of each taxon. The table Status makes a list of all the possible names for the species entered. It is also used to create the content of the table Species-Valid, directly inherited from Species-Nomenclature. Taxonomical data for the genera have the same structure. The table 'Classification-Higher' draws the supra-generic ranks. The table Plants contains all information for host plants, including classification ranging from phylum to

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species. Representation of geographic knowledge allows queries ranging from country to biogeographical area. The relations between taxa, host plants (or geographical region), and references are included in link tables to ease the navigation between these notions.

THE DATA

References

A list of all references included is available in the site, via a pdf document. Spider Mites Web currently includes 1,280 references.

Nomenclatural information

One of the major difficulties encountered when building the database was to model the nomenclature and taxonomic information. We have chosen to use the valid species name as output for any query. This constitutes the taxonomic base. For each of these species, nomenclatural information is available. To describe this nomenclatural information we attributed a status to any nomenclatural publication (Table 1). This status is derived from the International Code of Zoological Nomenclature (1999) allowing to keep standard definitions. A total of 1,257 valid species and 3,886 records are included in the database. A systematic list of all species is available.

Geographical distribution

Our data input is in accordance with the Taxonomic Database Working Group (TDWG) recommendations (Hollis & Brummit, 2001). We used the maximal precision for data entry up to level 4. This TDWG level represents 609 basic

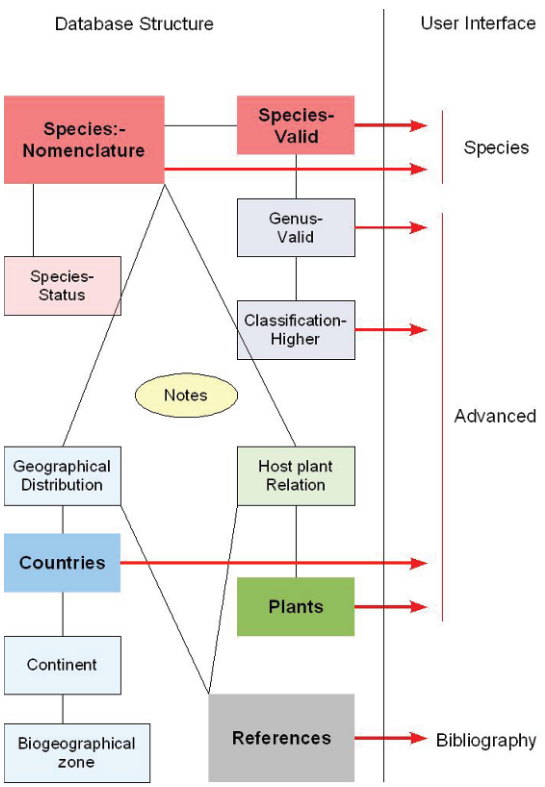


Figure 1 Scheme of the database showing the structure of the data in relation to user interface.

Table 1 The status used for recording names in Species-Nomenclature.

Valid species status:
Valid name: the name used for taxonomical information
Valid nomenclatural act: the publication of a valid name
Valid name in invalid publication: the publication of a valid name
Change of status: a change to subspecies or to species
New combination: change of generic assignment
Emendation: a justified change in the original spelling
Replacement name: a new name for a valid taxon with an invalid name
Synonyms status:
Synonym description: the publication of a junior synonym
Reinstatement: a name considered as junior synonym and now valid species
Synonymy by: reference for a synonymising work
Miscellaneous status:
Homonym: an already used valid name
Unjustified emendation: an unjustified change in the original spelling
Misspelling: a valid citation with an erroneous spelling
Misidentification: an erroneous citation
Corrected identification: a correction of above

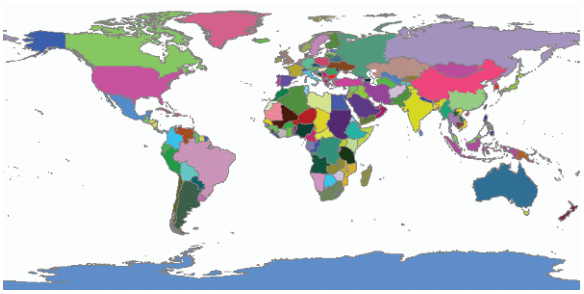


Figure 2 World map of the countries used in Spider Mites Web. Note that Russia is split into Western and Eastern, China into Palaearctic and Oriental.

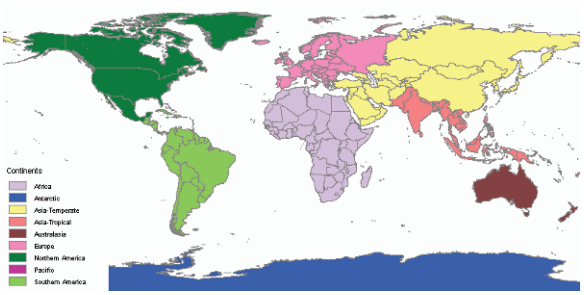


Figure 3 Map of the continents used in Spider Mites Web.

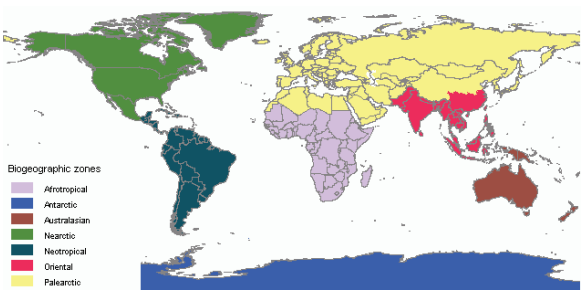


Figure 4 Map of the biogeographical areas used in Spider Mites Web.

units, grouped in 364 next units (TDWG level 3), 264 'countries' (Fig. 2), nine continents (Fig. 3), and seven biogeographical areas (Fig. 4). Russia is divided into Western Russia, which includes European and Caucasian Russia, and Eastern Russia (east of the Urals). This country is in Europe as well as in Asia. China is divided into a Palaearctic and an Oriental area. For both regions, searches can be performed on the whole country. To keep the names compatible with old records, we have used the following former countries: USSR, Czechoslovakia, and Yugoslavia. The queries can be made on former countries or on actual ones. 5,381 taxon-geographical unit-reference links are present in the database.

Host plants

The host plants are organized in families. We give the list of all the 3,914 plants recorded in the database. Sometimes information is limited to a family (e.g., Poaceae) or a genus (e.g., *Poa*). An up-to-date plant nomenclature has been used as much as possible by using the IPNI (International Plant Names Index, 2004). A total of 11,745 links to taxon-host plant-reference are present in the database.

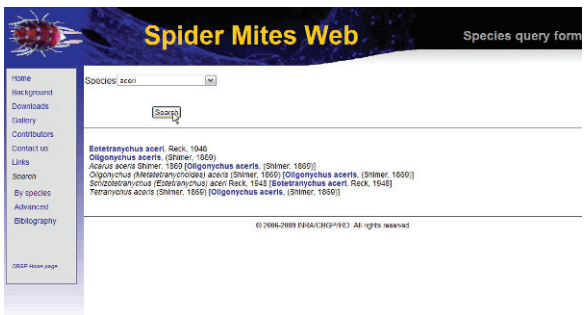


Figure 5 Screen copy of By Species search, showing the results for *aceri*.

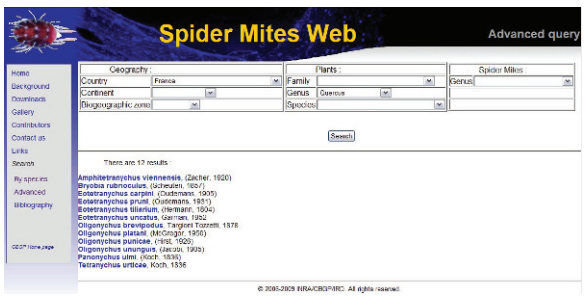


Figure 6 Screen copy of Advanced search, showing results for all the species living in France and already recorded on the genus *Quercus* (Fagaceae).

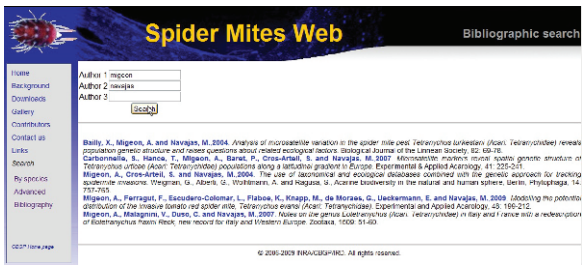


Figure 7 Screen copy of Bibliographic search showing the results with the combinations of two authors, Migeon and Navajas.

THE USER INTERFACE

The user interface was built for an easy and intuitive use. Only three search types are possible, but cross-search allows many combinations.

Search by species

A screen copy of this query is shown in Figure 5. Search can be performed by entering a species name, valid or not. The results, here displayed for *aceri*, show the valid names in blue with hypertext link. The other names are in black and the valid name is indicated in parentheses. Clicking the hypertext link brings the user to the species page.

Advanced search

A screen copy of this query is shown in Figure 6. The user can perform a cross-search including taxonomical, geographical, and host-plant information. At least one of all the fields have to be completed. For example, Figure 6 shows the species that are present in France and have been reported from *Quercus* as a host plant. Only the valid names are displayed in the results. Hypertext links bring the user to the species page, just as for the 'By Species' search.

Bibliographic search

This query allows to search references combining one, two, or three authors (Fig. 7). The result is a list of references with hypertext link for each one.

RESULTS

Species page

The species page is divided into four parts. The first is dedicated to summarizing the original data (Fig. 8 gives an example on *Eotetranychus rubiphilus*). Nomenclatural data are displayed in a second part (Fig. 9; *E. rubiphilus*). Host-plants, alphabetically sorted by families (Fig. 10; *Eurytetranychus admes*) are in the third part. Finally, geographical distribution, ordered by biogeographical zones and countries is

Figure 8 Screen copy of the head of species page, with original description reference, country type, and host plant type.

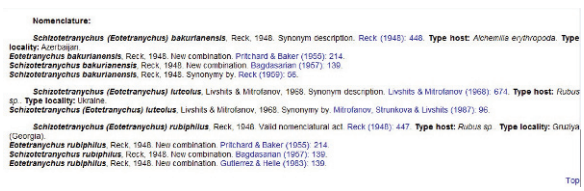


Figure 9 Screen copy of nomenclature data for *Eotetranychus rubiphilus*.

Figure 10 Screen copy of host plant data for *Eurytetranychus admes*.

given (Fig. 11; *Tetranychus evansi*). References are indicated in brackets for each record (nomenclature, host-plant or geographical data).

Reference page

The structure of the reference page is the same as in the species page. Complete reference is given at the top of the page. Nomenclatural data, host plants and geographical data are given with a hypertext link to the Species page.

Add-ons

Spider Mites Web includes a photo-gallery. We welcome new images! A list of all contributors of reprints or images is also given. The authors thank all contributors!

CONCLUSION

The site is aimed at providing comprehensive information on all spider mites of the world, including their taxonomy, distribution and host plants as can be retreived from the literature. In addition to the interactivity and cross analyses of the electronic database, this site gives a synthetic view of the biodiversity of the Tetranychidae, covering a wide scale ranging from continents to host plants. In the present context of global trade, such a synthesis is an essential facility that quickly provides lists of potentially harmful organisms. Such information should contribute to the prediction of bio-invasion risk and guide management issues.

We hope that acarlogists will try Spider Mites Web and welcome any comments and further information. The database is designed for use by the scientific community and it will be improved by the responses of its users.

Distribution (37 results):

Afrotropical: Congo [Bolland, Gutierrez & Flechtmann (1998); Bonato (1999)]; Congo (RDC ex Zaire) [Knapp, Saunyama, Sarr, et al. (2003); Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Gambia, The [Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Kenya [Knapp (2002); Knapp, Saunyama, Sarr, et al. (2003); Torolitch, Navajas & Knapp (2008); Migeon, Ferragut, Escudero-Colomar, et al. (2009); Malawi [Knapp, Saunyama, Sarr, et al. (2003)]; Mauritius [Moutia (1958); Baker & Pritchard (1960); Gutierrez (1963); Moraes, McMurtry & Baker (1987)]; Mozambique [Meyer (1987); Knapp, Saunyama, Sarr, et al. (2003)]; Namibia [Keizer & Zuurbier (2001); Knapp, Saunyama, Sarr, et al. (2003); Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Niger [Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Reunion (France) [Gutierrez (1963); Gutierrez & Etienne (1986); Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Rodrigues I. (Mauritius) [Gutierrez (1974); Gutierrez (1963)]; Senegal [Duverney & Ngueye-Ndiaye (2005)]; Seychelles [Gutierrez (1974); Gutierrez (1963)]; Somalia [Knapp, Saunyama, Sarr, et al. (2003); Sotl, Varela & Nyambo (2003)]; South Africa [Meyer (1996); Knapp, Saunyama, Sarr, et al. (2003); Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Tanzania [Torolitch, Navajas & Knapp (2008)]; Zambia [Jensen & Mingochi (1958); Bolland, Gutierrez & Flechtmann (1998); Knapp & Luchen (2000); Knapp, Saunyama, Sarr, et al. (2003)]; Zimbabwe [Anonymous (1982); Blair (1983); Meyer (1987); Moraes, McMurtry & Baker (1987); Blair (1989); Knapp & Luchen (2000); Knapp, Saunyama, Sarr, et al. (2003); Saunyama & Knapp (2003); Migeon, Ferragut, Escudero-Colomar, et al. (2009)].

Australasian: Hawaiian is. (Usa) [Kumashiro (1996)].

Nearctic: United States [Wiene (1952); Schuster (1959); Wollenberger & Gebhi (1964); Harper (1966); Oatman, Flechtner & McMurtry (1967); Qureshi, Oatman & Flechtner (1969); Denmark (1973); Moraes & McMurtry (1985); Moraes & McMurtry (1980); Moraes & McMurtry (1988); Moraes & McMurtry (1987); Moraes, McMurtry & Baker (1987); Halbert (1999)].

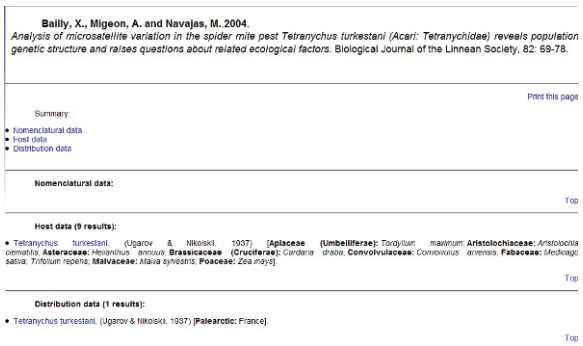
Neotropical: Argentina [Rossi Simons (1951); Rossi Simons (1971); Furtado (2006); Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Brazil [Silva (1954); Flechtmann (1967); Flechtmann (1967); Paschoal (1968); Paschoal & Reis (1965); Chiavegato & Reis (1969); Paschoal & Carvalho (1969); Flechtmann & Baker (1970); Paschoal (1970); Flechtmann & Abreu (1973); Flechtmann & Baker (1975); Tuttle, Baker & Sales (1977); Ramalho & Flechtmann (1979); Moraes, Santos & Humber (1980); Humber, Moraes & Santos (1981); Moraes & Flechtmann (1981); Moraes & McMurtry (1983); Feres & Hirose (1986); Moraes, McMurtry & Baker (1987); Lopes Filho (1990); Knapp, Saunyama, Sarr, et al. (2003); Leite, Picanco, Zanuncio, et al. (2003); Rosa, Gondim, Fiaboe, et al. (2005); Furtado (2006)]; Furtado, Moraes, Kreiter, et al. (2006); Ferrero, Moraes, Kreiter, et al. (2007); Fiaboe, Gondim, Moraes, et al. (2007); Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Puerto Rico (Usa) [Medina Gaud & Garcia Tuduri (1977)]; Virgin Is (American) [Moraes, McMurtry & Baker (1987)].

Oriental: China (Oriental) [Ho, Wang & Chien (2004)]; Taiwan [Ho, Wang & Chien (2004)].

Palearctic: Algeria [Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Canary Is. (Spain) [Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; France [Migeon (2005); Migeon (2007); Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Greece [Tsagkarakou, Cros-Arteil & Navajas (2007)]; Israel [Anonymous (2006); Ben-David, Melamed, Gerson, et al. (2007)]; Italy [Castagnoli, Nannelli & Simoni (2006)]; Madeira I. (Portugal) [Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Morocco [El-Jaouani (1988); Knapp, Saunyama, Sarr, et al. (2003)]; Portugal [Ferreira & Carmona (1995); Bolland & Vala (2000)]; Spain [Ferragut, Escudero & Olmeda (1987); Ferragut & Escudero (1999); Bolland & Vala (2000); Aucejo, Foo, Gimeno, et al. (2003); Escudero & Ferragut (2005); Migeon, Ferragut, Escudero-Colomar, et al. (2009)]; Tunisia [Bolland, Gutierrez & Flechtmann (1998); Kreiter, Auger, Lebdi Grissa, et al. (2002); Knapp, Saunyama, Sarr, et al. (2003)].

Top

Figure 11 Screen copy of geographical distribution data for *Tetranychus evansi*.



Bailly, X., Migeon, A. and Navajas, M. 2004.
Analysis of microsatellite variation in the spider mite pest Tetranychus turkestani (Acari: Tetranychidae) reveals population genetic structure and raises questions about related ecological factors. Biological Journal of the Linnean Society, 82: 69-78.

Print this page

Summary:

- Nomenclatural data
- Host data
- Distribution data

Nomenclatural data:

Top

Host data (9 results):

- Tetranychus turkestani, (Ugarov & Nikolskii, 1937) [**Apiaceae (Umbelliferae):** *Torilyllum maximum*; **Aristolochiaceae:** *Aristolochia clematitis*; **Asteraceae:** *Helianthus annuus*; **Brassicaceae (Cruciferae):** *Cardaria draba*; **Convolvulaceae:** *Convolvulus arvensis*; **Fabaceae:** *Medicago sativa*; *Trifolium repens*; **Malvaceae:** *Malva sylvestris*; **Poaceae:** *Zea mays*].

Top

Distribution data (1 results):

- Tetranychus turkestani, (Ugarov & Nikolskii, 1937) [**Palearctic:** France].

Top

Figure 12 Screen copy of Bibliography results. The reference is in the head of the page.

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Author index

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Subject and organism index

Page numbers refer to the first page of a chapter in which an index entry is treated