Diversity of acaropathogenic fungi in Poland and other European countries

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Abstract The occurrence, species diversity and some aspects of taxonomical affinity and host selectivity of acaropathogenic fungi associated with phytophagous, saprotrophic and predacious mites in Poland and other European countries were investigated on wild and cultivated plants, in insect feeding sites under the bark and in decayed wood. From among 33 species of fungi affecting mites only five species of Entomophthorales were separated and the most numerous were *Neozygites floridana* mostly on *Tetranychus urticae*, *N. abacaridis* on a few eriophyid species, and *Conidiobolus coronatus* attacking gamasid mites most frequently of the genus *Dendrolaelaps*. The most frequent mite pathogens occurring in mite communities on plants and in wood infested by insects were of the genus *Hirsutella*. Until now 13 of their form-species have been recognized in these habitats, but only *H. kirchneri*, *H. necatrix* and *H. thompsonii* (including its variety *synnematosa*) can be treated as exclusive oligophagous pathogens of phytophagous mites, though their potential host range seems to embrace only selected eriophyid or tarsonemid mites. Taxonomical differentiation of fungal strains was based on close morphological observations and molecular analysis of ITS region sequences. Two new species of acaropathogenic fungi were described in these studies. *Hirsutella danubiensis* sp. nov. was found in the tetranychid *T. urticae*, whereas *H. vandergeesti* sp. nov. affected phytoseiid mites of the genera *Amblyseius*, *Neoseiulus*, *Seiulus* and *Typhlodromus*, and the tarsonemid *Tarsonemus lacustris*.

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Introduction

Among the relatively small number of microbiological agents that cause infective diseases
of mites the entomopathogenic fungi constitute the most numerous group (Lipa 1971; Van
der Geest 1985; McCoy 1996; Chandler et al. 2000; Van der Geest et al. 2000). Although
fungi affecting mites and insects generally belong to the same taxonomical entities, only
little more than 50 species show acaropathogenic capabilities, compared to >1,000 actu-
ally known insect pathogenic species excluding Laboulbeniales (Bałazy 2000; Van der
Geest et al. 2000). The first cases of mite mycoses were described about 70 years after the
recognition of the infective character of insect mycoses and this delay remained until the
1970s, except for a few subtropical and tropical institutes, where mycoses of mites had
been included into some research, but usually on a very limited scale (e.g., Fisher 1950,
1951). In Poland the first records on mite mycoses appeared in the 1970s, but during the
last three decades they have been developed and widened continuously (Miętkiewski et al.
2000). The political and economical changes of the turn of the 1980s made it possible not
only to accelerate these studies in Poland but also to enter into international cooperation
and to stimulate acaropathogenic research in other European countries (a.o., Austria,
France, Germany, Great Britain, Greece, The Netherlands, Spain). Until now the mycoses
of two groups of mites have been partly recognized: (1) phytophagous spider and gall
mites, in gardens, orchards and grasslands, and (2) saprotrophic and predacious mites,
common in subcortical insects' feeding grounds in forests and mid-field afforestations on
trees and decayed wood.

   Data on mite pathogens are scarce, hence recent investigations on mycoses of the two
groups of host mites widens our knowledge about the diversity of fungal pathogens as well
as their taxonomical affinity and host selectivity. Most material included here has been
collected or identified in 2003–2007, and this paper is supplementary to three previous
publications (Miętkiewski and Bałazy 2003; Miętkiewski et al. 2003; Bałazy et al. 2008).

Materials and methods

Bark and wood samples were preliminarily investigated and selected in local laboratories.
Further rearing and periodical checking for the appearance of mycosed arthropod indi-
viduals, and subsequent analytical processing (isolation and identification of disease
agents, their frequency, duration and succession) were performed in the Research Centre
for Agricultural and Forest Environment of the Polish Academy of Sciences in Poznań.

   Materials were kept in rearing containers for several months and periodically checked
for the presence of fungal insect and mite pathogens at 2–4-week intervals. Fungi were
isolated by common insect pathology techniques (Bałazy et al. 2008). The standard potato-
dextrose agar (PDA), Sabouraud-dextrose agar (SDA) and SDA enriched with egg-yolk
(SDEYA) media were used, the latter sometimes supplemented with powdered milk and/or
antibiotics. Quantitative estimations of the fungal pathogens' share in mite mortality were
done only in high prevalence cases. Fungi were preserved alive, but in cases of epizootics
only representative strains were retained. For details on sampling methods and further

treatment of collected material we refer to previous publications (Miętkiewski et al. 2000, 2003; Bałazy et al. 2008). In recent years attempts have been made to verify the pathogenicity of particular strains by artificial infection of experimental host mites or other invertebrates (Bałazy et al. 2008). On a small scale this research has also concerned mites from forest litter, moss and lichens covering tree logs and trunks, decayed tree hollows and occasionally abandoned bird nests.

The acaropathogenic fungal species considered in this paper have been mostly collected in orchards, meadows and settlements in the vicinity of Siedlce, Białowieża Forest, Wielkopolski National Park and the coniferous or mixed forests of the Wielkopolska region, spruce forests and shelterbelts of southern Bavaria (Germany), similar ecosystems in central and eastern Austria, Parisian Basin (Picardie, Gâtinais, in north-eastern France) and single localities in other countries. In the Siedlce area systematic collections were continued in 2003–2007 on different plants, mostly grasses, weeds and fruit trees. Most samples were taken during the vegetation seasons, but hibernating forms were also studied in dead mites on fruit trees (Tkaczuk et al. 2003). Sometimes so little was left of the host mites, that species identification was impossible.

For taxonomical differentiation of fungal strains, the macro- and micromorphological features of the mycelium and sporulation on the host mites and in cultures were compared, including molecular markers of the whole ITS region (ITS 1, 5.8S and ITS 2). Twenty of them were obtained by ourselves, others were received from GenBank. The total alignment used in this study has 636 bp. Table 1 contains information on the origin of four sequences of recently isolated *Hirsutella* strains and one strain of *Paecilomyces farinosus*, newly introduced as an outgroup. The sequences obtained from GenBank are characterized by accession numbers following on the species names. Accession numbers of 15 strains earlier characterized by the authors can be found in Bałazy et al. (2008). *Cordyceps* strains considered in this analysis were included as the closest teleomorphic relatives based on BLAST search.

Total genomic DNA was extracted from fresh mycelium using the Plant DNeasy Extraction Kit (Qiagen, Valencia, CA, USA). The whole ITS region was amplified using universal primers ITS 4 and ITS 5 proposed by White et al. (1990). The sequences with some ambiguous positions were amplified with the primers ITS 2 and ITS 3 as well. PCR reactions were performed using a Mastercycler personal (Eppendorf), according to Stensrud et al. (2005). Amplified fragments were visualized on 1% agarose gels. The DNA band was subsequently excised and eluted using the QIAEX II Gel Extraction Kit (Qiagen). Cycle sequencing reactions were performed using the purified PCR product, AmpliTaq DNA polymerase and fluorescent dye-labeled terminators BigDye v.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacture's instructions. The ITS was sequenced bidirectionally using the same primers as before. The products were resolved by electrophoresis using Applied Biosystems' 310 DNA sequencing system.

Table 1 New *Hirsutella* and *Paecilomyces* strains used in this study

Species	Strain number	Host	Geographic origin
H. kirchneri	3661/1a	*Abacarus hystrix*	Fontainebleau, France
H. gregis	0207	*Abacarus hystrix*	Siedlce, Poland
H. tydeicola	1107	*Tydeus* sp.	Siedlce, Poland
H. cf. *brownorum*	3787m	Tarsonemid larva	Strass, Bavaria, Germany
P. farinosus	P5	Unidentified caterpillar	Białowieża Forest, Poland

Sequences (about 510 bp long) were aligned using the program CLUSTAL X (Thompson et al. 1994) and thereafter manually adjusted by the eye. The ITS region made as contig from two or more singular sequences from both strands of DNA were compared with those from GenBank NCBI using BLASTN 2.2.5 (Altschul et al. 1997). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 (Kumar et al. 2004) and MrBayes (Ronquist and Huelsenbeck 2003).

Results and discussion

Diversity of mite pathogenic fungi and phylogenetic relationships of *Hirsutella* species

From among several dozen species of fungi affecting mites (Table 2) only five species of Entomophthorales were separated, i.e., two *Conidiobolus* and three *Neozygites* species. *Conidiobolus coronatus* is characterized by low host selectivity and strong post-infection aggressiveness caused by highly toxic metabolites (Boguś et al. 1998; Boguś and Szczepanik 2000). Apart from frequent infections of gamasids, mostly *Dendrolaelaps* spp., it was isolated from pupae and adults of predacious beetles including *Crypturgus* spp., all instars of sciarid flies, a few juvenile springtails and one larva of the longicorn beetle *Pogonochaerus hispidus*. Although *C. coronatus* is a constant component of soil fungi especially in dump habitats (Domsch et al. 1980), it has only exceptionally been isolated from mites on plants. In turn, *Neozygites* species show very limited spectra of infected hosts, often restricted to one or few closely related species. For instance, *N. floridana* hosts are about 10 closely related spider mites (Tetranychidae), and often the fungus causes severe epizootics in their populations. However, Delalibera et al. (2004) found that the pathogenic specificity is (or can be) reflected in molecular structure (18S rDNA). On this basis these authors described the new species *N. tanajoae*, strictly adapted to infect the spider mite *Mononychellus tanajoae*, though the micromorphology of this pathogen did not differ from *N. floridana*. Another species, *N. abacaridis*, infects only a few eriophyids of the genera *Abacarus*, *Aculodes* (Miętkiewski and Bałazy 2003) and *Aculus fockeui*—the latter host is newly reported here. Its incidence in populations of the latter host often exceeded 50% in autumn 2007. Only a few species of the genus *Neozygites* are known as mite pathogens (Keller and Petrini 2005).

The most frequent pathogens occurring in mite communities on plants and in wood infested by insects are *Hirsutella* species. Until now 13 of their form species have been recognized in the habitats under study, among them three undescribed species. On the basis of accessible data and the authors' own observations only the species *H. kirchneri*, *H. necatrix* and *H. thompsonii* (including its variety *synnematosa*) can be treated as effective oligophagous pathogens of phytophagous mites, though their potential host ranges seem to embrace only selected eriophyid and/or tarsonemid mites, and sometimes tetranychids. In the Central and Western European climate conditions, only *H. thompsonii* and *H. kirchneri* seem to significantly reduce some eriophyid populations (Minter et al. 1983; Miętkiewski et al. 2003). *Hirsutella thompsonii* was discovered in the Tropics and principally considered a tropical species (Fisher 1950, 1951; McCoy 1996). Recent studies showed that this fungus is the common pathogen of phytophagous mites, mainly eriophyids, in central Europe on grasses and fruit trees (Minter and Brady 1980; Minter et al. 1983; Miętkiewski et al. 2003). In Poland both its synnematous and mononematous forms commonly occur and some isolates also produce stromatic outgrowths, though perithecia were not obtained.

Table 2 Acaropathogenic fungi collected in the years 2003–2007 on phytophagous, predacious and saproxylic mites in European countries, and unpublished data of some earlier collections

Fungus species	Host mite	Host plant or substrate, country, date, other remarks
Entomophthorales		
Conidiobolus coronatus (Costantin) Batko	Dendrolaelaps spp.	Picea abies, Pinus sylvestris, Quercus robur; subcortical detritus; single individuals found in several months rearings; GE 2003–2006, PO 2003–2007
Conidiobolus sp.	Bryobia sp.	Potentilla anserina; leaf underside; PO, Jun. 2004
Neozygites abacaridis Miętkiewski et Bałazy	Abacarus hystrix, Aculodes spp., Aculus fockeui[a]	Grasses, young cereals, fruit trees; leaves, exrescences, PO, Aug.–Nov., 2006–2007
Neozygites floridana (Weiser et Muma) Remaudière et Keller	Tetranychus urticae, Bryobia rubrioculus	Many cultivated species, some weeds; AU, August 2006, DK, Sep. 2001; PO, Jun.–Oct. (max. in Sep.) 2003–2007, SP, Apr. 2005
Neozygites sp.	Two infected gamasids	Lolium perenne; PO, Wielkopolski Nat. Park, Jul. 2002; scarce material
Hypocreales—Clavicipitaceous anamorphs		
Haptocillium sp.	Abacarus hystrix	L. perenne; PO, Aug. 2003, AU, Aug. 2006
Hirsutella cf. brownorum Minter et Brady	Tarsonemus potentillae, Tarsonemus sp., Dendrolaelaps sp. and juvenile acaridids	P. anserina, leaves; PO, Aug. 2004. Subcortical insect feeding galleries on P. abies and P. sylvestris (laboratory rearings); GE and PO, 2003–2007; sometimes epizootic in deteriorated cambium; Q. robur; FR, Jul. 1997
H. danubiensis sp. nov.	Tetranychus urticae[a]	P. anserina; Danube Valley, AU, Aug. 2007
H. gregis Minter, Brady et Hall	Abacarus hystrix	On grasses; GB, FR, GE, PO, Jul.–Oct. 2003–2007
H. haptospora Bałazy et Wiśniewski	Dendrolaelaps nostricornutus, Proctolaelaps sp.	P. sylvestris; galleries of bark beetles; PO, 1997–2007
H. kirchneri (Rostrup) Minter Brady et Hall	Abacarus hystrix, Aculodes dubius, A. mckenziei, Aceria tussilagofoliae,[a] Aceria sp.,[a] Phyllocoptes sp.,[a] Oribatid mite[a]	Common on grasses in Central and West European countries. On Tussilago farfara, Arctium lappa and Viola sylvestris; PO, Sep.–Nov. 2006–2007. On a branch of Corylus avellana; FR, Dec. 2004
H. minnesotensis Chen, Liu et Chen	Tarsonemus sp.	P. abies; subcortical detritus; GE, PO, 2003–2006; usually in strongly deteriorated material; rare
H. necatrix Miner, Brady et Hall	Abacarus hystrix[a]	L. perenne; FR, Sep. 2005–2007; PO, 2003–2007

Table 2 continued

Fungus species	Host mite	Host plant or substrate, country, date, other remarks
H. nodulosa Petch	Phytonemus pallidus ssp. Fragariae, Tarsonemus potentillae	On leaf underside of Fragaria grandiflora, PO. In Abies alba, Larix dahurica, P. abies, Pinus halepensis, P. maritima, P. sylvestris, Q. robur; on leaves of P. anserina; in subcortical detritus of AU, Aug. 2006–2007, GE, GR, Apr. 2004, FI, Sep. 2005, FR, PO, 2003–2007, RU, Aug. 1990
H. rostrata Bałazy et Wiśniewski	Dendrolaelaps spp., some uropodids and parasitids	Subcortical detritus P. sylvestris, P. abies, Q. robur, of common species, PO, GE, 2003–2007
H. thompsonii Samson, McCoy et O'Donnell	Abacarus hystrix, Aculodes spp., Aceria sp., A. tussilagofoliae[a]	Central-, West- and South-European countries, on grasses, coltsfoot and rosaceous shrub leaves, Jul.–Dec. 2003–2007; GR, Apr. 2004; SP, Apr. 2007
H. thompsonii var. synnematosa Fisher: Samson, McCoy et O'Donnell	Eriophyes pyri	Pyrus communis, Malus domestica, on leaves Jun.–Oct. 2003 in flower and leaf buds, PO, Nov.–Mar. 2003; DK, Sep. 2001; GB, Sep. 2004
H. tydeicola Samson et McCoy	Aceria phloeocoptes[a], Phyllocoptes abaenus[a], Tydeus sp. (Eriophyidae, Tydeidae)	Prunus domestica; PO, in bruises, Jan. 2007; on leaves, Sep.–Oct. 2007
H. vandergeesti sp. nov.	Amblyseius sp.[a], Neoseiulus sp., Seiulus sp., Typhlodromus sp., Tarsonemus lucustris	Stachys sylvatica; on leaf underside; FR, Sep.–Oct. 2007
Paecilomyces farinosus (Holm ex S. F. Gray) Fr.	Phylocoptes gracilis (Eriophyidae)	Rubus idaeus; in buds; PO, Oct. 2007
P. fumosoroseus (Wize)	Remaining of an oribatid carapace	Forest floor vegetation and litter; AU, Aug. 2007
Lecanicillium dimorphum (J. D. Chen) Zare et W. Gams	Dendrolaelaps spp. and some other gamasids	P. sylvestris, P. abies, Ulmus spp.; in galleries of subcortical insects; AU, GR, GE, FR, PO, Apr.–Nov. 2003–2007
Lecanicillium cf. lecanii (Zimmermann) Zare et W. Gams	Aceria phyllocoptes Dendrolaelaps sp., some gamasids and oribatids	Forest and meadow litter, plant leaves and subcortical detritus; GE, FR, PO; few singular isolates, rare; Apr.–Nov. 2004–2007
Lecanicillium cf. longisporum (Petch) Zare et W. Gams	Gamasids and tetranychids	In subcortical galleries and on leaves; GE, FR, PO; few isolates; Jun.–Dec. 2003–2006
Lecanicillium muscarium (Petch) Zare et W. Gams	Acaridids, eriophyids, gamasids, uropodids, tetranychids	On plants and in subcortical galleries, common in moist habitats or during wet periods on insects; AU, FR, GE, PO, Apr.–Nov. 2003–2007; RU, Aug. 1990; mycoses of mites singular.

Table 2 continued

Fungus species	Host mite	Host plant or substrate, country, date, other remarks
Lecanicillium psalliotae (Treschow) Zare et W. Gams	*Dendrolaelaps* spp.	In subcortical material; AU, GE, FR, PO, 2004–2007; occasionally isolated even in dry conditions
Lecanicillium spp. (various strains)	*Abacarus hystrix, Aculodes* sp., *Euseius finlandicus*,[a] eriophyids, gamasids, and other oribatids	On plants, in subcortical detritus and in organic soil or litter; not rare; AU, FR, GE, PO, Jul.–Nov. 2004–2007; RU, Aug. 1990, singular isolates
Simplicillium lanosoniveum (van Beyma) Zare et W. Gams	*Proctolaelaps* sp., uropodids	In subcortical detritus and litter from a meadow; GE, PO, Jun.–Nov. 2003–2006; singular isolates
Simplicillium spp. (various strains)	Gamasid mites	In subcortical materials; GE, PO, Jun.–Nov. 2003–2007; singular isolates
Other hyphomycetales		
Acrodontium crateriforme (van Beyma) de Hoog	*Neotetranychus rubi, Dendrolaelaps* sp., uropodids	On plant stems and leaves and under bark; often numerous, suggesting pathogenicity to arthropods; GE, FR, PO, Jul.–Sep. 2004–2005, 2007
Cladosporium cladosporoides (Fres.) de Vries	*Abacarus hystrix, Aculus schlechtendali*,[a] *Eriophyes pyri*[a]	*Agropyron repens, P. communis, P. domestica*; most common on plants and tree branchlets; all countries and sites; Jul.–Nov. 2003–2007
Ramularia ludoviciana Minter, Brady et Hall	*Abacarus hystrix, Aculodes mckenziei*[a]	On *L. perenne, Festuca rubra, Agrostis stolonifera*; AU, GE, FR, PO, Aug.–Dec. 2003–2007; in late summer and autumn
Sporothrix schenki Hektoen ex Parkins	Eriophyids, gamasids, acaridids and others	On grasses, in subcortical detritus and feeding galleries in wood; GE, FR, PO, Jul.–Nov., every year; pathogenic to eriophyids; not rare; particularly on juvenile instars

AU Austria, *DK* Denmark, *FI* Finland, *FR* France, *GE* Germany, *GB* Great Britain, *GR* Greece, *PO* Poland, *RU* Russia, *SP* Spain

[a] Newly recorded host mites

ITS sequences indicated practically no differences between *H. kirchneri* and *H. necatrix* on the genetic level; these species form together with *H. gregis* a well separated clade (Figs. 1, 2). The strains of *H. thompsonii* display much greater heterogeneity. A tree made with Neighbor Joining only with *H. thompsonii* strains and *H. necatrix* as an outgroup reveals close affinity of all three specimens (not shown). The strain Dq345579 (Xiang et al. 2007) was isolated in the USA by C. W. McCoy in 1981 from citrus rust mite *Phylloc-optruta oleivora*. The strain 3699 came from *Eriophes pyri* (Łosice, Poland) and was described as *H. thompsonii* cf. var. *synnematosa*. Data on the origin or morphology of strain Af293899 are missing in the GenBank Database, as in publications. Hodge (1998) obtained two sequences of *H. thompsonii* var. *synnematosa*. Both are similar to the 3699 strain sequence and form one clade also with Dq345579, Af293844. A similar result for other DNA regions could be a reason for separation of a new taxon from the large and divergent *H. thompsonii*. The closest to this group appeared the rather homogenous *H. minnesotensis* clade (Figs. 1, 2). This latter species is nematopathogenic, and has been isolated repeatedly from juvenile and mature individuals of tarsonemid and probably anoetid mites, reared in rotten wood of Norway spruce and black cherry in the laboratory (Bałazy et al. 2008). Single individuals have been found continuously in material from Bavarian spruce forests over a period of up to 2 years of rearing.

Hirsutella tydeicola found on *Tydeus* sp., *Phyllocoptes abaenus* and *Aceria phloeo-coptes* on plum trees, is newly recorded from Poland and perhaps from Europe. In phylogenetic trees it is situated near the nematopathogenic *H. rhossiliensis*, but at con-spicuous distance. We found no information on its artificial culturing, so we inserted mycelium of the obtained strains on SDA medium. The fungus grew slowly, attaining 2 cm colony diameter during 1 month. Aerial mycelium forms a hard and wrinkled layer of about 2–3 mm thick, in the centre elevated up to 5 mm of the surface, floccose and white with a slight navy-blue tint. The hyphae are evenly thick (1.3–3.8 μm in diameter). Phialides usually perpendicular or nearly so, protruding singly, 21–37 μm long, rarely attaining up to 50 μm; their basal parts are 3.8–5 μm thick, pretty abruptly narrowing near the half of their height into needle-like smooth necks, 0.5–0.7 μm thick at the tip. Phia-lospores fusiform, a little bent with the distal end subacute, 4.8–6.2 × 2–3.2 μm, thickest in the central part, set in twos stuck by mucus on the neck ends. Culture reverse brown with the stain diffusing to the medium at over 2 cm zone around the colony margin.

A much wider host range represents *H. nodulosa*, till now reported from insect larvae and several saproxylic or predacious mite species (Bałazy et al. 2008). Since some of this species' strains showed the ability to produce string-like or cylindrical synnemata of the phialides unlike those protruding on their mononematous hyphae (Fig. 3), we selected them for comparative molecular analysis (work in progress). An acaropathogenic strain of this fungus isolated from a tarsonemid mite displays closest genetic affinity to *H. aphidis* from the aphid *Dysaphis plantaginea* (bootstrap support 98%, posterior probabilities 1.0). The closest teleomorphic state for both is *Cordyceps cochlidiicola*, whereas *H. vermicola* and *H.* cf. *brownorum* are placed in the same clade (bootstrap support 96%, posterior probabilities 1.0).

Similar polyphagous aspects are suspected in *H. haptospora* and *H. rostrata*. The first was originally isolated from uropodid mites found in ant nests (*Formica polyctena*), but morphologically identical strains were found on digamasellid mite species (mostly *Den-drolaelaps*) and sciarid midge larvae inhabiting subcortical galleries of cambiophagous insects on Scots pine logs and branches in Poland (Notecka Forest). Recently it was also found on a sciarid larva in subcortical detritus of *Mimosa* wood near Alès, in southern France. Contrary to the easily isolated strains from midges, the fungus on mites appeared
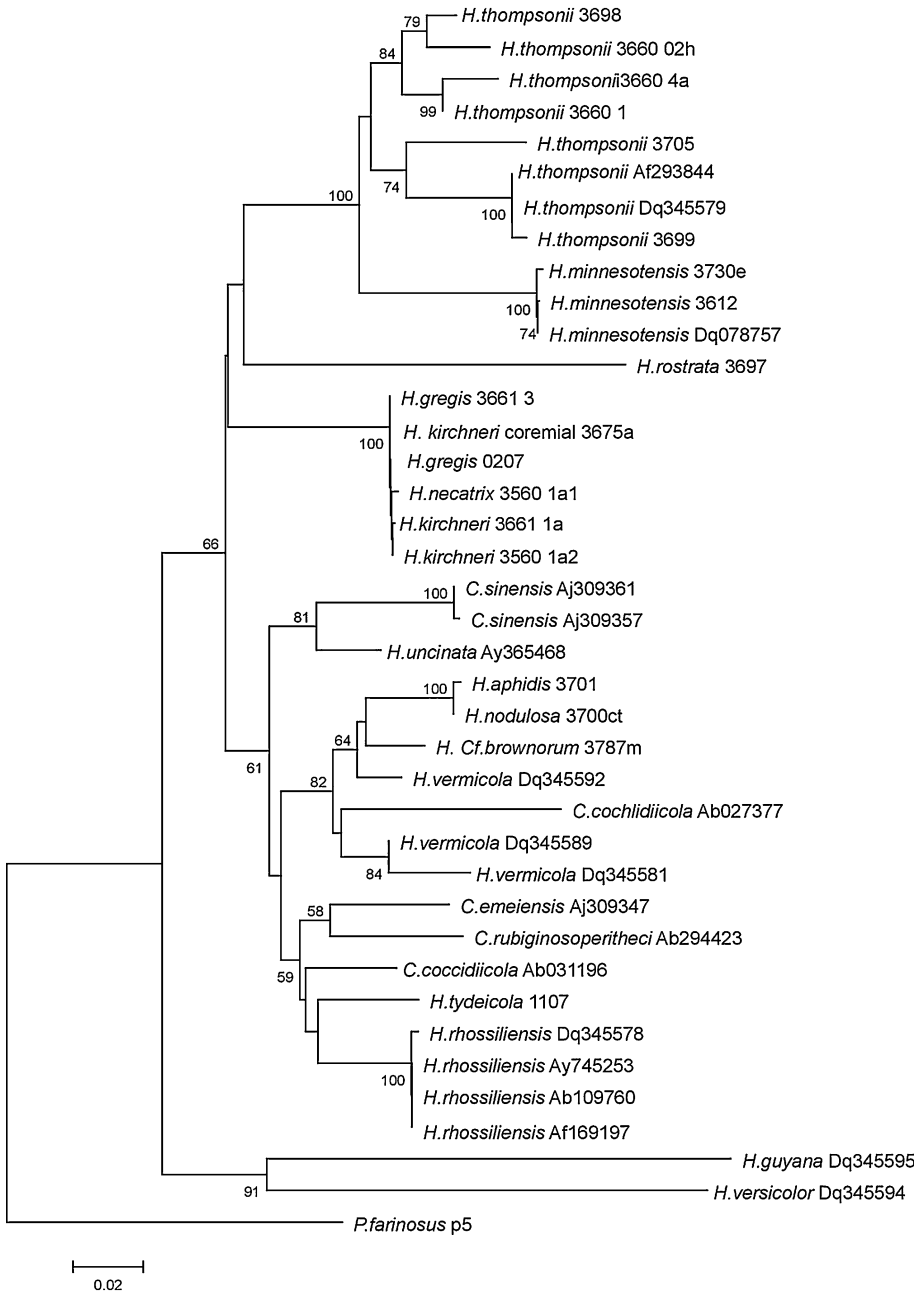
Fig. 1 Phylogenetic tree of *Hirsutella* and *Cordyceps* species obtained with Neighbor Joining method (Kimura, two parameters model). Numbers below the branches are bootstrap percentage values based on 10,000 replicates
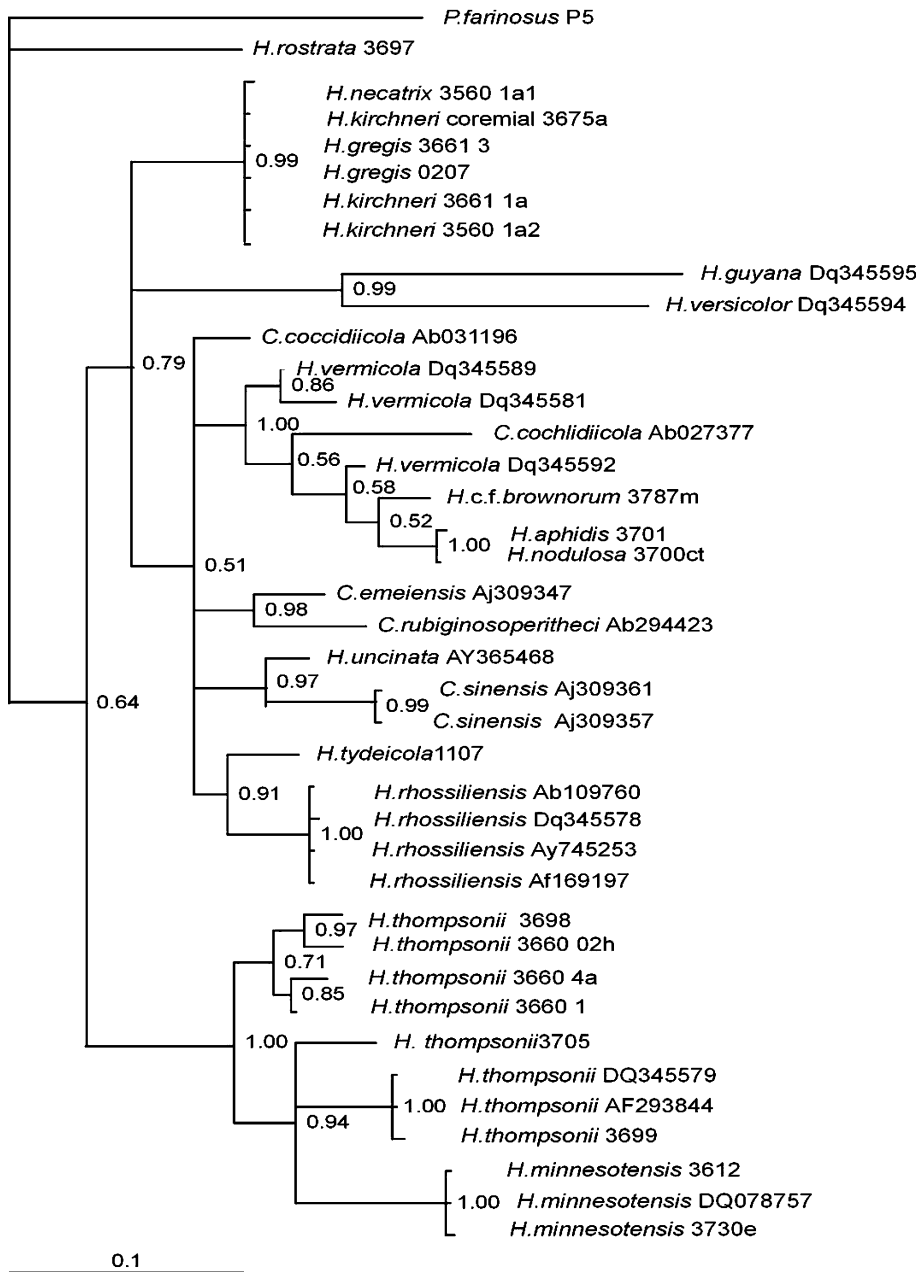
Fig. 2 Phylogeny (consensus tree) of *Hirsutella* and *Cordyceps* species derived from the ITS region data set using Bayesian analysis. Bayesian posterior probabilities are given on nodes

recalcitrant for culture isolation, which hinders a comparison of their genetical markers. *H. rostrata*, the species common on dead mites found in bark beetle galleries, has lately been found on a beetle larva, but attempts to artificially infect birch bark beetle (*Scotylus*

Fig. 3 *Hirsutella nodulosa*, sporulation on synnemata

ratzeburgi) larvae and codling moth (*Cydia pomonella*) caterpillars with its spores failed (Bałazy et al. 2008).

A fungus close to *H. brownorum* often occurs on instars of tarsonemids and, supposedly, acarid, anoetid and parasitid larval forms (seldom also adults) in rotting cambium among the galleries of bark beetle and sciarid larvae under the bark of coniferous trees. However, the shape of its phialospores and its hyphal diameter hardly match the original description of *H. brownorum* (Minter and Brady 1980). The only culture obtained yet allowed us to establish its position in the phylogenetic tree between *H. nodulosa* and *H. vermicola* (Figs. 1, 2). We apply for it the designation *H.* cf. *brownorum* until its nomenclatural position is solved, which can be difficult because of the scarcity of original material of *H. brownorum*. The fungus seems to be widely distributed and it affected over 50–60% of juvenile mites in some bark and wood laboratory rearings. Mycelium on host mites is rather scarce, with delicate hyphae (2–2.8 μm thick) protruding mostly from mouthparts and tarsal segments of the legs, procumbent among the cambium fibres and insect excrements at 3–6 mm distance from the host body. Phialides usually 12–25 long (extreme 32 μm), with the basal ampulliform part 6–12 × 4–5 μm, with single or bi- to four-furcate thin necks of the terminal parts delicately twisted. They protrude singly or oppositely, sometimes in groups of three, laterally from the hyphae in rather distant intervals ca. 70–150 μm. Phialoconidia of somewhat asymmetrical outline 4.5–5.8 × 2.8–3.5 μm, smooth, with a small wart-like projection on the distal end directed to the side; the spores are produced singly or in twos on the necks; on furcate or polyphialidic sporogenous cells normal conidia developed only on one neck, on the second one and further they were underdeveloped or not produced at all. The culture was obtained only once on SDEYA agar, and difficult to isolate; at first mycelium was delicate, white, non-sporulating, and velvety. After enrichment with additional egg yolks consecutive secondary subcultures were growing a little faster and in some of them single conidiogenous cells with very weak sporulation were produced.

Although we investigated entomopathogenic fungi in broad context, the common polyphagous insect pathogens, e.g., *Beauveria*, *Metarhizium*, *Paecilomyces* (or *Isaria* as proposed by Sung et al. (2007)) or the spider-pathogenic *Gibellula* species, did not or

rarely appear on mites. Some higher infection rates appeared in cases of a few *Lecanicillium* species, but only in favorable humidity conditions and at simultaneous high prevalence rates of aphid or scale insects diseases, which seem to be the primary sources of infective material. Among the most common of these pathogens, *L. muscarium* appeared in autumnal months on *Stachys sylvatica* leaves infested by *Cryptomyzus ribis* aphids, but the infections of mites were sporadical. More *Lecanicillium* and *Simplicillium* strains were isolated from mites from the subcortical communities, but their pathogenicity to mites needs to be verified experimentally. Apart from the most common forms close to *Lecanicillium* cf. *lecanii*, also *L. psalliotae*, *L. polymorphum* and *Simplicillium lanosoniveum* were isolated repeatedly. Although some *Lecanicillium* strains isolated from mites showed micromorphological features identical to isolates from insects, their cultures could differ considerably in macromorphological aspects, e.g., mycelium texture, growth intensity, medium staining and others. This suggests that the biological diversity of these organisms is greater than mentioned in recent monographs (Zare and Gams 2001). In two grass samples strains of *Haptocillium* were obtained from dead eriophyids. Mainly in autumn, *Cladosporium* spp. were also isolated from dead mites, though their pathogenicity to mites and insects has as yet been documented weakly.

The constant component of fungal communities of eriophyid mites feeding on grasses is *Ramularia ludoviciana*. Its pathogenic abilities to eriophyids have been confirmed lately in trials by R. Miętkiewski (unpublished). As most acaropathogenic fungi it begins to appear in mid-summer and peak mite mortality falls in October and the first half of November.

Description of two new *Hirsutella* species

Two of the obtained *Hirsutella* forms could not be identified, so we decided to give their full morphological characteristics as new species. Unfortunately, numerous and careful trials to isolate cultures on artificial media failed.

Hirsutella danubiensis Tkaczuk, Bałazy et Wegensteiner, spec. nov. (Fig. 4a, b)

Fungus acaropathogenicus. Mycelium in acaris copiosum, album ex longis et aequoangustis hyphis crassitate 2.5–5.0 μm constans, cum septis in spatia 15–55 μm collocatis. Hyphae ex acarorum mortuorum corporibus radiate excrescunt et circiter eorum sub Potentilla anserina foliis extenduntur. Phialides multae, tenuiconicales ad basim 2.5–3.7 crassae, 35–62.5 μm longae (longitudine media 48.5 μm), modulate in summum exile collum, angustatum ad 0.8–1 μm crassum, nonnumaquam furcillatum, cuius superficies minimum aspera, attenuantur. Phialoconidia parva, dimensionibus 4.5–6.6 × 2.0–2.7 μm (in medio 5.2 × 2.4 μm), instar citri sinensis fructus segmentorum, in apicali parte paulo fortius attenuata, bilateraliter symmetrica, singula vel bina in summis collis formantur.

In acaris mortuis Tetranychidarum (Tetranychus urticae) ex Potentilla anserina foliis in Danubii fluminis valle prope Vindobonam die 12 mensis Augusti anno 2007 collectis. Holotypus: specimen numero 1208 designatum, in collectione Universitatis Podlasiensis in Siedlce.

Hirsutella danubiensis Tkaczuk, Bałazy et Wegensteiner, sp. nov. (Fig. 4a, b)

Mycelium abundant dirty-white of long equally-narrow hyphae 2.5–5.0 μm, with the septa distributed in uneven distances 15–55 μm, protruding radially from dead mites and
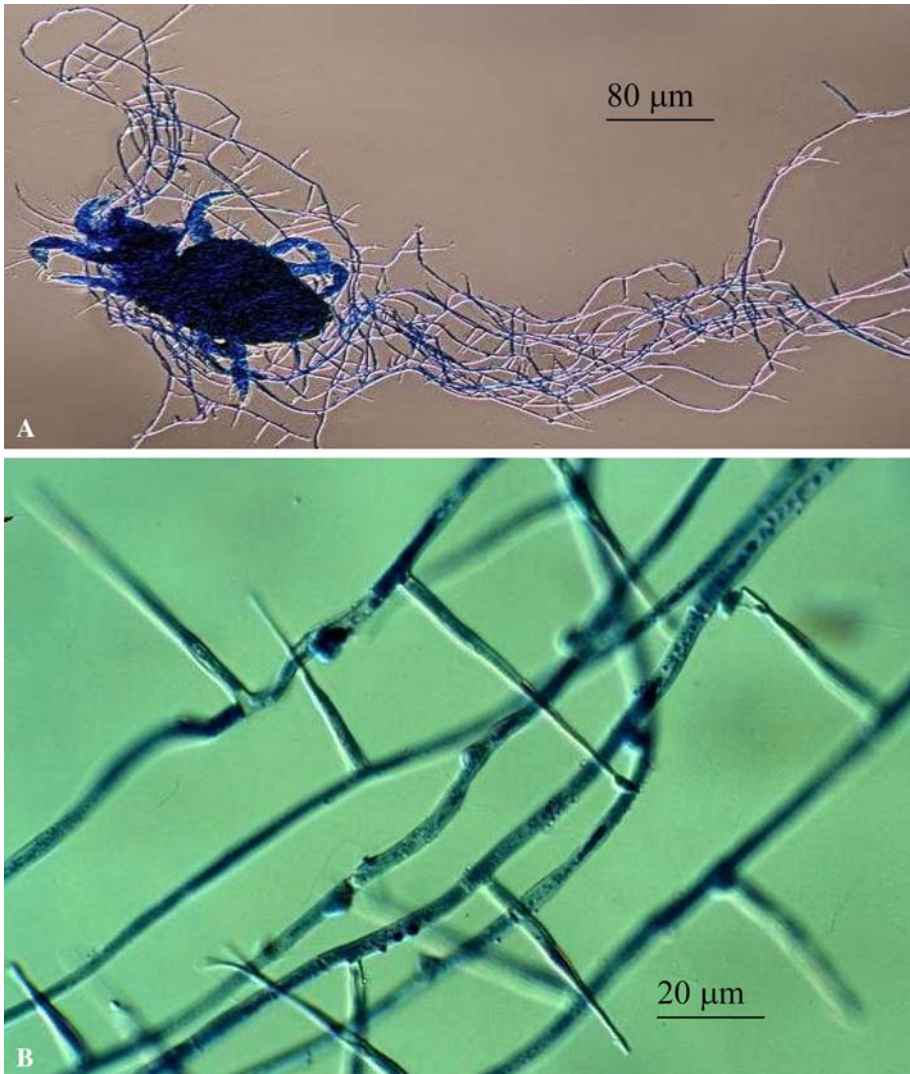
Fig. 4 *Hirsutella danubiensis* sp. nov. **a** Mycelium on spider mite *Tetranychus urticae*, **b** fialides and conidia

outspread around them on the leaf surface. Phialides numerous, very long, arising singly at right angles from hyphae, 2.5–3.7 μm wide at the base, 35–62.5 μm long, (average 48.4 μm), sometimes forked, slender, and from approximately 2/3 of their total height gradually tapering into a slightly warted equally narrow neck, about 0.8–1 μm thick, on the summit. Phialoconidia small, 4.5–6.6 × 1.9–2.7 μm of the shape of orange segment, more strongly narrowed at the distal end and bilaterally symmetrical, produced and persisting singly or stuck in twos by a thin layer of mucus on the top of necks.

On dead *Tetranychus urticae* (Tetranychidae) collected on *Potentilla anserina* leaves in the Danube river valley near Vienna (Austria), in August 2007. The specimen nr 1207 in

Fig. 5 *Hirsutella vandergeesti* sp. nov. **a** Mycelium on phytoseid mite, **b** hyphae, phialides and phialospores

the collection of the Department of Plant Protection of the University of Podlasie in Siedlce, found on 12 August 2007 is designed as a holotype. *Hirsutella danubiensis* could be easily distinguished from the other *Hirsutella* species producing small conidia, and by its very long phialides of general narrowly conical appearance, without conspicuous basal distension. Etymology: related to the Danube valley as the site of its occurrence.

Hirsutella vandergeesti Bałazy, Miętkiewski et Tkaczuk, spec. nov. (Fig. 5a, b)

Fungus acarorum pathogenicus. Acari mortui corpus textis hypharum, e cellulis elongatis vel ovoidaeis, 2–6 µm crassis constantium implent. Hyphae irregulares ramos formant et in basis partibus coxarum atque chelicarum inhaerescunt. Hyphis aerinis copiose increscentibus cellulae mycelii intra hospitis corpus ovoideae aut subglobosae diametro 4–7 µm, continent mycelii hyphalis reliquias, cuius cellulae in cruribus elongatae sunt. Hyphae aerinae aequotenues, crassitudine 3–4 (–4.5) µm, exigue ramosae, ad longitudinem

3–4 mm radiate excrescunt, maxime inter scutum dorsale et ventrale atque circum partes buccales, anales et inter basis particula coxarum. Hyphae perveniunt ad longitudinem 2.5– 3 mm, plerumque circum acaros mortuos mycelii canoalbi texta densa formantes. Parietes hypharum externi clarofusci superficie polita, septa hyalina, paululum distincta, regulariter in spatia 10–18 μm collocantur. Phialides tenuiconicales, maxime crassae ad basim, ex hyphis directe, aliquando in hypharum finibus paulo oblique excrescunt. Quarum dimensiones (27–) 30–37 (–43) × 4–4.5 μm ad basim, usque ad circa 4/5 suae longitudinis totaliter recte ad crassitudinem 1.5–2 μm attenuantur. Adeo paries clarofuscus et crassus phialides protegit. In phialidis apice tenuissimus (∼0.5 μm), achromaticus processus plasmaticus, 4–7 μm longus invenitur, in cuius apice phialospora formatur. Raro ex una phialide duo colla crescunt, quorum alterum saepe – sed non semper – brevius est. Raro etiam collum supra phialidis segmentum crassoparietalis furcatam formam habet, quamquam duarum sporarum formatio in collis bifurcatis rarissime notabatur. Phialoconidia parva dimensionibus (4.0–) 4.2–5.5 × 2.1–2.5 (–3.0) μm, instar fructus citri sinensis segmentorum, in parte apicali magis attenuata, saepe cum fine paululum acuto, bilateraliter symmetrica, fere semper singula et mucotecta formantur.

In acaris mortuis Phytoseiidarum (Amblyseius sp. Neoseiulus, Seiulus sp., Typhlodromus sp.), et Tarsonemidarum (Tarsonemus lacustris) ex Stachydis sylvaticae foliis collectis, in silva humida Saint Gobin prope Cessières, in Francogallia, Septembri et Octobri mensibus anno 2007. Folia ab insectis Aphidarum et Coccodearum atque ab acaris Tetranychidarum indefinitis, quae non sunt infecta, inhabitantur. Holotypus: specimen numero 4200 g designatum, in collectione mycologica Insituti Agrariae et Silvestris Oecologiae Academiae Scientiarum Polonorum Posnaniae depositum.

Hirsutella vandergeesti Bałazy, Miętkiewski et Tkaczuk sp. nov. (Fig. 5a, b)

Acaropathogenic fungus; the body of a dead mite filled with hyphae consisting of elongated or oval cells 2–6 μm thick, irregularly branched and penetrating also into basal parts of legs and chelicers. During the period of the abundant growth of aerial mycelium, the cells of the internal hyphae take ovoid or subglobose forms 4–7 μm in diameter and steadily disappear with age, apart from some elongated cells only in legs. Aerial hyphae equally narrow 3–4 (–4.5) μm thick, poorly branched and up to 3–4 mm long, grow radially around the mite between dorsal and ventral discs, a little more abundant at the mouth parts and between the basal segments of legs. Outer walls of the hyphae light brownish, smooth; septa hyaline, distributed regularly in distances of 10–18 μm. Phialides narrowly conical, thickest at the base, protruding perpendicularly, sometimes somewhat obliquely in the terminal parts of hyphae. Their measurements are (27–) 30–36 (–43) × 4– 4.5 μm at the base, converging rectilinearly up to the height of approximately 4/5 of their total length where they attain the thickness of 1.5–2 μm. Up to this point they are covered with a thick light-brownish wall as in hyphae and never forking. The apical part of each phialide forms a very thin (0.5 μm) and thin walled, colourless plasmatic outgrowth 4– 7 μm long, with a phialosphore at its tip. Very seldom two such necks grow on one phialide, one of them almost always shorter. The formation of two normal phialospores on forked necks only very rarely observed. Phialoconidia small, (4–) 4.2–5.5 × 2.1–2.5 (– 3.0) μm, shaped like an orange segment, more strongly narrowed at the distal end and ventrally sinuous beneath subapiculate tip. The spores are covered with a thin layer of mucus.

On dead mites of the families Phytoseiidae (*Amblyseius* sp., *Neoseiulus*, *Seiulus* sp., *Typhlodromus* sp.) and Tarsonemidae (*Tarsonemus lucustris*), collected on *Stachys*

sylvatica leaves in the flood plain forest St. Gobin near Laniscourt (France), in September and October 2007. The plants were infested by aphids, coccids and tetranychid mites, which were never found to be infected by this fungus. Specimen nr. 4200 g in the collection of the Research Centre for Agricultural and Forest Environment, found on 1 September 2007, is designated as holotype. Etymology: to honour Dr. Leo Van der Geest's merits in acarology.

Conclusions

Fungal diseases of mites occurring on plants, under bark (in feeding galleries of cambio-xylophagous insects), and in decayed wood are widespread in (semi)natural habitats. The fungal pathogens of mites are closely related to most insect pathogenic fungi but only few species are capable to infect both insects and mites. Among five reported representatives of the order of Entomophthorales only one pleophagous species, *C. coronatus*, appears capable to infect hosts of both groups, whereas from among 13 acaropathogenic *Hirsutella*-species, classified as hyphomycetous clavicipitalean anamorphs of ascomycetes only for two (*H. nodulosa* and *H. rostrata*), single insect species have so far been reported as hosts. Alternatively, from the common and typically entomopathogenic anamorphs only two species of the genus *Paecilomyces* were found on single mites within this study.

A preliminary attempt to determine the affinity of particular acaropathogenic species of *Hirsutella* by the analysis of ITS region sequences of the genomic DNA showed two distinct groups infecting some phytophagous eriophyid, tarsonemid and tetranychid mites. The first is formed by three very close species, *H. gregis*, *H. kirchneri* and *H. necatrix*; the second group, containing *H. thompsonii* and its variety *H. t.* var. *synnematosa*, appears more variable. Other species are scattered singly in dendrograms, neighboring with the entomopathogenic (*H. nodulosa*, *H.* cf. *brownorum*) or nematopathogenic (*H. thompsonii*, *H. tydeicola*) clades, except *H. rostrata* which is clearly separated. Two newly described species, basing on the differences in morphology (*H. danubensis* and *H. vandergeesti*), appeared too fragile for culture isolation, therefore could not be included in ITS sequencing. Currently *Lecanicillium*, *Simplicillium* and allied taxa are subjected to insect and mite pathogenicity bioassays, as well as morphological analysis including exact biometrics and successive nucleic acid sequencing.

The majority of the recorded acaropathogenic fungi affecting mites on plants appear from about mid-summer, increasing in density till the first frost. Some of them, e.g., species inhabiting subcortical insect galleries and those hibernating on branches or in buds and excrescences (zoocecidia) on twigs, may be collected for laboratory studies all through the year. Resting spores of *Neozygites* species may be collected from fruit tree branches. The species *H. kirchneri* and *Ramularia ludoviciana* can also be found on grasses during winter, under the snow.

Fungal acaropathogens on plants appear characteristically in very small patches, distributed randomly over the area of potential host distribution. Though the local incidence rates may be high, e.g., *H. thompsonii*, *H. kirchneri* or *N. abacaridis* on eriophyids feeding on grasses or plum leaves, this does not seem to have much effect on the general distribution and density of their host mites. In fresh subcortical insects' feeding grounds, mycosed mites were seen only singly, and their density increases usually after 2–3 weeks of rearing.

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