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Gnathosomal interlocking apparatus and remarks on functional morphology of frontal lobes of eriophyoid mites (Acariformes, Eriophyoidea)

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Abstract Comparative study of eriophyoid mite gnathosomata using different microscopic techniques shows presence of new dorsoproximal structures (here described as "interlocking apparatus"), which could consolidate mouthparts in rest (non-feeding) position and during feeding actions. Four types of the interlocking apparatus are described. Differences in the interlocking mechanisms coincide with the results of recent molecular phylogenetic studies of Eriophyoidea and provide additional support for the major clades revealed in those studies. Comparative analysis on the morphology of the frontal lobe of the prodorsal shield shows that mechanical protection of the motivator surrounding area and interlocking apparatus are possibly the primary function of the frontal lobe. Unusual shapes of the frontal lobe in selected eriophyoids (elongated, broadened, notched lobes) might result from additional functions that the frontal lobe can perform, e.g. providing additional guide for palps and stylets, or hermeticity of the space between dorsoproximal palpcoxae during feeding.

Keywords Confocal microscopy · LTSEM · Comparative morphology · Arthropod phylogeny · Feeding behavior · Phytophagous mites

Introduction

Eriophyoid mites are an ancient group of highly specialized plant parasites with distinctive mouthparts (gnathosoma) adapted for piercing plant cells, injecting saliva and sucking their cell sap (Lindquist 1996; de Lillo et al. 2002; de Lillo and Monfreda 2004; Schmidt et al.

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2012; Sidorchuk et al. 2014). The eriophyoid gnathosoma includes paired palps and seven or nine (according to Lindquist 1996, p.5) stylets enclosed in a subcapitular sheath. In slide mounted mites these structures usually are deformed/displaced (or vice versa, merged tightly) and only rarely they are exposed for observation under a light microscope in flattened specimens with accidentally disjointed mouthparts (e.g. Fig. 1b). However, using only phase contrast light microscopy, Keifer (1959) analyzed a variety of gnathosomal structures in eriophyoid mites and argued for the importance of gnathosomal morphology for classifying Eriophyoidea. He also found a peculiar medial protuberance, dorsoproximal on the palpcoxae in some eriophyoids, which he called the "cheliceral retainer" (Keifer 1959, p. 280). General gnathosomal morphology was studied using various modifications of scanning electron microscope (SEM) techniques in the second half of the twentieth century (Nuzzaci and Alberti 1996 and papers cited therein). Recently, current information on eriophyoid gnathosomata was reviewed and supplemented in a separate chapter of the PhD thesis of Craemer (2010).

In the course of our recent collaborative study of *Mackiella reclinata* Chetverikov and Craemer 2014 (Chetverikov et al. 2014b) we analyzed numerous micrographs of
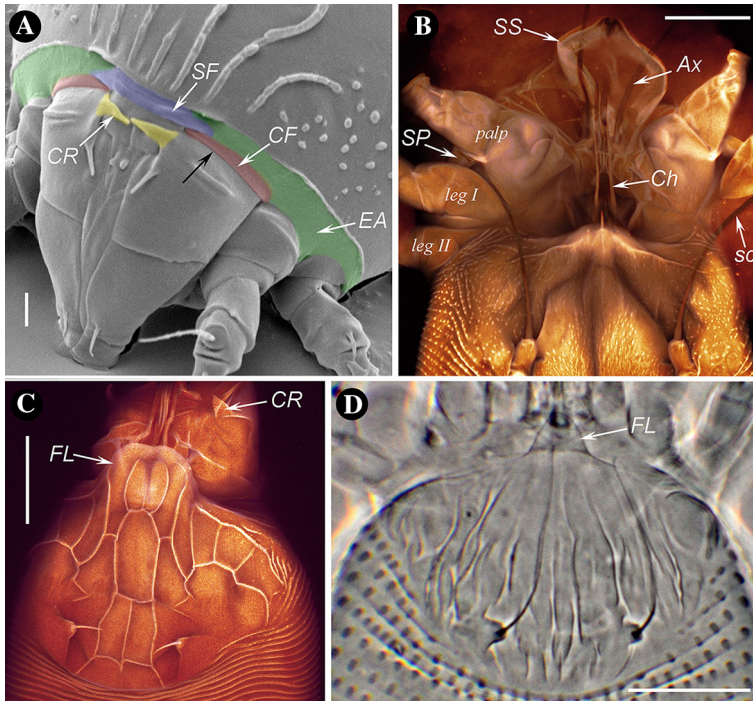

Fig. 1 Selected structures in proximal gnathosoma and adjacent regions of eriophyoid mites. **A** Anterior prosoma of larva of *Tergilatus sparsus* (Meyer and Ueckermann 1995) (LTSEM); **B** dorsal aspect of female prosoma of *Nalepella tsugifoliae* (Keifer 1953) with disjointed mouthparts (CLSM); **C** prodorsal shield of *Keiferella juniperici* (Boczek 1964b) (CLSM); **D** prodorsal shield of *Colomerus* cf *vitis* (Pagenstecher 1857) (PCLM). Notations: *CF* circumcapitular flange (coloured *pink*), *EA* epicoxal area (coloured *green*), *SF* supracapitular fold (coloured *blue*), *CR* cheliceral retainer (coloured *yellow*), *FL* frontal lobe, *SP* fragment of suboral plate, *SS* subcapitular sheath, *Ax* auxiliary stylet, *Ch* chelicera, *sc* scapular seta of prodorsal shield. *Black arrow* on **a** indicates circumcapitular furrow. *Scale bar* **a** = 2 μm; **b**, **c** = 30 μm; **d** = 15 μm. (Color figure online)

gnathosomata of various European and South African eriophyoids captured by us over the last 15 years. These images were results of different microscopic techniques (mainly SEM and CLSM). We studied the micrographs to especially evaluate the unusual shape of the frontal lobe of *M. reclinata*. We additionally concurred that several new (previously not described) minute gnathosomal structures were present in the images which seem to be involved in keeping the mouthparts in position. These structures are associated with the basal parts of the chelicerae, medioproximal palpcoxae and the antero-dorsal margin of the prodorsal shield. We provisionally name this complex of gnathosomal structures the "gnathosomal interlocking[1] apparatus" (see details in "Discussion"). We noticed that morphology of this apparatus considerably varies among different eriophyoid taxa and hypothesized that there are several types of interlocking mechanisms in Eriophyoidea. We also anticipated that the differences between these types might correspond with major taxonomic groups of eriophyoid mites, e.g. families of eriophyoids proposed by Amrine et al. (2003) and large clades found by Chetverikov et al. (2014c) and Li et al. (2014) in their molecular phylogenetic analyses. In this study we primarily aimed to test these two hypotheses based on the available material (digital images of mites from our personal databases). In this paper we (1) describe the new structures found in the proximal gnathosomata of selected eriophyoids, (2) briefly discuss different types of interlocking structures and inferred mechanisms and their possible phylogenetic significance, and (3) give some remarks on the functional morphology of eriophyoid frontal lobes.

Materials and methods

Taxon sampling

In this study we analyzed digital images (SEM, CLSM, differential interference contrast light microscopy (DICLM) and phase contrast light microscopy (PCLM), see below for methodology) of about 80 mite species from the three currently recognized eriophyoid families (Phytoptidae, Eriophyidae and Diptilomiopidae). These images had been captured in the course of extensive study of the diversity of eriophyoid mites in 2002–2003 in South Africa (and one species from France), and in 2010–2014 in the USA and the European part of Russia (performed by C.C. and P.C., respectively). We compared prodorsal shields and proximal gnathosomata of these mites in order to find morphological differences in gnathosomal morphology, which might be used for testing the current classification of Eriophyoidea at family level. We were especially interested in (1) studying gnathosomata of phytoptids (including the recently described early-derivative mite *Loboquintus sub-squamatus* Chetverikov and Petanovic 2013 (in Chetverikov et al. 2013) as they were presumed to be an ancient assemblage of putatively archaic eriophyoids, and (2) comparing gnathosomal structures of eriophyids and diptilomiopids in order to test if they comprise a monophyletic group (as was suggested in recent molecular phylogenetic studies by Li et al. (2014) and Chetverikov et al. (2014c)) or if they are two separate groups (sensu Amrine et al. 2003). All the mite species mentioned below in the text were studied using SEM, LM or CLSM (if not mentioned otherwise). Those collected in South Africa, and *Trisetacus* cf *pinastri* from France, were studied by C.C. (16), the others collected in Europe were studied by P.C. (15). Mites of the following genera were studied. Family Phytoptidae:

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[1] The term "interlocking" was initially proposed in the PhD thesis by Craemer (2010) where she described two interlocking mechanisms discussed as types I and III in this paper (see below).

Loboquintus (1 sp.), *Mackiella* (1 sp.), *Nalepella* (1 sp.), *Oziella* (2 spp.), *Phytoptus* (2 spp.), *Setoptus* (2 spp.), *Trisetacus* (5 spp.). Family Eriophyidae: *Aceria* (3 spp.), *Acalitus* (1 sp.), *Calacarus* (1 sp.), *Colomerus* (1 sp.), *Ectomerus* (1 sp.), *Keiferella* (1 sp.), *Leipothrix* (1 sp.), *Phyllocoptes* (1 sp.), *Platyphytoptus* (1 sp.), *Tergilatus* (1 sp.). Family Diptilomiopidae: *Diptacus* (1 sp.), *Diptilomiopus* (3 spp.), *Rhyncaphytoptus* (1 sp.).

DICLM and PCLM

In addition to conventional SEM and CLSM observations, the specimens were slide mounted in two different chloral hydrate based media: Hoyer's (Dobrivojevic and Petanovic 1982) and modified Berlese (Amrine and Manson 1996). The slide mounts were studied in the Laboratory of Parasitology of the Zoological Institute of RAS (Russia) under phase contrast (PC) and differential interference contrast (DIC) light microscopy (LM) using a Leica DM750 (under Hi-plan 100x/1.25 oil Ph3 objective) and a Leica DM5000 (under HCX PL Fluotar 100x/1.30 oil DIC objective) compound scopes, associated with digital microscope cameras (ToupCam and Leica DFC320). Specimens were identified and images captured using ToupCam and Leica DFC320 Imaging Software. All the slides are deposited in the National Collection of Arachnida-Acari (NCA-Acari), Plant Protection Research Institute (ARC-PPRI, South Africa) and the Acarological Collection of the Zoological Institute of Russian Academy of Sciences (ZIN RAS).

SEM

Two SEM techniques were used: (1) low temperature SEM (LTSEM) and (2) conventional SEM. The LTSEM study was performed by C.C. in the Laboratory for Microscopy and Microanalysis of the University of Pretoria (South Africa) under a conventional JEOL JSM 840 SEM with a cryo-stage using the protocol described in Chetverikov et al. (2014b). Conventional SEM study of mites was performed by P.C. in the Laboratory of electronic microscopy of ZIN RAS (Russia). The live mites were sputter-coated with platinum in the chamber of Eiko Engineering Co., Model IB-5 (1984-2), and observed under SEM in the vacuum chamber of a Quanta 250 SEM using a previously described protocol (Chetverikov et al. 2013). SEM images were captured digitally in TIFF format using a frame grabber controlled by Orion[®] 6.6, Belgium and the proper Quanta frame grabber.

CLSM

CLSM acquisition was carried out by P.C. in the Center of Microscopy and Microanalysis of Saint-Petersburg State University (Russia) using the Leica TCS SP2 Spectral confocal and multiphoton system. The CLSM technique applied in this study followed those described in Chetverikov (2012, 2014b), Chetverikov et al. (2012) and Kirejtshuk et al. (2014). CLSM images were carried out using Amira[®] 5.3.2 software according to Amira User's Guides based on the confocal stacks. All 3-dimensional images were recorded using "PrintScreen" keyboard function or "Snapshot" command embedded in Amira[®]. The presented CLSM images were obtained by using a combination of Orthoslice module and several Voltex modules with different Amira[®] colormap filters, which was very useful to emphasize the structures of interest (by accentuating their contrast) (for details see Chetverikov 2014a, p. 134). All images were optimized with Photoshop[®] CS11 version 12.1 (Adobe Systems)

Results

New structures observed in proximal gnathosoma and adjacent regions

Circumcapitular flange and epicoxal area (Fig. 1)

The prosoma of eriophyoid mites includes the gnathosoma and two pairs of legs. Dorsally it bears a prodorsal shield and is ventrally represented by coxisternae and the suboral plate. A ring of soft cuticle separates the basal gnathosoma from the idiosoma dorsally and forms a flexible articulation joining the gnathosoma to the body. This articulation corresponds to the circumcapitular furrow (sensu Walter 2005), surrounds the basal gnathosoma and is usually distinctly visible in SEM images, mostly in immatures (Fig. 1a, black arrow). Only the dorsal part of this furrow is observable because the ventral part is usually obscured under the suboral plate. We observed two distinct cuticular areas in the antero-lateral walls of the prosoma. We name these areas the *circumcapitular flange* and the *epicoxal* area to allow unambiguous reference to them. The circumcapitular flange is situated adjacent and posterior to the dorsoproximal palpcoxae behind the circumcapitular furrow and looks like a thin semicircular cuticular ring (Fig. 1A, CF). The epicoxal area is situated between the anterolateral margins of the prodorsal shield and coxae I and II (Fig. 1A, EA). This area is better developed in immatures, whereas in adults it is usually reduced because of the enlarged prodorsal shield and coxae. The boundary between the epicoxal area and circumcapitular flange is obscure, but in many species (especially in vagrants) these areas are well separated from the prodorsum by the thickened margin of the prodorsal shield.

Supracapitular folds and suboral plate

Our SEM images show the presence of additional thin folds in some species between the circumcapitular flange and frontal lobe of the prodorsal shield (or anterior margin of prodorsal shield if the lobe is not developed). We term them *supracapitular folds* (Fig. 1A, SF). The eriophyoid gnathosoma bears a ventral rigid plate, usually covering the ventro-proximal palpcoxae. This structure has been referred to as the *infracapitular*, *subcapitular* or *suboral plate* (Chetverikov et al. 2013, p. 10, footnote 6). Although some authors believe this plate to be an anterior fusion product of the anterior coxisterna of legs I (J. Amrine, *pers. comm.*), we consider it to be the fused ventral palpcoxae. In this case this plate is a gnathosomal structure, thus the term "suboral" appears more appropriate.

Subcapitular sheath and proboscis

In our slide collection we found one slide containing a female of *Nalepella tsugifoliae* with disjointed mouthparts (Fig. 1B). CLSM investigation of this slide reveals a leaf-like, likely flexible structure between the separated palps, which at first glance resembles the suboral plate in shape, and is in a position to which this plate can be displaced in slide-mounted specimens. However, during careful examination of this sample, at a level lower (more ventral) than this structure, we found fragments of the broken suboral plate ventrally to the left palp (Fig. 1B, SP). We compared our CLSM images with illustrations from the literature and concluded that the leaf-like structure represents the unfolded subcapitular sheath (sensu Lindquist 1996, p. 7, fig. 1.1.1.3.5; designated as "STS" in fig. 1.2.17 and fig. 1.2.18 from Nuzzaci and Alberti 1996). According to the schematic reconstruction of

eriophyoid gnathosoma from Nuzzaci and Alberti (1996, fig. 1.2.16), when this sheath is folded, it forms a trough-like structure with two longitudinal channels: dorsal (contains stylets) and ventral (contains oral cavity leading to the mouth). Lindquist (in Schmidt et al. 2012) and later Sidorchuk et al. (2014) used the term "proboscis" to designate this complex of gnathosomal structures (stylets + sheath) in triasacaroids (the eriophyoid-like mites found in Triassic amber) and eriophyoids. In triasacaroids the proboscis is projecting freely below and apically beyond other gnathosomatic elements, whereas in eriophyoids the proboscis is usually hidden between palps, which are closely appressed to each other.

Protuberances and depressions on palps and chelicerae

In accordance with previous observations (e.g. de Lillo et al. 2002) our data suggest that the proximal chelicerae of eriophyoid mites are commonly bulbous and enlarged; they are attached to the motivator to form a composit cheliceral arrangement. In phytoptid genera *Trisetacus*, *Setoptus* and *Phytoptus* we discovered differently shaped lateral processes of the proximal chelicerae interlocking with the palps (Figs. 2Cc, 3Ac, Ec). In various eriophyoids we also often observed two tiny dorsal tubercules on the proximal part of the cheliceral shaft (Figs. 3Aa, 4A white arrows), their origin and function is uncertain. In phytoptids (e.g. *Trisetacus*, *Setoptus* and possibly *Oziella*) we found a narrow groove which goes dorsally along the adoral (inner) margin of palpcoxae (Fig. 2Aa, Da), whereas in members of the families Eriophyidae and Diptilomiopidae this groove was not found. However, in most eriophyids and diptilomiopids a distinct process, the so-called "cheliceral retainer" (sensu Keifer 1959), is present on the basal palpcoxae (Figs. 1A, C, 4), which is absent in phytoptids.

Gnathosomal interlocking apparatus in eriophyoid mites

In species of eriophyoid mites lacking a frontal lobe, the area ahead of the anterior dorsal edge of the prodorsal shield is observable with SEM. It can also be observed in eriophyoid immatures because they usually do not have large frontal lobes, even if adults possess them. Under SEM, LTSEM and CLSM we observed tiny structures in the dorsoproximal region of the gnathosoma, constituting a functional unit which we call "interlocking apparatus". We observed four types of the interlocking apparatus and resultant mechanisms in the studied eriophyoids.
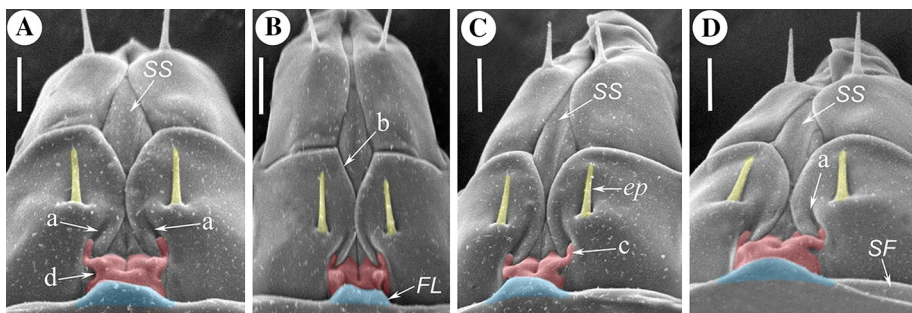

Fig. 2 LTSEM images of gnathosoma of four different females of *Trisetacus* cf *pinastri* (Nuzzaci 1975). Note: Frontal lobe (*FL*), *blue*; basal chelicerae and cheliceral process are *red*; setae *ep* are *yellow*. Notation: *a* groove (or sulcus) to accomodate lateral cheliceral process, *b* dorsomedial palpcoxal margin, *c* hook-shaped lateral process to interlock with dorsomedial palpcoxal margin, *d* notch, *ep* palpcoxal seta *ep*, *SS* subcapitular sheath, *SF* supracapitular fold. *Scale bar* = 3 μm. (Color figure online)
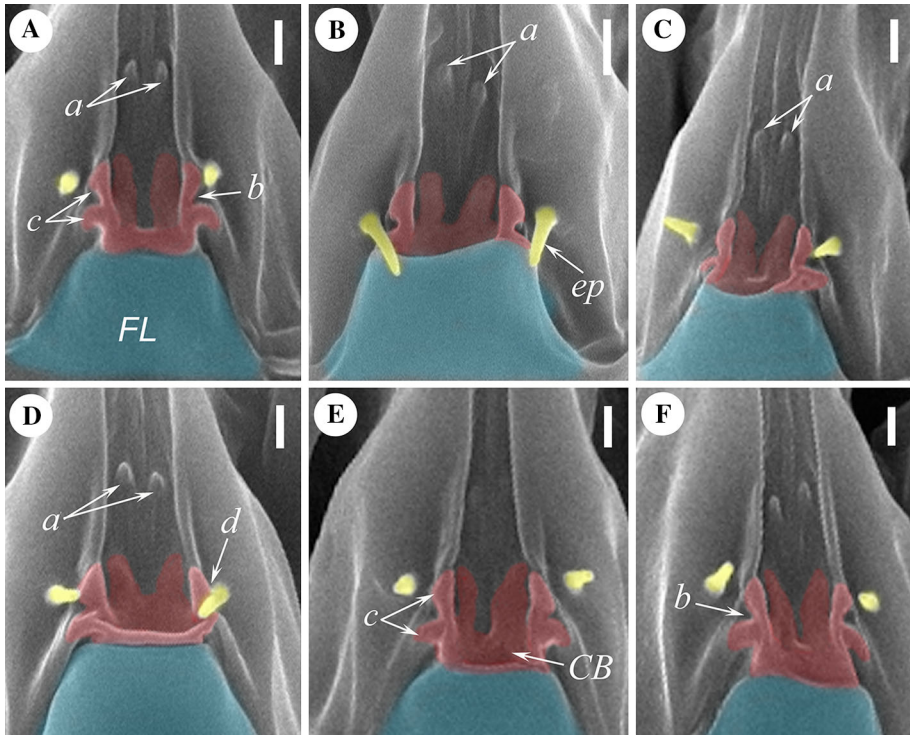
Fig. 3 Conventional SEM images of gnathosomata of six females of *Phytoptus monthalensis* (Meyer 1991). Notation: *a* two dorsoproximal cheliceral tubercles, *b* notch, *c* forked lateral cheliceral process (coloured *red*), *d* seta *ep* (coloured *yellow*) and notch interlocked, *CB* cheliceral base (coloured *red*), *FL* frontal lobe (coloured *blue*). *Scale bar* = 1 μm. (Color figure online)

Type I (Fig. 2)

The dorsomedial margins of the palpcoxae either touch each other or slightly overlap (Fig. 2B*b*); a thin groove or sulcus runs along the dorsomedial palpcoxal margin (Fig. 2A*a*, D*a*); the proximal cheliceral base has two hook-shaped lateral processes (corresponding to the above mentioned groove, Fig. 2C*c*) and a small notch (fitting to the margin of palpcoxae, Fig. 2A*d*). We found this type of interlocking mechanism in *Trisetacus* (*T.* cf *pinastri* Nuzzaci 1975, *T. sylvestris* Castagnoli 1973, *T. juniperinus* (Nalepa 1911), *T. bioti* Sekerskaya and Sharonov 1982 (in Vasilieva et al. 1982) and *Setoptus* (*S. radiatae* Meyer 1991 and *S. pini* Boczek 1964a). The SEM images of *Oziella atherodes* (Chetverikov 2011) and *O. liroi* (Roivainen 1947) were not clear enough to provide a definitive conclusion, but it is likely that *Oziella* mites also possess a similar type of interlocking mechanism.

Type II (Fig. 3)

Proximal cheliceral base (Fig. 3E) with two-forked (two-pronged) lateral process (Fig. 3A*c*, E*c*) with a U-shaped lateral notch (Fig. 3A*b*, F*b*); the short stout palpcoxal seta *ep* (Fig. 3B*ep*) presumably slots into this notch (Fig. 3D*d*). We suggest that when the mite is feeding, the palps slightly fold (like a telescope), so that palpcoxae become wider and
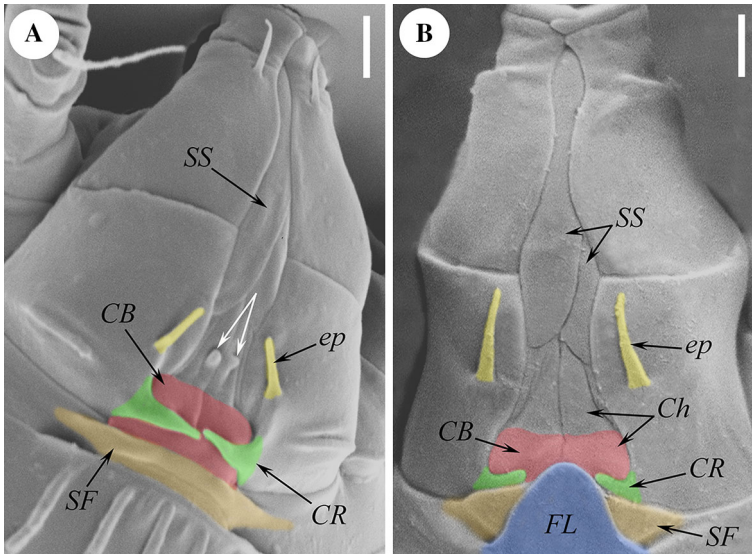
Fig. 4 LTSEM images of gnathosoma of larva of *Tergilatus sparsus* (Meyer and Ueckermann 1995) (**A**) and a female of *Acalitus mallyi* (Tucker 1926) (**B**). Notations: *FL* frontal lobe (coloured *blue*), *Ch* chelicera, *CB* enlarged cheliceral base (coloured *red*), *SF* supracapitular folds (coloured *orange*), *CR* cheliceral retainer (coloured *green*), *SS* subcapitular sheath *ep* palpcoxal seta *ep* (coloured *yellow*). *Two white arrows* in **A** indicate two dorsoproximal cheliceral tubercles. *Scale bar* = 2 μm. (Color figure online)

swollen and setae *ep* interlock with the notches of the lateral cheliceral process (Fig. 3D*d*); when at rest the setae *ep* dislodge from the notches and palps take their usual (unfolded) form. We only found this type of interlocking mechanism in *Phytoptus monthalensis*. Because we observed under SEM all the structures described in this paragraph in six females, and additionally, DICLM confirmed the shapes of these proximal cheliceral base structures (DICLM images not shown here) we believe that the structures comprising Type II are not the result of any kind of deformation or artefact.

Type III (Fig. 4)

The cheliceral retainer interlocks with the enlarged dorsoproximal cheliceral base; supracapitular folds and the frontal lobe of the prodorsal shield, if present, cover dorsally the exposed part of the proximal chelicerae (anterior to the hidden cheliceral-motivator articulation). We found this type of interlocking mechanism in various members of the families Eriophyidae (e.g. in *Acalitus*, *Aceria*, *Calacarus*, *Keiferella*, *Tergilatus*) and Diptilomiopidae (in *Diptilomiopus* and *Diptacus*).

Type IV (Fig. 5)

Palpcoxae broadened and elongated, forming a "tube-like structure" tightly covering the internal gnathosomal structures (Fig. 5C, G). The facing medial palpcoxal surfaces are pressed together, their mediodorsal edges thin, overlapping, exposed in immatures, and covered by a tongue-shaped frontal lobe bearing seta *vi* in adults (Chetverikov et al. 2013, fig. 2, p. 10; Fig. 5C). Two distinct apodemes that strengthen the palpcoxae form a
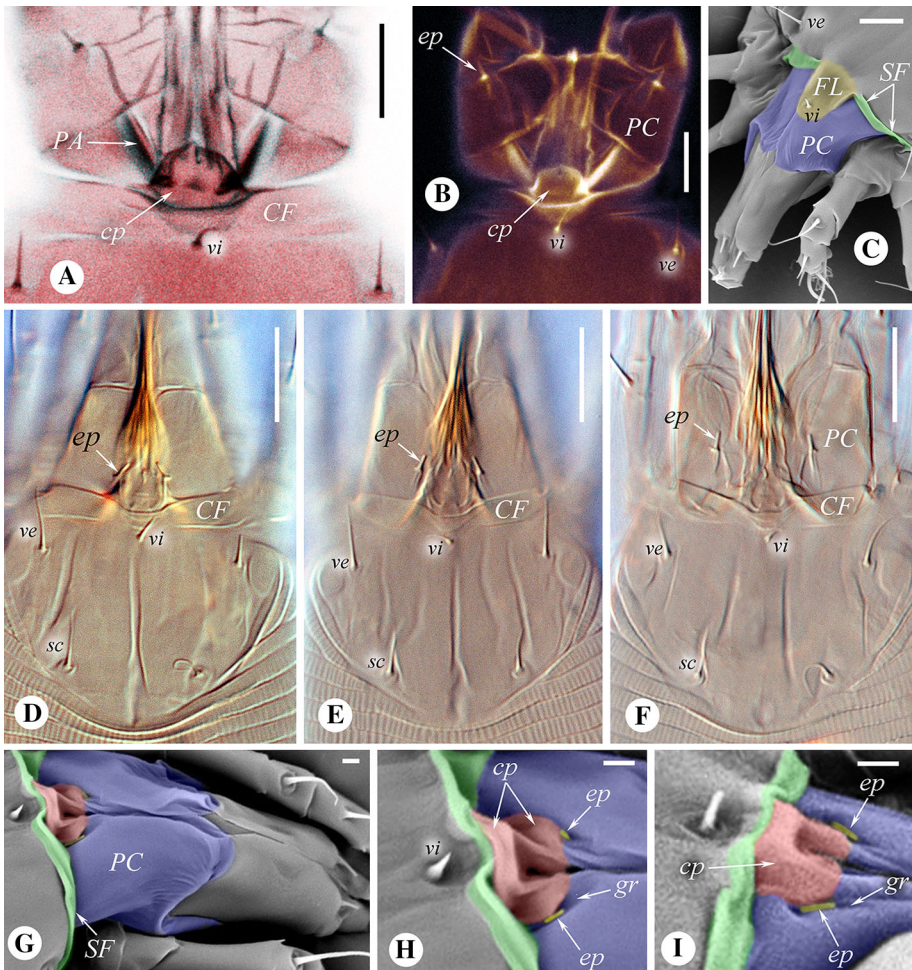
Fig. 5 CLSM (**A**, **B**), conventional SEM (**C**, **G–I**), and DICLM (**D–F**) images of nymphs (**A**, **D–I**), larva (**B**) and female (**C**) of *Loboquintus subsquamatus*. Notations: *PC* palpcoxae forming a "tube-like structure" (coloured *blue*); *FL* tongue-shaped frontal lobe of prodorsal shield (coloured *orange*) bearing seta *vi*; *CF* circumcapitular flange; *SF* folded anterior margin of prodorsal shield and supracapitular folds (coloured *green*); *PA* palpcoxal apodeme; *ep* palpcoxal seta *ep* (coloured *yellow* in **H**, **I**); *vi* internal vertical seta; *ve* external vertical seta; *sc* scapular seta on prodorsal shield; *gr* tiny groove on mediodorsal palpcoxa; *cp* "cap" (coloured *pink* in **G**, **H**, **I**). *Scale bar* **A**, **B**, **C** = 5 μm; **D**, **E**, **F** = 10 μm; **G**, **H**, **I** = 2 μm. (Color figure online)

V-shape figure in slide-mounted specimens (Fig. 5A, B). A rounded erythrocyte-like rigid structure, resembling a depressed cap or a pileus of agaric fungi (e.g. *Rassula* or *Lactarius*), is inserted between proximal palpcoxae, both in immatures and adults (Fig. 5A*cp*). CLSM suggests that this complex structure might be a result of a tight junction, an overlap or even a fusion between a tiny supracapitular fold and the proximal cheliceral base (Fig. 5A). Seta *ep* is located dorsally on the medioproximal part of the palpcoxa,[2] usually

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[2] Chetverikov et al. (2013, p. 25) wrongly mentioned that seta *ep* is not seen in SEM images because it is situated on the medial surface of palpcoxae.

concealed in a small groove (Fig. 5H, I) under the lateral edge of the "cap", rarely seen in SEM images if the palps are not telescoped (Fig. 5G, H, I). In slide mounted specimens both the dorsolateral and anterior position of seta *ep* depends on how much the specimen was flattened during the slide making process (Fig. 5D, E, F). Up to now we have only found this type of interlocking mechanism in *Loboquintus subsquamatus*.

Discussion

In this study we primarily focused on the morphology of the proximal gnathosoma of eriophyoid mites in order to try to understand the various structures and to obtain new, potentially important phylogenetic characters for comparison with the current classification of Eriophyoidea at the family level. We also revised the interpretation by Chetverikov et al. (2013) of the proximal gnathosomal structures of the early-derivative mite genus *Loboquintus*, proposed new terminology, and described new structures of the dorsoproximal gnathosoma and adjacent regions of eriophyoids. Our observations suggest that eriophyoid mites possess an interlocking apparatus on the dorsoproximal gnathosoma. Although functional interpretation of structures comprising this apparatus is problematic, we suggest that they can (1) support and assist the chelicerae relative to the palpcoxae and the prodorsal shield during movements of the palps and (2) adjust angular flexion of the stylets if they are bent during feeding. Therefore, the interlocking apparatus contributes in maintaining/keeping the bunch of gnathosomal stylets together and thus assists the subcapitular sheath to consolidate/integrate gnathosomal components into a proboscis.

Phylogenetic significance of the interlocking gnathosomal structures

Differences in shape, size and direction of the entire gnathosoma and its constituents have been used to support the hypothesis that Diptilomiopidae is monophyletic and separate from Eriophyidae and Phytoptidae (Keifer 1959; Lindquist 1996; Amrine et al. 2003). Although cladistics suggest that Diptilomiopidae is monophyletic, based on synapomorphic gnathosomal morphology (Craemer 2010), the results of recent molecular phylogenetic studies suggest the opposite. Li et al. (2014) demonstrated that both Diptilomiopidae and Eriophyidae are paraphyletic, and the so-called "big-beaked" (Lindquist and Oldfield 1996, p. 278) type of diptilomiopid gnathosoma could have evolved independently in different lineages of vagrant eriophyids and diptilomiopids. However, these two families belong to the clade Eriophyidae *s.l.*, which is supported by results of molecular phylogenetic analyses and comparison of their internal genitalic anatomy (Chetverikov et al. 2014c). Three other groups were found in the above mentioned molecular phylogenetic analyses (Fig. 6): ancestral paraphyletic pentasetacines (*Loboquintus* and *Pentasetacus*)
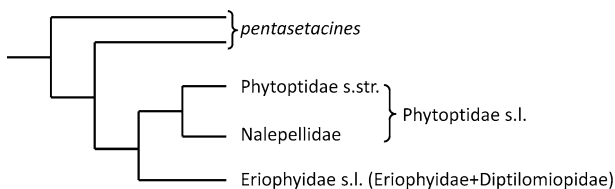

Fig. 6 Phylogeny of Eriophyoidea (retrieved from Chetverikov et al. 2014c; Li et al. 2014)

plus two highly supported clades, Phytoptidae *s. str.* (=Phytoptidae sensu Boczek et al. 1989) and Nalepellidae sensu Boczek et al. 1989, constituting the monophyletic Phytoptidae *s.l.* (=Phytoptidae sensu Amrine et al. 2003 excluding pentasetacines), sister to Eriophyidae *s.l.*

In this study we defined four types (I, II, III and IV) of gnathosomal interlocking mechanisms which are congruent with the results of gene sequence studies (Fig. 6) with respect to the basal relations within the Eriophyoidea. According to our data and previous observations (e.g. Keifer 1959), many eriophyids and selected diptilomiopids have cheliceral retainers on their dorsoproximal palpcoxae, which interlock with the prominent proximal cheliceral bases (Figs. 1C, 4). A cheliceral retainer has never been found in phytoptid mites; our observations on early derivative mite genera *Loboquintus* (Fig. 5) and *Pentasetacus* (Chetverikov et al. 2014a, p. 131, fig. 5) suggest that they also do not have this structure. Thus the presence of the cheliceral retainer might be a synapomorphic character supporting monophyly of Eriophyidae *s.l.*, and all members of this clade possibly share a similar type (III) of this interlocking apparatus or its derivatives. As opposed to eriophyids and diptilomiopids, mites of the clade Phytoptidae *s.l.* have differently shaped lateral processes of the cheliceral base (Figs. 2, 3) which interlock with a mediodorsal palpcoxal groove (in members of Nalepellidae) or with palpcoxal seta *ep* (in *Phytoptus monthalensis*, belonging to Phytoptidae *s. str.*). Our data suggest that the presence of cheliceral processes and their involvement in the interlocking apparatus (types I and II) might provide additional support for the monophyly of Phytoptidae *s.l.* Remarkably, *Trisetacus, Setoptus* and *Oziella* mites possess well developed overlapping mesal dorsal flaps of the palpcoxae, which might be the functional analogue of the cheliceral retainer in Eriophyidae and Diptilomiopidae (Fig. 2B*b*). These flaps are especially well developed in *L. subsquamatus*, which has a peculiar complex interlocking mechanism (type IV, Fig. 5) significantly differing from what we found in other eriophyoids. The uncommon characteristics of the interlocking apparatus in *Loboquintus* reinforce isolation of this taxon from other members of Eriophyoidea and support its ancestral position, which was revealed using molecular markers (Chetverikov et al. 2014c). In conclusion to this section, we would like to mention that the ideas described above on the interlocking mechanisms, and their potential phylogenetic significance, obviously need future testing. We predict that the accumulation of new data on gnathosomal morphology, obtained using various microscopic techniques, will result in finding new types of gnathosomal interlocking mechanisms which might assist in unraveling eriophyoid phylogeny.

Remarks on the functional morphology of the frontal lobes of eriophyoid mites

The prodorsal shield of most eriophyoids has an anteromedian extension, usually called a "frontal lobe" in species descriptions (Lindquist 1996, p.10). The frontal lobe is largely an attribute of adults; it is absent, or very small, in larvae studied so far and it is usually weakly developed in nymphs. Many eriophyoid species which were described as lacking a frontal lobe, or in which a frontal lobe was not depicted and/or reported, actually have it (e.g. shown at Fig. 1C, D). This is probably because the frontal lobe may be very small, thin or directed vertically, and thus indistinct or transparent in slide-mounted specimens when studied with conventional LM. The frontal lobes vary in shape (triangular, semicircular, acuminate, notched etc., Fig. 7), outlines (e.g. smooth, spiny, emarginated, crenate), thickness (from thin and flexible to thick and stout) and size (from minute to well-developed). The prominent frontal lobe is sometimes considered to be a typical attribute of
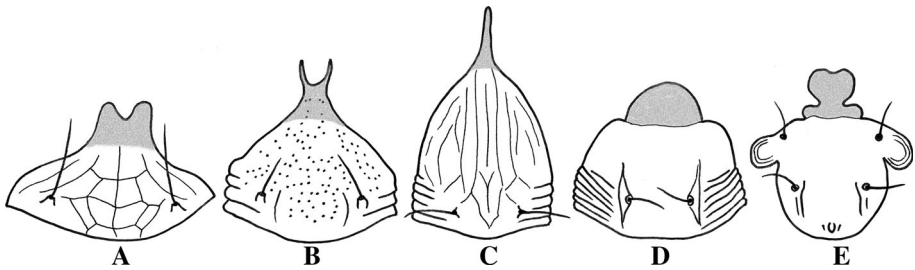
Fig. 7 Notched (**a**, **b**), elongated (**c**) and broadened (**d**, **e**) frontal lobes in selected eriophyoids: **a** *Brevulacus reticulatus* (Manson 1984), **b** *Acadicrus bifurcates* (Keifer 1965), **c** *Stenarhynchus aristidus* (Mohanasundaram 1983), **d** *Platyphytoptus sabinianae* (Keifer 1938), **e** *Mackiella reclinata* (schematically redrawn from images from the original descriptions)

vagrant mites,[3] which also usually have dorsoventrally differentiated annuli, sometimes with ridges and a large prodorsal shield (Amrine et al. 2003). But there are many exceptions, e.g. various concealed mites (refuge seeking and gall-making) might have rather prominent frontal lobes, so their presence or absence might not strictly reflect the lifestyle of the mite (Sukhareva and Chetverikov 2013 and papers cited therein). However, the frontal lobe shape is probably correlated with lifestyle: vagrant mites usually have well-developed rigid, thick lobes, whereas concealed mites usually possess thinner, probably more flexible lobes (Amrine et al. 2003).

According to our observations, moderately thickened and relatively short (<30 % length of prodorsal shield) subtriangular or semielliptical frontal lobes represent the most common type of frontal lobes in Eriophyoidea (e.g. Figs. 1D, 4B). Such lobes are present in numerous eriophyids, in many sierraphytoptines and rhyncaphytoptines (but the lobe usually is absent in diptilomiopines), and in *Pentasetacus* and *Boczekella*. Enlargement and thickening of the anterior portion of the prodorsal shields observed in free-living eriophyoids (e.g. in anthocoptines and acaricalines) resulted in massive frontal lobes, which can be explained by the assumption that such lobes in vagrant mites "…may provide rigid support for gnathosoma" (Lindquist 1996, p. 10). In comparison to the widely distributed subtriangular and semielliptical frontal lobes (discussed above), there are at least three groups of much more rarely observed shapes of frontal lobe: notched or emarginate lobes, narrow elongated lobes and large broadened lobes (Fig. 7). In several phyllocoptines (e.g. in genera *Acadicrus* Keifer 1965, *Neoepitrimerus* Kuang and Li 1994 (in Kuang et al. 1994) or *Siamina* Boczek 1993, and in the rhyncaphytoptine mites of the genera *Asetacus* (Keifer 1952), *Konola* (Keifer 1979) and *Brevulacus* (Manson 1984), the frontal lobe is notched or distinctly emarginated (Amrine et al. 2003, figs. 155, 156, 146, 299, 300 and 308 respectively). We propose this notch accomodates the basal parts of the stylets, which are upthrusted between the palps when the mite is feeding (e.g. shown in Keifer 1965, plate 9, p. 18), providing them a sort of bilateral guide.

In adults of *Loboquintus*, the frontal lobe is elongated and looks like a thin flexible tongue-shaped extension of the anterior prodorsal shield. The frontal lobe covers the space between overlapping mediodorsal flaps of the proximal palpcoxae, strengthening the hermeticity of the "tube-like" structure (Fig. 5C). Mites *Stenarhynchus aristidus*

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[3] The idea has been incorporated in the generic key by Amrine et al. (2003) as one of the characters distinguishing Phyllocoptinae and Eriophyinae subfamilies comprising mainly of usually vagrant fusiform and usually concealed vermiform mites respectively.

(Mohanasundaram 1983) possess a similar but more acuminate frontal lobe (Amrine et al. 2003, fig. 304). Meyer (1990) reports that selected South African mites, *Afromerus* (Meyer 1990), *Ectomerus* (Meyer 1990) and some *Aceria* (Keifer 1944) (formerly in *Artacris*), have elongated, thin triangular frontal lobes with sharp anterior processes, which are especially long in *Ectomerus systenus* Meyer (1990) and *Aceria clutiae* Meyer (1990). According to the LTSEM observation of the second author on *Ectomerus systenus* (Fig. 8C), it can be deduced that the shape of the lobe ideally fits the space between the dorsomedial edges of the palps if the gnathosoma is telescoped during feeding. Thus, elongation of the frontal lobes is possibly necessary to fill/cover the fissure-like space between the palps in order to provide additional hermeticity in the dorsal area of the gnathosoma, i.e. to cover the overlapping edges of stylet sheath between distal palps.

A few mite genera (e.g. *Platyphytoptus* and *Mackiella* mites) possess very thin, broadened, subrectangular or semicircular frontal lobes, which can entirely cover the cheliceral bases, the dorsoproximal and, partially, the lateroproximal part of palps as well. These lobes might keep the dorsoproximal parts of palps together. In *Phytoptus mon-thalensis* the obtuse, subtrapezoidal frontal lobe apparently presses against the cheliceral base when the mite is feeding (Fig. 3). In this case the frontal lobe might provide additional support for the cheliceral bases. Consequently, the movement of the chelicerae is constrained, helping to ensure that they do not bend or twist out of position when they pierce plant tissue. Mites *Mackiella reclinata* possess a frontal lobe with a unique shape; it is divided into two parts (basal and apical) by a deep lateral notch (Fig. 8A). When the *Mackiella* gnathosoma is at rest, the anterodorsal palpcoxal margin is situated underneath the apical part of the frontal lobe (Fig. 8A). When the palps are telescoped backwards (feeding position), the anterior palpcoxal margin interlocks with the lateral notch of the frontal lobe (Fig. 7B); in the feeding position, the palp coxa is entirely covered by the basal part of the frontal lobe, whereas the next palpal segment (the trochantero-femuro-genual segment sensu Lindquist 1996) is hidden under the apical part of the lobe (Fig. 7B). According to our LTSEM observations on *Mackiella reclinata*, the frontal lobe remains
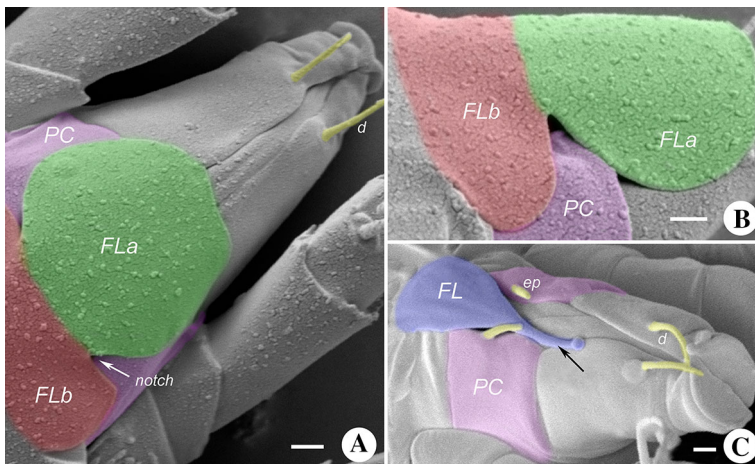

Fig. 8 LTSEM images of *Mackiella reclinata* (**A**, **B**) and *Ectomerus systenus* Meyer (1990) (**C**). Notations: *PC* palpcoxa (colored *violet*), *FL* frontal lobe (colored *blue*), *FLa* apical part of frontal lobe (coloured *green*), *FLb* basal part of frontal lobe (coloured *red*). Remark: palpal setae *ep* and *d* both coloured *yellow*; *black arrow* in **C** indicates anterior process of frontal lobe. *Scale bar* = 1 μm. (Color figure online)

straight and does not change its form when the palps are telescoped for feeding (Fig. 7B). Thus, the lobe might function like a guide for the palps, forming a kind of dorsolateral trough and thus preventing their disjunction, or structurally reinforcing the palps during feeding.

This brief review indicates that the frontal lobe appears to be closely involved in the consolidation of proximal gnathosomal structures, control of their functioning, and mechanical protection or support. When the frontal lobe is distinctly developed, the space between the dorsomedial surfaces of the proximal palpcoxae is hidden under the lobe. This space is typically somewhat triangular, which is probably the reason why subtriangular frontal lobes are so common in Eriophyoidea. This functionally and anatomically complex triangular area is the connection point between the palps, the motivator, cheliceral bases and other interlocking structures. We suggest mechanical protection of this area is the primary function of the frontal lobe. Unusual shapes of the frontal lobe in selected eriophyoids (elongated, broadened, notched lobes) might result from additional functions which the lobe can perform (e.g. providing hermeticity in the gnathosomal channel and providing additional support for palps and stylets, or for accommodating peculiar feeding attributes).

The variation in the shapes of the frontal lobes and their connection with basal gnathosomal elements (e.g. dorsoproximal palpcoxae, cheliceral base and their derivatives) within Eriophyoidea might reflect the complex evolution of eriophyoid mouthparts, in the course of which different interlocking mechanism types evolved. In accordance with Shevchenko (1970), we agree that the frontal lobe shape might depend on the difference in thickness of epidermal tissue in exposed and hidden parts of plants. Characteristics of epidermal tissues greatly vary not only in different parts of a plant specimen, but among different plant taxa as well (Dickison 2000). Obviously, in the course of evolution, mites have had to adapt their mouthparts to the epidermis of their host-plants. Thus one of the intriguing goals for future study is to compare the epidermal characteristics with the gnathosomal morphology of eriophyoids in order to test the hypothesis that there may be correlations between them. Another hypothesis which could be tested in the future is whether the shape of the frontal lobe and other structures, either associated with, or part of the gnathosoma, are of phylogenetic importance or not. Comparative study using various modern microscopic techniques (like LTSEM and CLSM), cladistic analyses of the morphological data, and mapping morphological characters on molecular phylogenetic trees are believed to be the most appropriate approaches for such a study.

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