



Comprehensive phylogeny of acariform mites (Acariformes) provides insights on the origin of the four-legged mites (Eriophyoidea), a long branch

Pavel B. Klimov^{a,b,*}, Barry M. O'Connor^a, Philipp E. Chetverikov^c, Samuel J. Bolton^d, Amir R. Pepato^e, Abdolazim L. Mortazavi^a, Andrey V. Tolstikov^b, Gary R. Bauman^f, Ronald Ochoa^g

^a Department of Ecology and Evolutionary Biology, University of Michigan, 1109 Geddes Ave, Ann Arbor, MI 48109-1079, USA

^b Tyumen State University, Faculty of Biology, 10 Semakova Str., Tyumen 625003, Russia

^c Saint-Petersburg State University, Universitetskaya nab., 7/9, 199034 St. Petersburg, Russia

^d University of Arkansas, Fayetteville, AR 72701, USA

^e Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Brazil

^f USDA, ARS, Electron and Confocal Microscopy Unit, Beltsville, MD 20705, USA

^g USDA, ARS, Systematic Entomology Laboratory, Beltsville, MD 20705, USA

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ABSTRACT

Eriophyoid, or four-legged mites, represent a large and ancient radiation of exclusively phytophagous organisms known from the Triassic (230 Mya). Hypothesizing phylogenetic relatedness of Eriophyoidea among mites is a major challenge due to the absence of unambiguous morphological synapomorphies, resulting in ten published hypotheses placing eriophyoids in various places in the acariform tree of life. Here we test the evolutionary relationships of eriophyoids using six genes and a representative taxonomic sampling of acariform mites. The total evidence analysis places eriophyoids as the sister group of the deep soil-dwelling, vermiform family Nematalycidae (Endeostigmata). This arrangement was supported by the rDNA and CO1 partitions. In contrast, the nuclear protein partition (genes EF1- α , SRP54, HSP70) suggests that Eriophyoidea is sister to a lineage including Tydeidae, Ereynetidae, and Eupodidae (Eupodina: Trombidiformes). On both of these alternative topologies, eriophyoids appear as a long branch, probably involving the loss of basal diversity in early evolution. We analyze this result by using phylogenetically explicit hypothesis testing, investigating the phylogenetic signal from individual genes and rDNA stem and loop regions, and removing long branches and rogue taxa. Regardless of the two alternative placements, (i) the cheliceral morphology of eriophyoids, one of the traits deemed phylogenetically important, was likely derived directly from the plesiomorphic acariform chelicerae rather than from the modified chelicerae of some trombidiform lineages with a reduced fixed digit; and (ii) two potential synapomorphies of Eriophyoidea + Raphignathina (Trombidiformes) related to the reduction of genital papillae and to the terminal position of PS segment can be dismissed as result of convergent evolution. Our analyses substantially narrow the remaining available hypotheses on eriophyoid relationships and provide insights on the early evolution of acariform mites.

1. Introduction

Eriophyoid mites (3 extant families, over 350 described genera and 4400 species) are an exclusively phytophagous lineage representing one of the largest chelicerate radiations, with fossils known from the Triassic (Schmidt et al., 2012). Morphologically, eriophyoids are distinguishable from other mites by being vermiform, four-legged organisms (hence, the vernacular name, 'four-legged mites'). The chelicerae of eriophyoids are modified into stylets adapted for insertion into plant

cells and sucking up their liquid contents (Lindquist and Amrine, 1996). Feeding activities of many species cause formation of characteristic galls and other plant tissue abnormalities (hence, another vernacular name, 'gall mites'). Species that do not form galls are free-living on plant surfaces and, less often, live inside plant tissues (Chetverikov, 2015; Chetverikov and Petanović, 2016; Keifer, 1975). The host plants include mostly angiosperms (flowering plants) and gymnosperms (e.g., conifers). Gymnosperms (Gerson, 1996), paleozoic progymnosperms or early seed ferns have been cited as ancestral host plants (Bagnjuk et al.,

* Corresponding author at: Department of Ecology and Evolutionary Biology, University of Michigan, 1109 Geddes Ave, Ann Arbor, MI 48109-1079, USA.
E-mail address: pklimov@umich.edu (P.B. Klimov).

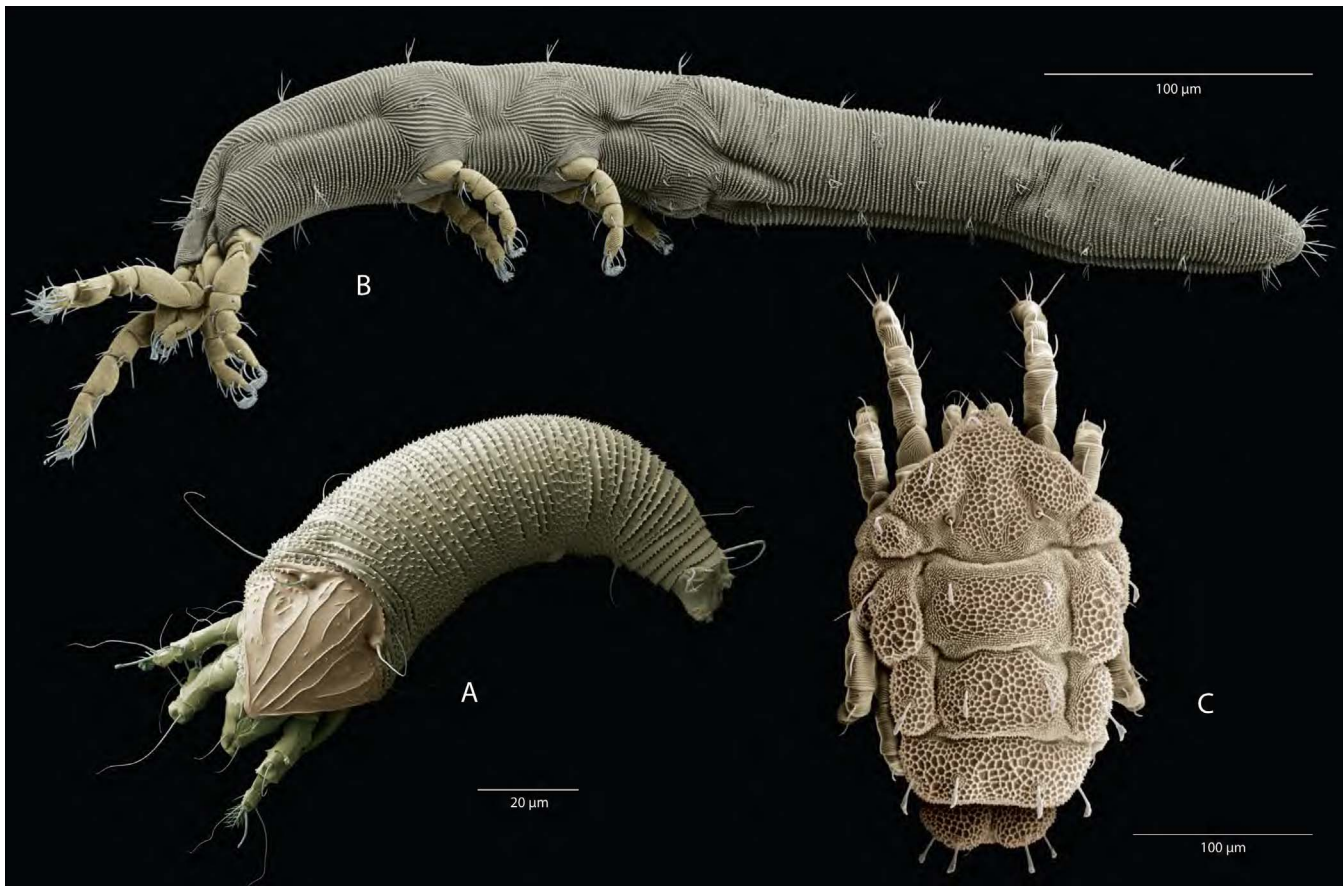


Fig. 1. A member of Eriophyoidea, (A) wheat curl mite, *Aceria erecti* (female) and representatives of two acariform lineages considered to be closest relatives of eriophyoids: the deep soil mite (B) *Cunliffea* sp. (Endeostigmata: Nematalycidae) and the citrus yellow mite, (C) *Brachytydeus formosa* (Trombidiformes: Eupodina: Tydeidae). Photo credit: Gary Bauman (USDA ARS Electron and Confocal Microscope Unit, Beltsville, Maryland), Ronald Ochoa (USDA ARS Systematic Entomology Laboratory, Beltsville, Maryland).

1998; Schmidt et al., 2012). Secondary associations, resulting from recent host shifts from one of the main host lineages, involve modern horsetails and ferns (Gerson, 1996; Petanović et al., 2015). The economic importance of eriophyoid mites is linked to their ability to transmit pathogenic viruses among hosts plants (Oldfield and Proeseler, 1996) and to the formation of galls (e. g., erineum patches, deformation of buds), and other symptoms (e.g., leaf spotting), which are associated with changes in plant physiology (Westphal and Manson, 1996). The most important pests attack grain, bulb, berry and nut crops as well as an array of ornamental plants (Lindquist and Amrine, 1996).

The general morphology of eriophyoids is highly specialized for plant feeding, especially the mouthparts; eriophyoids also developed a distinctly vermiform body (Fig. 1A), a feature known for a few other specialized mite lineages, e.g., those mimicking ant larvae (*Perperipes*, *Larvamima*), living in mammalian hair follicles or dermal glands (Demodecidae), interstitial spaces of deep soil (Nematalycidae) or the hyporheic zone of rivers (*Pseudowandesia*, *Stygothrombium*). At the same time, the eriophyoid morphology is highly simplified, especially with regard to the number of legs (two pairs instead of four) and fundamental setae/solenidia, probably due to the mites' remarkably small size (100–500, although usually 150–250 µm). As a result, eriophyoids display a combination of numerous autapomorphies and plesiomorphies, some of which may be due to reductive character losses influenced by extreme miniaturization. There is no unambiguous morphological synapomorphy allowing placement of Eriophyoidea in a major acariform lineage, such as Trombidiformes or any monophyletic group of Endeostigmata (Lindquist, 1996, 2017).

For over a century, phylogenetic placement of Eriophyoidea has been a major challenge and the subject of active debates, with authors

proposing an array of disparate acariform sister groups: Alycidae, Nematalycidae (Endeostigmata), tydeoid families Tydeidae + Ereyneidae, Penthaleidae, Tarsonemoidea, Raphignathae, Demodecidae, Tenuipalpidae, Tetranychoidae (Trombidiformes), or Astigmata (Sarcoptiformes) (reviewed in Lindquist, 1996, 1998). Some of these hypotheses were based on a single, conspicuous morphological character or ecological characteristic. These include: the reduction of the posterior legs (hence Tarsonemoidea and Tenuipalpidae), vermiform shape (hence Demodecidae and Nematalycidae), the absence of a tracheal system (hence Astigmata), or obligate phytophagy (hence Tetranychoidae). Other hypotheses involve more in-depth analyses of multiple character states and their evolutionary polarities (e.g., Endeostigmata and the trombidiform families belonging to Eupodina). However, all evidence comes from ambiguous synapomorphies, including characters representing evolutionary transformation series, where the eriophyoid state is the hypothetical ultimate evolutionary step (e.g., evolutionary “trend” toward elongation of chelicerae in presumably derived Alycidae), characters where the ancestral state cannot be determined based on comparison with other lineages (e.g., the absence of a tracheal system), using characters with too broad or perhaps imprecise definitions (suppression of eugenital setae in females but not in males), or ones that may be highly correlated with the vermiform body and hence are likely to be homoplastic due to functional constraints (e.g., the presence of opisthosomal annuli). The currently accepted classification, which suggests that the Eriophyoidea and Tydeoidae are related superfamilies, places both of these superfamilies into Eupodina, a higher-level lineage in Trombidiformes (Lindquist et al., 2009; Zhang et al., 2011).

Recent molecular studies have presented contradictory evidence on

the origin of eriophyoids. Mitochondrial DNA suggested that eriophyoids are either a basal divergence from all acariform mites (Xue et al., 2016) or sister to Sarcoptriformes (Oribatida + Astigmata) (Xue et al., 2017). Those studies included no sampling of basal acariform lineages (Endeostigmata) and even essential basal diversity of Trombidiformes (including critical taxa, e.g., families of Eupodina). Analysis based on sequences of ribosomal nuclear gene 18S suggested a somewhat similar scenario, with eriophyoids occupying a basal position on the tree and forming a sister-group relationship with Astigmata or Astigmata + Alycidae (Li et al., 2016; Xue et al., 2017). The grouping with Astigmata is likely caused by a long branch attraction artifact. This gene has been already shown to infer a spurious basal position of Astigmata + Endeostigmata (Domes et al., 2007) within Acariformes, instead of placing Astigmata within Oribatida (which are mostly soil-dwelling mites) as suggested by morphology (Norton, 1998) and numerous subsequent molecular studies (Dabert et al., 2010; Klimov and O'Connor, 2008; Klimov and O'Connor, 2013; Pepato and Klimov, 2015). Positively misleading bias in 18S phylogenies was also reported elsewhere (Duvall and Ervin, 2004). Curiously, the basal position of Astigmata + Eriophyoidea (Li et al., 2016 [18S]; Xue et al., 2017 [18S]) is reminiscent of the old and short-lived morphological hypothesis suggesting a close relationship of Astigmata and eriophyoids (Oudemans, 1923; Reuter, 1909), with both lacking a tracheal system. The question of whether the absence of a tracheal system in eriophyoids is an ancestral state or a secondary loss, however, is very important. Its definitive resolution would allow for the placement of eriophyoids either in Endeostigmata (where the tracheal system is ancestrally absent) or Trombidiformes (tracheal system is almost always present or lost secondarily) (O'Connor, 1984). Cheliceral morphology is another interesting and phylogenetically important character complex of eriophyoids (Lindquist, 1998). In many higher-order trombidiform lineages (including families of Eupodina that have been proposed to be related to eriophyoids), attenuation of the movable digit is accompanied by a reduction in length of the fixed cheliceral digit. But in Eriophyoidea the fixed digit is elongate and styliform, similar in shape to the movable digit. This poses a serious obstacle in deriving the eriophyoid form of the cheliceral stylets from these trombidiform lineages (Lindquist, 1998), making this character potentially decisive in morphology-based phylogenetic inferences.

Here we investigate the phylogenetic position of eriophyoid mites using six loci, representing two ribosomal RNA genes (18S, 28S), one mitochondrial protein-coding gene (CO1: cytochrome c oxidase subunit I), and three nuclear protein-coding genes: elongation factor 1 α EF1 α , signal recognition particle protein 54 k (SRP54), Hsc70-5 heat shock protein cognate 5 (HSP70); 6302 positions (4495 nt for rDNA and 1807 amino acids for proteins) and a nearly complete sampling of all key lineages (113 families, 198 terminals). We included a large diversity of Endeostigmata (basal acariform mites, many of which live in deep soil and are difficult to collect) and Heterostigmata, a major trombidiform lineage that has never been included in a phylogenetic analysis before. Our study aims to test the hypotheses of eriophyoid relationships proposed previously (see above) in an explicit phylogenetic context. Given our trees, we also test whether the styliform movable digits of Eriophyoidea (see above) are either derived from the styliform movable digits of many Trombidiformes, or from the robust movable digits of ancestral chelate-dentate chelicerae.

2. Material and methods

2.1. Taxon sampling, DNA isolation and sequencing

For 198 taxa from 113 families (supplementary Table S1), six loci were sequenced: 18S, 28S, EF1- α , SRP54, HSP70, CO1; a total of 34,602 aligned nt before translation and character exclusion; in analysis: 6302 positions, with 4495 nt of rDNA and 1807 amino acids (Table 1). Of these six loci, two are nuclear, encoding structural ribosomal RNA (18S,

28S); three are nuclear protein-coding (EF1- α , SRP54, HSP70); and one (CO1) is a mitochondrial, protein-coding gene. A total of 758 new sequences were deposited in GenBank, accession numbers KY921960–KY922717. Taxa were selected to account for all previously proposed molecular and morphological hypotheses on eriophyoid relationships (see Introduction) and to include a representative set of all known major lineages of Acariformes. The close acariform outgroups include a solifugid and 10 other chelicerate outgroups (Table S1, Figs. 2–4). For the distant outgroup we chose an insect because having very distantly related outgroups may identify problems related to long branch attraction artifacts because ingroup long branches are expected to be attracted to the root in this case (Wheeler, 1990). The semidestructive DNA extraction method used here, as well as rDNA secondary structure alignment, oligonucleotide primers, DNA amplification, and sequencing have been previously described (Bochkov et al., 2014; Klimov and O'Connor, 2008; Knowles and Klimov, 2011). Some primers were modified to amplify rDNA in certain Heterostigmata (Worksheet S2). All vouchers and co-vouchers are deposited in the University of Michigan, Museum of Zoology (UMMZ); accession numbers are listed in Table S1.

2.2. Phylogenetic analyses

Prior to analyses, protein-coding genes were translated to amino acids and their alignment was unambiguous as they have nearly identical length. Hypervariable regions of rDNA were unalignable due to the lack of common secondary structure, and therefore were excluded, resulting in a very conservative alignment containing almost no gaps. Matrices and trees from this study are available from TreeBASE (<http://www.treebase.org>) accession number 20900.

Substitution models and best partitioning strategies were estimated in PartitionFinder v1.1.1 (Lanfear et al., 2012). The best partitioning scheme was 18S stem, 18S loop, 28S stem, 28S loop for rDNA (Table 1), and EF1- α , SRP54, HSP70, CO1 for amino acids (Table 1, 10). Therefore, the eight-partition scheme, combining these two schemes, was used in the final, full analyses. The best substitution models were as follows: GTR + I + G (18S stem/loop, 28S loop), SYM + I + G (28S stem), LG + I + G (EF1- α , SRP54, HSP70), MtArtF (CO1) (Table 1, 16). If a particular model was not implemented in the downstream phylogenetic program, then the best fitting available model was chosen in PartitionFinder (Table 1). Phylogenetic relationships were inferred in a maximum likelihood and Bayesian framework in parallel versions of RAXML 8.2.9 (Stamatakis, 2014) and MrBayes 3.2.6 (Altekar et al., 2004; Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The Bayesian tree (Fig. S3: 12 analyses with 61,6700–64,7800 generations, 3727 post-burnin trees) was very similar to the Maximum likelihood tree (Fig. 3: Bayesian posterior probabilities for key divergences additionally indicated here). Therefore, we further report only Maximum Likelihood results. To estimate potential biases introduced by discordant gene genealogies on the total evidence tree (Degnan and Rosenberg, 2009), concatenated and separate analyses for each partition or their combinations were run (16 analyses total; Table 1, Fig. S4). Phylogenetic incongruence was estimated by the non-parametric bootstrap and information theory-based measures (Salichos and Rokas, 2013; Salichos et al., 2014).

2.3. Hypothesis testing

Evaluation of hypotheses of alternative placements of eriophyoids was done in Consel 1.20 using the AU statistic as the primary test (Shimodaira, 2002) for the three datasets: full dataset (rDNA + amino acids), rDNA only, and amino acids only. A total of 12 hypotheses were tested: 10 previously proposed and two general: eriophyoids are inside or outside Trombidiformes. To infer topologies representing these hypotheses, constrained RAXML analyses (flag “-g”) were run. For these topologies, site-wise likelihoods were calculated in RAXML (flag “-f G”).

Table 1

Molecular partitions, partitioning strategies, substitution models, phylogenetic conflict as indicated by tree certainty indices (TC, RTC), placement of Eriophyoidea and other major groups of acariform mites. Resulting topologies, with bootstrap support, are shown on Fig. S4.

id	Partition	Len1	Len2	Model	Partitions	TC	RTC	bootstrap	Erio + Endeo	Erio + Tromb	Astigmata	Eleutherengona
1	rDNA	13768	4495	4 models	5,6,8,9	113.8	0.584	66	0	75	–	–
2	rDNA stem	3451	2537	2 models	5,8	116.5	0.598	80	4 [*]	88	–	–
3	rDNA loop	10317	1958	2 models	6,9	79.6	0.408	52	1 [*]	–	–	–
4	18S	3824	1509	2 models	5,6	80.0	0.419	3–16 [*]	8 [*]	–	–	–
5	18S stem	1138	877	GTR + I + G	5	74.9	0.392	1	32 [*]	–	–	–
6	18S loop	2686	632	GTR + I + G	6	46.1	0.241	0	0	–	–	–
7	28S	9944	2986	2 models	8,9	102.4	0.528	63	5 [*]	82	–	–
8	28S stem	2313	1660	SYM + I + G ^b	8	97.6	0.503	38 [*]	8 [*]	83	–	–
9	28S loop	7631	1326	GTR + I + G	9	62.9	0.324	49	4 [*]	–	–	–
10	PCG aa ^a	20834	1807	4 models	12–15	109.9	0.567	2	55 [*]	41	84	–
11	PCG aa nu ^a	19247	1397	3 models	12–14	93.0	0.482	0	39 [*]	21	25	–
12	EF1-α aa	3743	372	LG + I + G ^c	12	25.4	0.140	0	15 [*]	–	1 [*]	–
13	SRP54 aa	7810	450	LG + I + G ^c	13	53.1	0.318	8 [*]	20 [*]	–	–	–
14	HSP70 aa	7694	575	LG + I + G ^c	14	67.2	0.367	6 [*]	32 [*]	13	25 [*]	–
15	CO1 aa	1587	410	MtArtF	15	49.8	0.298	6–33 [*]	25 [*]	5	48	–
16	rDNA + PCG aa	34602	6302	8 models	5,6,8,9,12–15	134.1	0.688	69	7 [*]	65	53	–

Len1 = alignment length (nt) before character exclusion; Len2 = analysis alignment length, nt (rDNA) or aa (protein); TC = Absolute Tree Certainty Index (includes all conflicting bipartitions); RTC = Relative Tree Certainty Index (includes all conflicting bipartitions); Erio + Endeo = Eriophyoidea + Nematalycidae; Erio + Tromb = Eriophyoidea + Trombidiformes; Astigmata = Astigmata nested inside Oribatida; Eleutherengona = (Heterostigmata, Raphignathina); rDNA = four partitions: 18S stem, 18S loop, 28S stem, 28S loop; rDNA stem = 2 partitions: 18S stem, 28S stem; rDNA loop = 2 partitions: 18S loop, 28S loop; PCG aa nu = 5 protein-coding partitions: EF1-α aa, SRP54 aa, SRP54 aa, HSP70 aa, CO1 aa; PCG aa nu = 4 nuclear protein-coding partitions: same as previous but no CO1 aa; – = not on best ML tree;

* = may include a few rogue taxa from other lineages;

^a = excludes one taxon (new family of Raphignathina) with a high proportion of missing sequence data;

^b = GTR + I + G in RAXML analyses;

^c = LG + I + G + F in RAXML analyses.

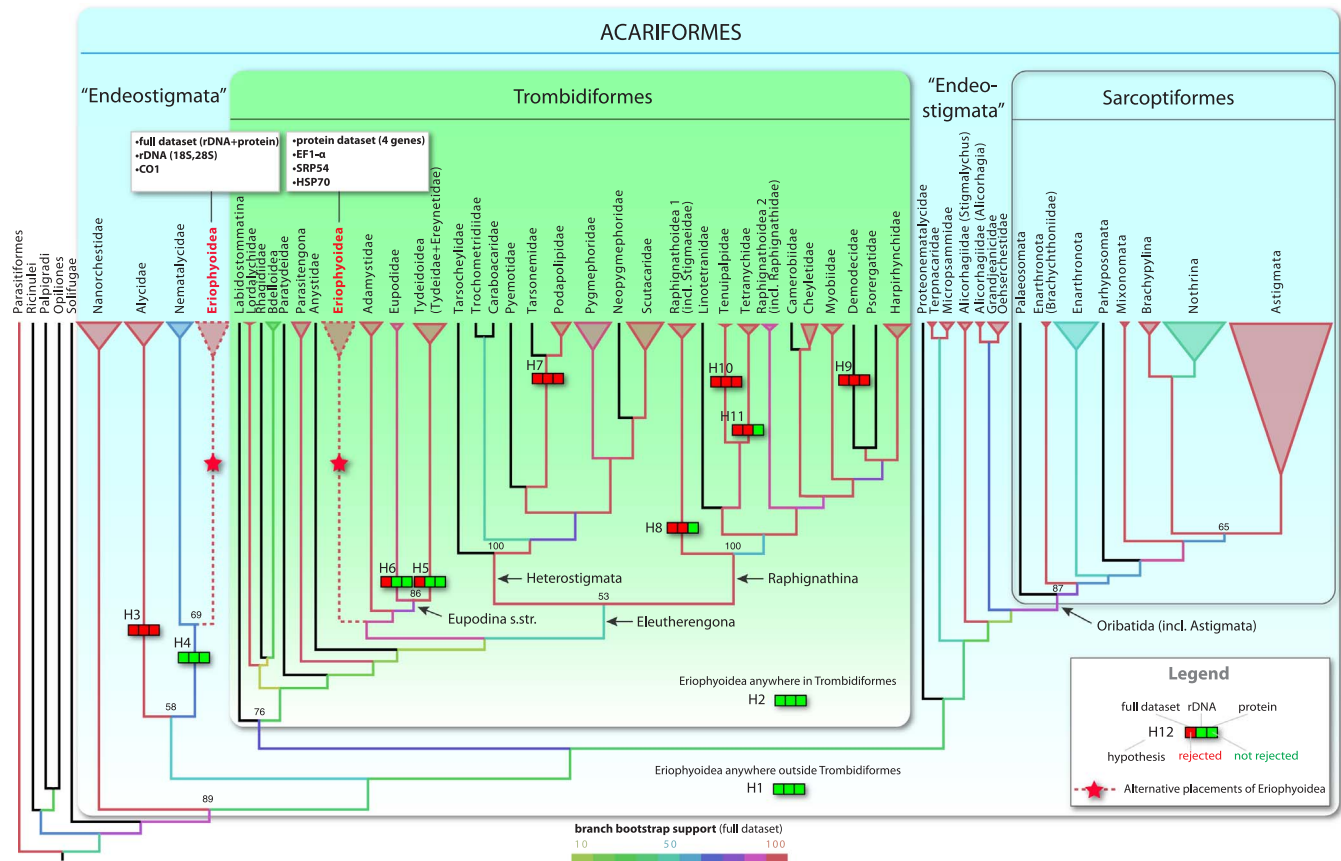


Fig. 2. Summary of phylogenetic analyses of Acariformes showing two alternative placements of Eriophyoidea (as found in this study) and statistical support for 12 previous major hypotheses of Eriophyoidea placement (Table 2). Hypotheses were evaluated using the approximately unbiased statistic (AU) at the 0.05 level for three molecular datasets (full, rDNA, protein). Bootstrap support (full analysis) is represented by color, and, for important lineages, is also given as numerical values. Complete set of bootstrap support values is shown in numerical format on Figs. S4.16 (full dataset), S4.1 (rDNA), and S4.10 (protein).

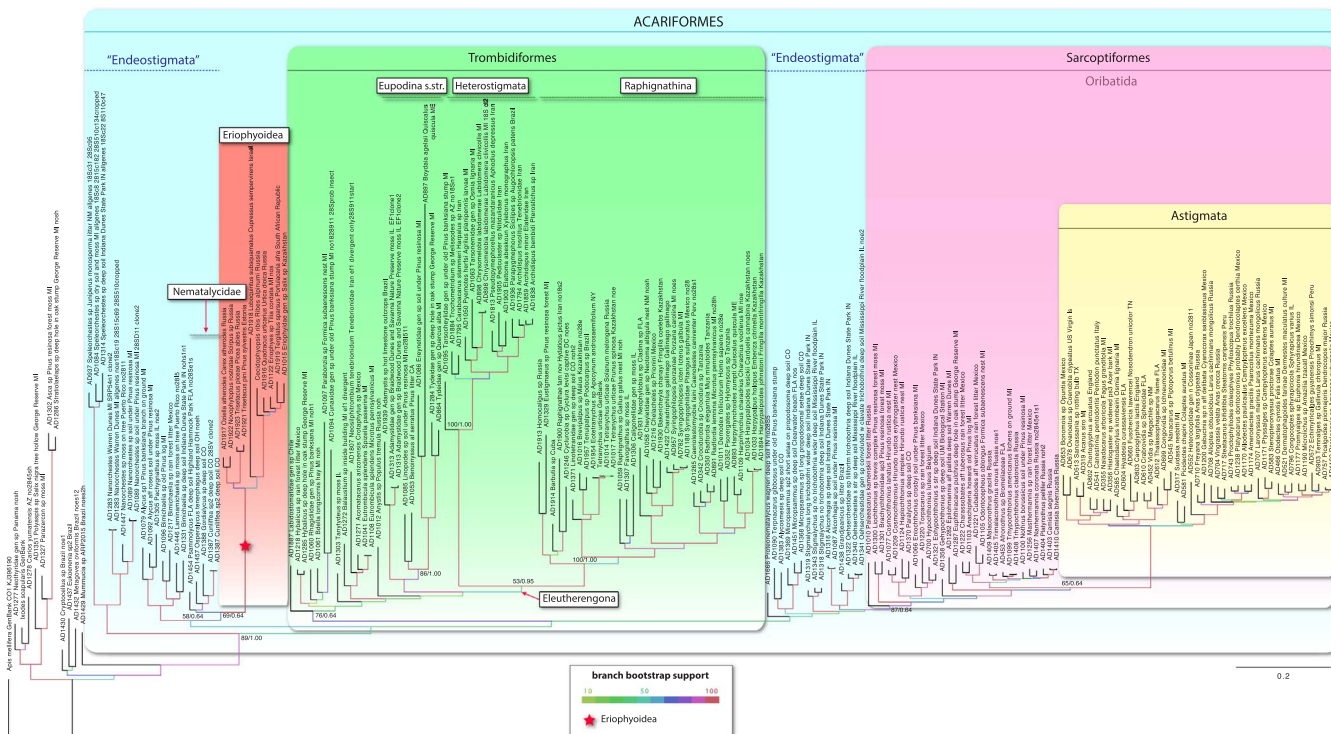


Fig. 3. Maximum Likelihood tree of Acariformes inferred from the full partition (rDNA + protein; Table 1, partition 16); the position of Eriophyoidea as sister-group of Nematalycidae is similar to that inferred by the rDNA-only partition. Bootstrap support is represented by color, and, for important lineages, is also given as numerical values. Complete set of bootstrap support values is shown in numerical format on Fig. S4.16. Posterior probabilities are also given for these important lineages (Fig. S3 shows a complete set of these values).

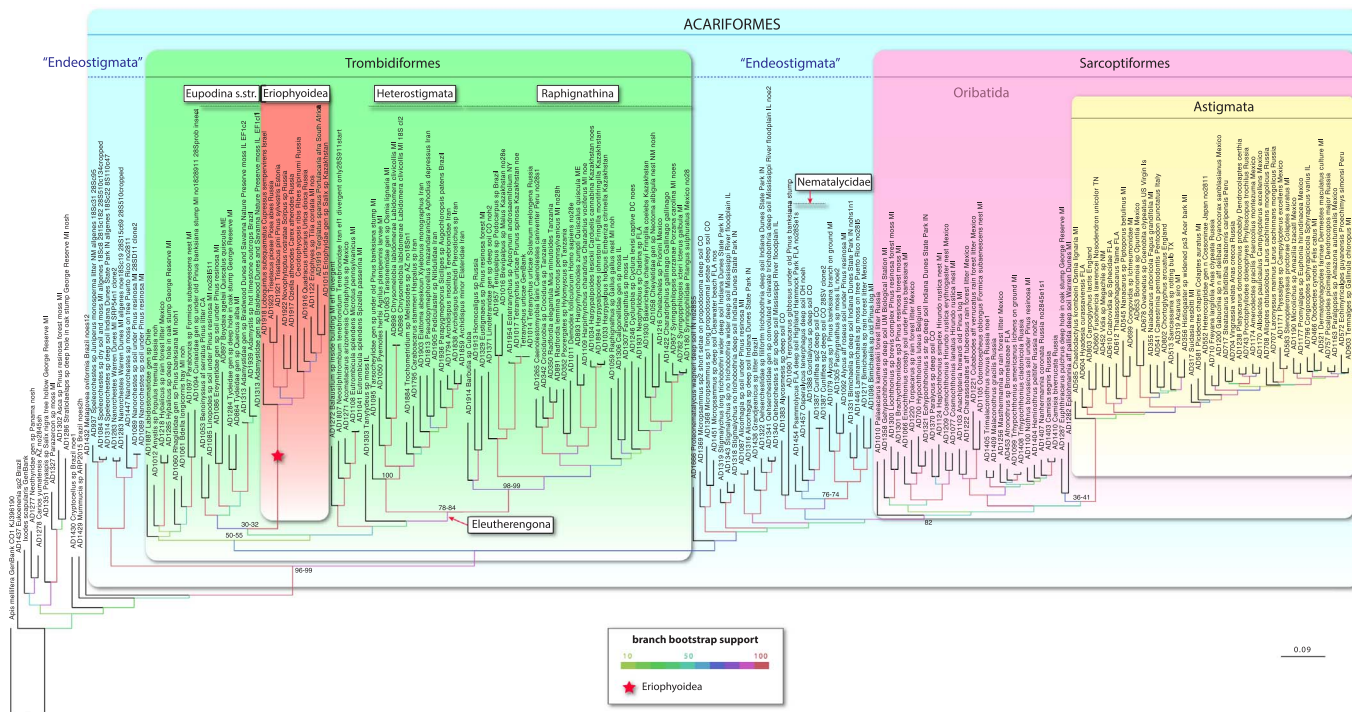


Fig. 4. Maximum Likelihood tree of Acariformes inferred from the protein-only partition (Table 1, partition 10), showing placement of Eriophyoidea within Trombidiformes. Bootstrap support is represented by color, and, for important lineages, is also given as numerical values for two similar analyses (Figs. S4.10 and S4.10b). Complete set of bootstrap support values is shown in numerical format on Figs. S4.10 and S4.10b (one taxon with large amount missing data is excluded).

Then, the RAxML per site log likelihoods were used by Consel to calculate the probability of a particular hypothesis.

2.4. Reducing systematic biases in phylogenetic inference: Removal of long branches, excluding rapidly evolved sites, and alternative rDNA coding

We tried removing various long branches and rogue taxa (Aberer et al., 2013; Bergsten, 2005; Brinkmann et al., 2005) to see how this can affect the position of Eriophyoidea. A total of 27 analyses were done (Fig. S5.1–27). For the rDNA partition, we also employed RY coding (Phillips et al., 2004) (Fig. S5.28). The latter did not alter the position of Eriophyoidea and even did not substantially change the bootstrap support for the clade Nematalycidae + Eriophyoidea (not reported further). To identify long branch attraction artifact, we tried the SAW method (Siddall and Whiting, 1999). Briefly, only one branch was removed and then only another branch was removed. If either of the branches appears at different places, then there is evidence of long branch attraction artifact. We also tried a technique based removal of rapidly evolving sites using the Tree Independent Generation of Evolutionary Rates algorithm (TIGER) (Cummins and McInerney, 2011) that has proven to be very useful in eliminating systematic biases (Qu et al., 2017). This approach analyzes the number of different character states in each column of an alignment and places characters to several custom bins, for example, slow-, medium- and fast-evolving. Then the user may exclude fast-evolving characters, which are deemed to be homoplasious, and see how this removal procedure affects the topology. Unfortunately, this analysis caused a major decrease in resolution among the main acariform lineages and for that reason was not explored further.

2.5. Interpreting bootstrap support

There is a strong opinion among some researchers that a high bootstrap support provides a definitive answer for accepting a particular phylogenetic hypothesis, while low-supported branches are not worth considering. High bootstrap support values, as often inferred in phylogenomic analyses, may be very misleading in the presence of conflict (Salichos and Rokas, 2013). For example, the same set of chelicerate taxa analyzed for 200 and 500 genes produced relatively well-supported trees, but only a few relationships on these trees matched (Sharma et al., 2014). Recent simulation and empirical analyses demonstrated that highly supported relationships recovered by genomic-scale datasets may rest on just a few genes (Shen et al., 2017). The strong support for conflicting relationships in phylogenomic data can be explained by the fact that common algorithms of deep phylogeny inference unrealistically assume that all individual gene trees are not independent and are forced to share the same underlying history. Because these algorithms do not estimate gene trees independently, the number of estimated parameters is small, hence parameter estimates tend to have a smaller variance (Liu et al., 2015). The result is overestimation of bootstrap support for conflicting relationships. Furthermore, a bootstrap value simply represents the proportion of the majority bipartition on the tree for a particular branch. It does not distinguish between cases where the remaining proportion of bipartitions is due to conflict (i.e., a single conflicting bipartition provides strong secondary signal) or uncertainty (i.e., disparate conflicting bipartitions are present, each at nearly equal proportion) (Salichos and Rokas, 2013; Salichos et al., 2014). Given these arguments, we also considered moderately and low supported branches and explicitly identified the presence of conflict by analyzing signal from individual gene/partition trees and bootstrap trees and using Internode Certainty metrics that distinguish between uncertainty and conflict (Salichos and Rokas, 2013; Salichos et al., 2014). Internode Certainty measures are based on Information theory and are designed to quantify phylogenetic incongruence, defined as the presence of several conflicting bipartitions for each node. Internode Certainty (IC) takes into consideration only

two most prevalent bipartitions, while Internode Certainty All (ICA) considers all conflicting bipartitions. An internode certainty value of 1 indicates an absolute support for the node by a single bipartition; while a value of zero indicates that two most prevalent (IC) or all conflicting partitions (ICA) are present at the same frequencies (absolute uncertainty). If, however, the frequencies of two most prevalent partitions are substantial, then this is indicative of conflict ($IC > 0$; $ICA > 0$). Negative values of both measures indicate that the internode of interest conflicts with the most prevalent bipartition; whereas values equal or approaching -1 indicate almost no support for that internode (Salichos et al., 2014). Calculations of IC and ICA are implemented in RAxML.

Coalescence methods, treating each gene tree independently and explaining phylogenetic conflict via incomplete lineage sorting, have been used successfully to resolve deep phylogenies (Linkem et al., 2016; McCormack et al., 2012; Song et al., 2015, but see Springer and Gatesy, 2016). These are potentially better alternatives to more commonly used concatenations approaches (see above), but to produce meaningful and statistically consistent results they require a large number of independent loci, i.e., genomic-scale data (Liu et al., 2015).

2.6. Ancestral character reconstruction

For character states related to cheliceral morphology (see Introduction), we used a model-based approach to estimate the ancestral states using, separately, two alternative topologies. The character has four states: (1) fixed digit not reduced and movable digit not modified; (2) fixed digit not reduced and movable digit styletiform; (3) fixed digit reduced and movable digit not modified; (4) fixed digit reduced and movable digit (sub)styletiform. For this number of states, there is a maximum of $4^2 - 4 = 12$ transition rate parameters and one state-at-root parameter. We estimated character transition rates and conducted ancestral character reconstruction analysis using the function rayDISC of the R package corHMM v. 1.20 (Beaulieu et al., 2013). We did four analyses for two trees inferred from the full and protein-only datasets and for the same set of trees ultrametricised based on penalized likelihood (Sanderson, 2002) by the R function chronopl of ape v4.1 (Paradis et al., 2004). For each tree, we ran a total of 14 corHMM analyses, with all sensical combinations of a particular state at the root, with two models of discrete trait evolution (equal rates and all-rates-different). The model log-likelihood and corrected Akaike Information Criterion (AICc) were recorded for each model. Equivalence of models was established as follows: $\Delta AICc = 0$ –2 substantial, 4 – 7 = weak, > 10 none (Anderson, 2008). To visualize state probabilities at each node we used the standard R function ‘plot’.

3. Results

3.1. Phylogeny of acariform mites

The maximum likelihood phylogeny based on the full dataset (rDNA + amino acids) inferred a monophyletic Acariformes with the closest sister group being Solifugae (Fig. 3). The earliest basal split involved the family Nanorchestidae vs. other acariform mites. The second basal branching was the grouping Alycidae + Nematalycidae + Eriophyoidea. The third basal divergence gave rise to the major clade, Trombidiformes, and a lineage that includes consecutive basal divergences of (Proteonematalycidae, (Terpnacaridae, Micropsammidae), Stigmalychus, (Oehserchestidae, Alicorhagiidae, Grandjeanicidae)), followed by the major clade of living mites, Sarcoptiformes (Fig. 3).

The ribosomal DNA partition inferred a similar pattern, except that Proteonematalycidae was placed as the most basal acariform divergence; Stigmalychus, Alicorhagiidae, Nanorchestidae, Alycidae + Nematalycidae + Eriophyoidea were rearranged to be basal branches leading to Trombidiformes (Fig. S4.1).

The protein-coding partition inferred Opiliones as sister-group to

Table 2

Testing hypotheses of eriophyoid placements using the Approximately Unbiased test, for the full (rDNA + PCG aa), ribosomal (rDNA), and protein (PCG aa) datasets.

id	Hypothesis (newick tree format) ^a	Full dataset			rDNA ^b			PCG aa		
		rank	AU	prob	rank	AU	prob	rank	AU	prob
H1	(Trombidiformes)(Erio)	1	– 13.6	0.775	2	17	0.318	2	7.4	0.638
H2	(Trombidiformes, Erio)	3	38.2	0.206	5	69.3	0.092	1	– 7.4	0.716
H3	(Alycidae, Erio)	9	313.6	0.00006	9	184	0.001	11	150.2	0.016
H4	(Nematolycidae, Erio)	2	13.6	0.44	1	– 17	0.861	3	16	0.383
H5	(Tydeidoidea, Erio)	5	69.1	0.043	4	64.5	0.086	4	20.5	0.334
H6	(Penthaeidae, Erio)	4	59.9	0.033	3	48.1	0.202	5	21.9	0.316
H7	(Tarsonemoidea, Erio)	12	1535.4	5E-38	12	1222.5	0.035	12	203.1	0.008
H8	(Raphignathae, Erio)	8	156.6	0.00001	8	136.6	9.00E-53	7	38.4	0.203
H9	(Demodecidae, Erio)	11	440.5	0.0002	11	342.9	0.01	9	104.5	0.003
H10	(Tetranychidae, (Tenuipalpidae, Erio)	10	341.4	3E-38	10	238.8	4.00E-06	10	105.6	0.009
H11	(Tetranychidae, Erio)	7	135.8	5E-33	7	124.8	3.00E-04	8	39	0.167
H12	(Astigmata, Erio)	6	112.3	0.008	6	90.5	0.005	6	22.2	0.335

rank = hypothesis rank by the AU test (the higher rank the more probable the hypothesis is relative to the others); AU = Approximately unbiased (AU) test statistic at the 0.05 significance level; prob = probability of a hypothesis, hypotheses that could not be rejected ($p \geq 0.05$) are highlighted in **bold**; Full, rDNA, and PCG datasets are defined in Table 1 (#16, 1, and 10, respectively);

^a = Erio = Eriophyoidea.

^b = Trombidiformes constraints do not include AD1094_Cunaxidae (a rogue taxon).

Acariformes; as before Nanorchestidae was the most basal acariform split, leading to the bifurcation involving Trombidiformes (where Eriophyoidea was a sister group to Eupodina s. str. + Adamysidae) and the remaining lineages (all the remaining Endeostigmata and Sarcoptiformes) (Fig. 4). Interestingly, the major trombidiform group Eleutherengona (= Heterostigmata + Raphignathina) was recovered by the full and protein datasets (Table 1, 10, 16), but not by the ribosomal dataset (Table 1, 1).

3.2. Phylogenetic placement of Eriophyoidea

3.2.1. Individual partitions

Analyses of single partitions or their biologically meaningful combinations (Table 1, Fig. S4) recovered two major placements: Eriophyoidea is sister to (i) Nematolycidae (Endeostigmata) as suggested by most ribosomal partitions, mitochondrial CO1, and the full analysis (Table 1, 1–4, 8–9, 15–16) or (ii) Eupodina s. str. + Adamysidae (Trombidiformes) as suggested by either combined or separately analyzed nuclear protein-coding genes, EF1- α , SRP54, HSP70, plus two rDNA partitions, 18S stems and 28S loop (Table 1, 5, 9–14). Bootstrap support for the former placement was low to moderate, 38–80% (Table 1), while for the latter placement it was much lower, 1–55%. In a single case (protein partition), the highest supported branch grouping Eriophyoidea within Trombidiformes was the majority bipartition (55% of bootstrap trees); Eriophyoidea outside monophyletic Trombidiformes was supported by 24% of bootstrap trees inferred by the protein partition analysis; and the grouping Eriophyoidea + Nematolycidae was supported only by 2% of trees resulted from the same analysis. By contrast, the latter grouping (Eriophyoidea + Nematolycidae) was recovered by 66% of rDNA bootstrap trees; while 44% of trees showed the nonsensical grouping of Trombidiformes + *Speleorchestes* (with the exclusion of one rogue cunaxid taxon). In conclusion, the protein partition suggested both placements (i–ii); rDNA partition suggested only the Eriophyoidea + Nematolycidae placement (i), however, when rDNA partitions were analyzed separately, some of them (18S stems and 28S loop) also suggested placement (ii) (Table 1).

3.2.2. Removal of long branches or rogue taxa

Another approach employed here was heuristic removal of apparently long branches and/or rogue taxa (Fig. S5.1–27). The following observations were made: (a) when *Speleorchestes* (Nanorchestidae) was removed from the rDNA dataset (Fig. S5.13–28), the support for Eriophyoidea + Nematolycidae dramatically increased (from 60 to 84%), while no placement of Eriophyoidea within Trombidiformes was

suggested; however, removing the entire family Nanorchestidae resulted in the grouping Eriophyoidea close to Eupodidae within Trombidiformes (i.e., at a nearly identical place as did the protein partition) on 8% of bootstrap trees vs 91% of trees that agree with the Eriophyoidea + Nematolycidae grouping. Removing Nematolycidae from the rDNA partition causes Eriophyoidea (and all Nanorchestidae) to be part of Trombidiformes on 23% of bootstrap trees; a further analysis with no Nematolycidae + Nanorchestidae increased support of Eriophyoidea being an internal group of Trombidiformes (49% bootstrap trees, IC/ICA = 0.214) while conflicting with alternative placement of Eriophyoidea within Endeostigmata (15% of bipartitions); additional removal of *Neosilphitrombium* (45.4% of missing data) increased support for the highest supported branch uniting Eriophyoidea + Trombidiformes to 66%. In contrast to removal of Nematolycidae, removal of Eriophyoidea (SAW method, Siddall and Whiting, 1999) did not affect the tree topology (Fig. S5.16). Removal of all Heterostigmata (a long branch in Trombidiformes) from the full rDNA dataset decreased bootstrap support for the highest node uniting Nematolycidae + Eriophyoidea from 66% to 44%, but did not make Eriophyoidea move within Trombidiformes. Removal of Astigmata (long branch in Sarcoptiformes) or Astigmata + Heterostigmata together had the same effect (i.e., drop in bootstrap support from 66% to 49% or 34%, respectively). A possible explanation for these results is that there are multiple, mutually attracting branches within both Endeostigmata and Trombidiformes, affecting the position of Eriophyoidea, which is itself a long branch. It may be possible that one of them is the real sister group to Eriophyoidea.

In the protein partition (Fig. S5.6–12), removing *Boydella* (Ereyneidae), *Parabonzia* (Cunaxidae), or both did not affect the position of Eriophyoidea within Trombidiformes, but slightly decreased support (from 55% to 38–49%). Removal of the entire cluster of families (Eupodina s.str. + Adamystinae) that could potentially artificially “attract” Eriophyoidea, again, did not change their position (Fig. S5.9, BS = 49%) as did further removal of taxa that formed the sister-group with Eriophyoidea on that tree (*Parabonzia* and unidentified Cunaxidae) (Fig. S5.10, BS = 34%). Removal of a relatively long basal endeostigmatid branch, *Speleorchestes*, also did not affect the position of Eriophyoidea. Removal of either Eriophyoidea or Nematolycidae (SAW method, Siddall and Whiting, 1999) did not affect the tree topology (Fig. S5.11, 12).

3.2.3. Phylogenetic hypothesis testing

Using the three partitions (full dataset, rDNA, protein), we tested 12 hypotheses suggesting a close relationship of Eriophyoidea with a

Table 3

Ancestral state reconstruction for Eriophyoidea cheliceral morphology (states 1–4, Fig. 5) based on two topologies suggesting two alternative placements of this group; evolutionary rates (q) of selected states related to cheliceral digits of eriophyoids and the probabilities (Pr) of a particular state (s1–4) to be at the root of Eriophyoidea are indicated.

id	Tree	Model	NRates	Root Pr	lnL	AICc	ΔAICc	q12	q21	q23	q32	Pr (s1)	Pr (s2)	Pr (s3)	Pr (s4)
1.4	full	ER*	1	1,0,0,0	−56.4	114.8	0.00	0.335	0.335	0.335	0.335	1.000	0.000	0.000	0.000
1.11	full	ARD	12	1,0,0,0	−46.1	117.8	3.03	0.118	0.000	0.000	0.467	1.000	0.000	0.000	0.000
2.4	full.ultra	ER*	1	1,0,0,0	−60.1	122.1	0.00	0.342	0.342	0.342	0.342	0.985	0.014	0.000	0.000
2.11	full.ultra	ARD	12	1,0,0,0	−48.3	122.2	0.10	0.088	0.000	0.000	0.645	0.962	0.000	0.038	0.000
3.4	PCG	ER*	1	1,0,0,0	−56.8	115.6	0.00	0.271	0.271	0.271	0.271	0.941	0.005	0.032	0.023
3.9	PCG	ARD	12	maddfitz	−47.9	121.5	5.86	0.000	0.000	0.000	0.839	0.001	0.000	0.999	0.000
4.4	PCG.ultra	ER	1	1,0,0,0	−62.8	127.6	6.44	1.176	1.176	1.176	1.176	0.701	0.172	0.067	0.060
4.11	PCG.ultra	ARD*	12	1,0,0,0	−47.7	121.2	0.00	0.000	0.000	1.965	3.288	0.000	0.167	0.804	0.029

id = run id, out of a total of 54 analyses, best models for both equal rate (ER) and all-rates-different (ARD) analyses are shown; Tree = trees inferred by ML analyses based on either full (full) or protein-only (PCG) partitions, these trees were converted to ultrametric trees (ultra) and then analyzed separately; Model = rate model, the best model across ER and ARD analyses based on the same tree is indicated by an asterisk (*); NRates = Number of rates; Root Pr = Root probability (1,0,0,0 means the root is fixed to state 1); lnL = log-likelihood of the solution; AICc = Corrected Akaike Information criterion value; ΔAICc = AICc difference across runs using the same tree; q12–q32 = selected rates related to the evolution of character states of Eriophyoidea (q12 means transition rate between state 1 to state 2); Pr (s1–4) = probabilities of states 1–4 at the ancestral node leading to Eriophyoidea, with the most probable state given in **bold**. Character states: (s1) fixed digit not reduced and movable digit not modified, (s2) fixed digit not reduced and movable digit styletiform, (s3) fixed digit reduced and movable digit not modified, (s4) fixed digit reduced and movable digit (sub) styletiform.

specific mite lineage (H3–H12) or more general placement inside (H2) or outside (H1) Trombidiformes (Table 2, Fig. 2). Among the tree partitions, the full partition was more restrictive, supporting only three hypotheses, H1, H3, H2 (in that order). The other two partitions suggested a wider range of statistically plausible hypotheses, among which were the above three hypotheses. As expected, rDNA favored relationships of Eriophyoidea outside Trombidiformes (H4, H1), however the next three hypotheses (H6, H5, H2) favored Eriophyoidea within Trombidiformes (Table 2, Fig. 2). The protein partition favored Eriophyoidea within Trombidiformes (H2), followed by the alternative hypotheses suggesting the opposite (H1) (Table 2, Fig. 2). Because of the relatively small number of included characters, this partition was able to reject only four hypotheses (H9, H10, H7, H3), three of which suggest various placements of Eriophyoidea within Eleutherengona (Table 2, Fig. 2).

3.2.4. Ancestral state reconstruction and evolutionary dynamics of cheliceral morphology

We ran two different sets of analyses using trees inferred by the full (rDNA + protein) and protein-only datasets (Fig. S6, runs 1, 3). These trees showed different placements of Eriophyoidea (see above). Another two sets of analyses used the same topologies converted to ultrametric trees (Fig. S6, runs 2, 4). Our *a priori* analyses suggest that state 1 (fixed digit not reduced and movable digit not modified, i.e. chelicerae unmodified chelate-dentate) was the ancestral condition (ΔAICc = 15.18–20.05 for alternative state root assignments for analyses based on the non-ultrametric tree and ΔAICc = 5.60–6.81 for the ultrametric tree analyses). In one case (Table 3, 3.9; Fig. S6, 3.9), the model with the root inferred by the *maddfitz* method (FitzJohn et al., 2009) was preferred over the model where the root was explicitly set to state 1 (ΔAICc = 5.54). All full-rate analyses (ARD) agreed that there was no back transition rate from the ancestral state (1) to state 2 (fixed digit not reduced and movable digit styletiform) in any portion of the tree (Table 3, rate q12).

All full-dataset analyses and protein equal-rate models suggested that the ancestor of Eriophyoidea had state 1 (Table 3, 1.4, 1.11, 2.4, 2.11, 3.4, 4.4; Fig. 5), while full-rate models inferred from the protein tree indicated that state 3 (fixed digit reduced and movable digit not modified) could be the ancestral state for Eriophyoidea (Table 3, 3.9, 4.11). These two contrasting solutions were ‘weakly’ equivalent (Anderson, 2008) to the alternative solutions suggesting that the ancestor of Eriophyoidea had state 1 (ΔAICc = 5.86–6.44). In addition, although having different transition rates is a biologically plausible scenario, it often has numerical difficulties when inferred in Maximum Likelihood. If these solutions do not offer a statistically better fit, then equal-rate models should be preferred (Pagel,

1999). This was the case for our non-ultrametric protein tree analysis where the single-rate models had a statistically better fit over the full-rate-model (Table 3, 3.4 vs 3.9). Only for the ultrametric protein tree analysis, the full-rate model offered a marginally better statistical fit (Table 3, 4.11 vs 4.4). The marginal preference of the full-rate model here was probably linked to the unusually short branches in the larger portion of the Acariform tree inferred by the Penalized Likelihood algorithm, and hence may be an artifact. We expect that a molecular clock analysis can infer an ultrametric tree with much more realistic branch lengths. In summary, our ancestral reconstruction analyses suggest that the eriophyoid ancestor probably had unmodified, plesiomorphic chelicerae (state 1) rather than chelicerae with the fixed digit reduced (state 3) (Fig. 5).

4. Discussion

Even with genomic-scale data, resolving relationships of a particular lineage, especially ones representing deep divergences, may be very difficult. This was the case for many lineages of arachnids, including Acariformes (Regier et al., 2010; Sharma et al., 2014). A total evidence phylogeny based on genomic data may appear to be highly resolved (Fernandez and Giribet, 2015; Starrett et al., 2016), but subsampling of loci based on different criteria (e.g., the amount of missing data) may then reveal strongly supported but conflicting relationships (Sharma et al., 2014). The difficulties in resolving chelicerate relationships may well be applicable to the early evolution of acariform mites, and, in particular, to the question of relationships of the four-legged mites, Eriophyoidea. This lineage is the only species-rich clade in acariform mites whose phylogenetic affinities among higher-order mite lineages remain essentially unresolved, with no clear indication of what are its closest relatives. There were multiple inferences based on either mitochondrial genes or 18S analyses (but not both), tending to place Eriophyoidea as a basal or nearly basal lineage of Acariformes (Li et al., 2016; Xue et al., 2017). For the mitochondrial data, the taxonomic sampling was incomplete for basal acariform lineages (i.e., Endeostigmata were not included); and the 18S relationships involved a nonsensical basal rearrangement of Astigmata (which is actually a derived lineage within Oribatida and a long branch) being sister to Alycidae + Eriophyoidea. Support for this relationship was 34% and 0.83 (bootstrap and posterior probability, respectively). Here we investigated phylogenetic relationships of Eriophyoidea using six genes and a representative taxonomic sampling of basal acariform groups. We applied molecular phylogenetic tools, particularly focusing on nuclear, protein-coding genes (along with rDNA and mitochondrial, protein coding genes) and using a representative taxonomic sampling, especially among the critical early derivative acariform and trombidiform taxa.

Probabilities of cheliceral states in the ancestor of Eriophyoidea

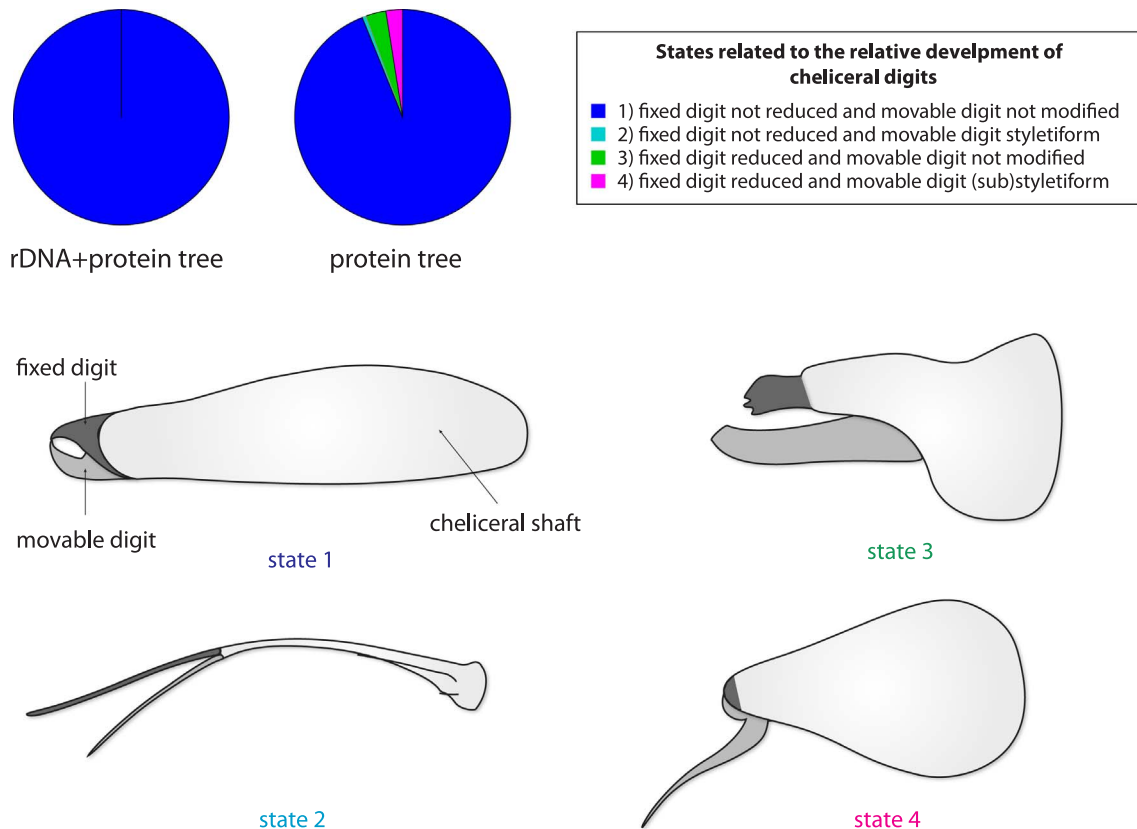


Fig. 5. Probabilities of states related to the relative development of cheliceral digits in the ancestor of Eriophyoidea based on ML topologies inferred from the full (rDNA + protein; Table 1, partition 16) and protein-only (Table 1, partition 10) datasets. Results of best-fitting ML character transition models (all equal-rates) are reported (Table 3, run id 1.4 and 3.4). States are schematically illustrated based on: (1) *Cunliffea strenzkei* (Endeostigmata: Nematalycidae); (2) Eriophyidae; (3) *Hybalicus fuscinus* (Trombidiformes: Lordalychidae); (4) *Proctotydaeus galapagosensis* (Trombidiformes: Eupodina: Tydeidae).

4.1. Robustness of phylogenetic inference

Our analyses show a remarkable, but not absolute, agreement with a previous morphological hypothesis (O'Connor, 1984) on the basal divergences leading to Sarcoptiformes (Fig. 3). The inferred relationships of Oribatida nearly precisely match the previous morphological hypothesis (Norton, 1998) and was able to resolve difficult clades, particularly, Paleosomata and Parhyposomata, which were misplaced by some studies (Maraun et al., 2009; Schaefer et al., 2010). As expected based on morphology, Astigmata is a nested lineage within Oribatida (Norton, 1998), forming a sistergroup to Brachypilina + Nothrina (total evidence, Fig. 3) or Mixonomata (protein-coding partition, Fig. 4), but not to the family Malaconothridae within Nothrina (Norton, 1998). Relationships of Heterostigmata agree well with a previous morphological hypothesis (Lindquist, 1986).

4.2. Conflict between data partitions with respect to the position of Eriophyoidea

The total evidence analysis (rDNA + nuclear and mitochondrial proteins) rendered Eriophyoidea as the sister group of Nematalycidae, the deep soil, vermiform endeostigmatid family (Fig. 1B). This basal position of Eriophyoidea was also supported by the rDNA partition and the single mitochondrial gene (CO1, as amino acids) when analyzed separately (Figs. 2, 3). Nuclear protein-coding genes (EF1- α , SRP54, HSP70) translated to amino acids, analyzed either together (Fig. 4) or separately (Fig. S4.12–14), placed Eriophyoidea within Trombidiformes, as a sister group to the clade including the families

Adamystidae, Eupodidae, Tydeidae (Fig. 1C), and Ereyneidae (Figs. 2, 4). Of these, the latter three families are currently classified in the trombidiform lineage Eupodina, where Eriophyoidea have also been placed based on morphological evidence (Lindquist, 1996, 1998). Remarkably, the potential relationship of Eriophyoidea with Nematalycidae was also proposed previously (Keifer, 1975; Shevchenko et al., 1991). In other words, the two main hypotheses suggested by our molecular analyses nearly mirror two previously published major morphological viewpoints on Eriophyoidea relationships.

The two alternative placements of eriophyoids were statistically corroborated by an Approximately Unbiased test (AU) (Table 2, H1, H2). This test consistently could not reject these two hypotheses across all three molecular partitions (full dataset, rDNA, proteins); more specifically: Eriophyoidea are *not* in Trombidiformes (H1, along with the nested hypothesis of Eriophyoidea + Nematalycidae, H4) and Eriophyoidea are *in* Trombidiformes (H4) (Table 2, Fig. 2). Similarly, hypotheses suggesting close relationships of Eriophyoidea with Eupodina (Trombidiformes) were supported by two partitions (rDNA, protein) but not by the full partition (H5, H6, Table 2, Fig. 2). However, some other hypotheses placing Eriophyoidea within specific lineages of the trombidiform lineage Eleutherengona, such as Tarsonemidae, Tenuipalpidae, or Demodecidae were strongly rejected by all our DNA partitions (H7, H9, H10, Table 2, Fig. 2). Other hypotheses, placing Eriophyoidea as sister to “Raphignathae” (paraphyletic, as expected, although the cluster of families including Stigmaeidae is monophyletic), Tetranychidae, and Astigmata could be rejected by the full and rDNA partitions but not by the protein partition, probably because of the relatively small number of characters in the latter (H8, H11, H12,

Table 2, Fig. 2).

Summarizing the above, in the rDNA partition, there is a strong phylogenetic signal placing Eriophyoidea as sister to Nematalycidae (but surprisingly not to other Endeostigmata), albeit with apparent conflict with the alternative placement near Eupodina when Nematalycidae are removed. CO1 renders nearly the same relationships, Nematalycidae + Eriophyoidea, but with weak support (BS = 6) explained by phylogenetic uncertainty rather than conflict. In contrast, the protein partition infers Eriophyoidea as sister to Eupodina s. str. (plus Adamystidae), while at the same time this partition cannot statistically reject the Nematalycidae + Eriophyoidea relationship.

4.3. Ribosomal DNA partition: Eriophyoidea, a long branch with position affected by removal of a short branch

One of the difficulties in placing Eriophyoidea is the fact that this lineage is an apparent long branch in the rDNA partition, and this partition, being the largest, provides most of the phylogenetic signal in our dataset (4495 nt vs 1807 aa). Experiments with removal of taxa from the dataset suggested that the eriophyoid long branch is “attracted” by a single short branch, Nematalycidae, but not to other lineages, even immediately related to Nematalycidae (Alycidae) or by other long branches in Endeostigmata (i.e., *Speleorchestes*). In contrast to the rDNA results, the position of Eriophyoidea was not affected by the removal of rogue taxa or long branches in the protein dataset.

Inferring the correct position of even a single long branch may be challenging as has been demonstrated on simulated datasets with a small number of terminals (Parks and Goldman, 2014). The effect of the presence of multiple long branches (as in our rDNA dataset) on a phylogenetic reconstruction is still poorly understood (Parks and Goldman, 2014). However, several important developments and practical recommendations on how to deal with long branches have been made (Bergsten, 2005; Boussau et al., 2014; O'Connor et al., 2010). The usual and easiest of these techniques is the removal of long branches (Aguinaldo et al., 1997) to see how this procedure changes the topology (Bergsten, 2005; Siddall and Whiting, 1999), but in our case, Eriophyoidea is a long branch by itself so its removal would make elucidating its position impossible.

Another group of techniques dealing with long branches is based on the removal of rapidly evolving sites (Goremykin et al., 2010; Pisani, 2004; Rivera-Rivera and Montoya-Burgos, 2016). We tried one of these techniques, Tree Independent Generation of Evolutionary Rates (TIGER) (Cummins and McInerney, 2011), but it produced a major decrease in resolution among main acariform lineages and for that reason was not explored further. This technique, among an array of other techniques dealing with long branch attraction, was recently evaluated empirically for its ability to correctly place a single, recalcitrant branch (Qu et al., 2017). However, in our dataset, which contains multiple long branches, a TIGER analysis was unsuccessful and produced inconclusive results.

4.4. Eriophyoidea: a long branch involving massive basal extinction?

The nature of some long branches in our dataset is perhaps in part due to extremely rapid substitution rates that occurred in their early evolution, but also probably due to complete extinctions of a substantial portion of their entire basal radiations. Indeed, Eriophyoidea is an ancient group, known from the Triassic, 235.0–221.5 mya (Schmidt et al., 2012; Sidorchuk et al., 2015). Both modern and Triassic eriophyoids have nine uniquely derived morphological character states (autapomorphies) (Lindquist, 1996). If these autapomorphies had evolved gradually and not in a single burst (punctuated equilibrium, Eldredge and Gould, 1997), then the stem eriophyoid taxa, having plesiomorphic states in these nine characters, must have been completely extinct. The massive extinction/loss of basal diversity scenario may also be suggested by recent paleontological findings: the entire group, the

superfamily Triasacaroidea, the stem group to the extant superfamily Eriophyoidea (Sidorchuk et al., 2015) has been extinct. However, the stem-group relationships of Triasacaroidea have been questioned by a cladistics analysis, which rendered them as a collection of mostly basal, unresolved lineages (Bolton et al., 2017). There was no support for this relationship, but if it is true then at least some Triasacaroidea may not be the stem group of the modern Eriophyoidea. Regardless of the status of Triasacaroidea, our argument about the loss of stem-group diversity of Eriophyoidea (plus Triasacaroidea) having the nine plesiomorphic states still holds.

We believe that the “long branch” properties of Astigmata are also due to a large-scale extinction of the basal diversity; although inclusion of the extremely rare, early derivative taxon *Schizoglyphus* in the future may break up this long branch (Graybeal, 1998; Hillis, 1998). However, the basal position of Astigmata, is only suggested by a single gene (18S), and unlike Eriophyoidea, can be easily resolved inside Oribatida if additional genes are used (Dabert et al., 2010; Klimov and O'Connor, 2008; Klimov and O'Connor, 2013; Pepato and Klimov, 2015). Given the possibility of massive extinction events responsible for generating long branches in our dataset, phylogenetic inference that models unusually rapid evolutionary rates by incorporating substitution rate heterogeneity across lineages (Pagel and Meade, 2008; Philippe et al., 2005) may not be justified to resolve the position of Eriophyoidea. We believe that resolving the position of Eriophyoidea will require generation of genomic or transcriptomic datasets and probably analyzing mitochondrial gene order or rare genetic events.

4.5. Morphological implications

Our work allows significant reduction to the hypothesis space concerning the origin of Eriophyoidea, pinpointing its position as two, nearly equally possible placements on the acariform tree. At the same time, we refined the taxonomic scope of its potential outgroups (i.e. Nematalycidae and Eupodina), making further morphological analyses easier, as certain spurious/conflicting character combinations can be confidently excluded. For example, the current morphological concept of the monophyletic Nematalycidae (families Nematalycidae, Micropsammidae, and Proteonematalycidae), based on the absence of prodorsal trichobothria, can now be rejected, to unite Nematalycidae and Alycidae as a monophyletic group. This grouping was consistently recovered with high or moderate support by the total evidence analysis and major partitions (Figs. 2, 3, and S4). If true, the consequence of this rearrangement is that conflicting character states arising from the presumed monophyly of Nematalycidae (as discussed in Lindquist, 1996) can be better explained by greater phylogenetic distances and the absence of common ancestry between Nematalycidae versus Micropsammidae and Proteonematalycidae. For example, Micropsammidae have paired setae *vi* (unlike Nematalycidae, Proteonematalycidae, and Eriophyoidea where they are unpaired), and Proteonematalycidae has a tracheal system (unlike Nematalycidae and Eriophyoidea, where a tracheal system is absent).

Similarly, hypotheses of potential trombidiform origins of Eriophyoidea can be refined to explain the origin of the unusual cheliceral morphology of Eriophyoidea. The ancestral eriophyoid chelicera is greatly elongated and has both nearly equally elongated fixed and movable digits (Chetverikov and Petanović, 2016; Lindquist and Amrine, 1996) (Fig. 5, state 2). This condition conflicts with the presumably derived ground plan of most modified trombidiform chelicerae where elongation of the movable digit is accompanied by a reduction of the fixed digit (Lindquist, 1998) (Fig. 5, states 2 and 5). Our amino acid topology suggests that the well-developed fixed digit as a possible ancestral state (Table 3, Figs. 5, and S6), in this case probably inherited from a trombidiform ancestor with an unmodified chelicera (Fig. 5, state 1) rather than from some trombidiform lineages where the morphology of the fixed digit is fundamentally different and apomorphic (as in Tydeidae and Ereyneidae) (Fig. 5, state 4). Indeed, many basal

Table 4
Key morphological characters for phylogenetic relationships of Eriophyoidea.

Possible synapomorphies for Eriophyoidea + Nematalycidae	Conflict	Possible synapomorphies for Eriophyoidea + Trombidiformes	Conflict
Loss of palp solenidia Unpaired vi setae	Nanorchestidae Proteonematalycidae, <i>Arhagidia</i> , some Astigmata	Highly attenuated movable digits Loss of setae <i>c</i> ₃	<i>Bimichaelia</i> All Endeostigmata have <i>c</i> ₃
Large distance between anus and genitalia Palp femur fused with palp trochanter	Demodecidae Labidostommatidae	Loss of setae <i>c</i> ₄ PS is terminal segment in adults ^a	Oehserchestidae Endeostigmata have terminal segment AN or PA
Annuli	Demodecidae	Loss of genital papillae in the adult stage ^a	All Endeostigmata have genital papillae

^a = possible synapomorphy for Eriophyoidea + Raphignathina.

trombidiform lineages have the unmodified fixed digit (Fig. 5, state 1): *Sphaerolichus* (but not *Hybolicus*), *Labidostomma*, Bdellidae (some species), Cunaxidae (some species), and Rhagidiidae (Figs. 2–4).

Our molecular topologies allow evaluation of additional potential apomorphies placing Eriophyoidea as either sister to Nematalycidae or Trombidiformes (Table 4). There are five presumed synapomorphies for the former placement (Table 4) and also five for the latter (Table 4). Of the five synapomorphies suggesting the Eriophyoidea + Trombidiformes grouping, two (namely, the loss of genital papillae and the terminally positioned segment PS) place them as sister to the trombidiform lineage Raphignathina (Table 4). Given our molecular results, either based on rDNA or protein, the relationship Raphignathina + Eriophyoidea is unlikely (Fig. 2). Hence these two potential synapomorphies probably resulted from convergent evolution. Thus, the majority of synapomorphies (5 vs 3) suggest the Nematalycidae + Eriophyoidea grouping. This grouping was also recovered, with high bootstrap support, by a formal phylogenetic analysis employing morphological characters (Bolton et al., 2017).

Based on our work, morphological hypotheses regarding potential relationships of Eriophyoidea can be, therefore, formulated more precisely, based only on a small set of endeostigmatid or trombidiform outgroups, and with a better understanding of character polarities in these putative outgroups.

4.6. Final remarks on the position of Eriophyoidea

Despite our combined, six-gene analyses having placed Eriophyoidea together with the basal endeostigmatid family Nematalycidae, it is impossible to ignore the incongruence among independent data partitions, suggesting two alternative placements to Eriophyoidea (Fig. 2). Two independent genes (18S + 28S rDNA, CO1) place Eriophyoidea together with the basal endeostigmatid family Nematalycidae, whereas three presumably independent nuclear genes (EF1- α , SRP54, HSP70), all suggest, albeit with lower support, that Eriophyoidea evolved within Trombidiformes and is related to Eupodina (Fig. 4). Thus, taking into account that the signal from rDNA is overrepresented (i.e. 4495 nt vs 1807 aa in the protein partitions), by the simple majority rule, the relationships suggested by the nuclear protein-coding genes can be considered as slightly preferred given data at hand. This conclusion is further weakly supported by the fact that (i) the position of Eriophyoidea on the amino acid-based tree is not affected by removal of other taxa; and (ii) several rDNA partitions (18S stem, 28 loop) also render eriophyoids at almost exactly the same place near Eupodina within Trombidiformes when certain taxa are removed. The arguments against the Eriophyoidea-inside-Trombidiformes hypothesis are that (i) alternative Eriophyoidea-outside-Trombidiformes placement cannot be statistically rejected with the data at hand, even by the amino acid dataset (Table 2, Fig. 2); (ii) by the fact that mitochondrial gene CO1 also agrees with the grouping of Eriophyoidea with Nematalycidae in the rDNA-based tree; and (iii) both rDNA and protein topologies reject several presumed morphological

synapomorphies for Eriophyoidea + Trombidiformes, and thus, favor the majority of synapomorphies suggesting the Eriophyoidea + Nematalycidae grouping. Additional large-scale sequencing coupled with a more thorough sampling of basal diversity of Trombidiformes and Eriophyoidea (e.g., *Pentasetacus*), and more robust analytical techniques can probably provide a definitive answer on the eriophyoid position in the future.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2017.10.017>.

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