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The phylogeny of Ptiliidae (Coleoptera: Staphylinoidea) – the smallest beetles and their evolutionary transformations

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Abstract. The smallest beetles and the smallest non-parasitic insects belong to the staphylinoid family Ptiliidae. Their adult body length can be as small as 0.325 mm and is generally smaller than 1 mm. Here we address the phylogenetic relationships within the family using formal analyses of adult morphological characters and molecular data, and also a combination of both for the first time. Strongly supported clades are Ptiliidae + Hydraenidae, Ptiliidae, Ptiliidae excl. *Nossidium*, *Motschulskium* and *Sindosium*, Nanosellini, and a clade comprising *Acrotrichis*, *Smicrus*, *Nephanes* and *Baeocrara*. A group comprising *Actidium*, *Oligella* and *Micridium* + *Ptilium* is also likely monophyletic. *Limulodes* + *Cephaloplectus*, strongly supported as a clade (*Cephaloplectus* included only in morphological analysis), are deeply nested within Ptiliidae in the morphology-only non-weighted and in the molecular analyses, but placed as sister to the remaining Ptiliidae (excl. *Nossidium*, *Motschulskium* and *Sindosium*) after successive reweighting. We propose that Ptiliidae should be taxonomically divided into two subfamilies: the most recently established Nossidiinae and Ptiliinae, the latter currently composed of seven tribes: Acrotrichini, Cephaloplectini, Discheramocephalini, Nanosellini, Ptenidiini, Ptiliini and Ptinellini, although the monophyly and taxonomic status is still uncertain in some cases. Important morphological innovations evolved in the stem group of Hydraenidae and Ptiliidae, including partly internalized mouthparts suitable for saprophagy and sporophagy, a labral-mandibular locking device, a specific elytral locking mechanism with elongated alacristae, wings with fringes of hairs, and a very unusual spermathecal pump. A complex feature of Ptiliidae linked with miniaturization is the transformation of the alae into "feather wings", with a highly efficient flight mechanism unique in beetles. Nanosellini include the smallest ptiliid species and display features correlated with extremely small body size, such as simplification of the endoskeleton of the head and thorax, far-reaching reduction of the wing venation, and a reduced number of antennomeres.

Key words. Ptiliidae, phylogeny, morphology, molecular data, combined analyses.

1. Introduction

The cosmopolitan Ptiliidae (featherwing beetles) are a comparatively small subunit of the megadiverse polyphagan clade Staphylinoidea of about 60,000 species. Nevertheless the family is interesting well beyond the mere scope of its species diversity for the extremely small body size. It includes the smallest beetles and the smallest non-parasitic insects. Adult body length of the majority of species does not exceed 1 mm, while the smallest is only 325 µm long (Fig. 1; Polilov 2015a). These morphologically diverse beetles (Figs. 2A–L, S1–S7) are often comparable in size to single-celled eukaryotes, for instance *Paramecium*. In recent years Ptiliidae became a model group for studying the effects of miniaturization on structural features of insects (Polilov 2015b, 2016). The external and internal morphology was described in detail for the adults (Sörensson 1997; Polilov 2005, 2008a; Polilov & Beutel 2009; Polilov 2016) and larvae (Dybas 1976; De Marzo 2002; Grebennikov & Beutel 2002; Polilov & Beutel 2009; Jałoszyński 2014, 2015; Polilov 2016; Sörensson & Delgado 2019). The mor-

phology of the brain was also investigated (Makarova & Polilov 2013; Polilov et al. 2019a), as well as the ultrastructure of the eyes (Makarova et al. 2019), the structure of the mouthparts (Yavorskaya et. al. 2017), structure and function of the flight apparatus (Polilov et al. 2019b; Yavorskaya et al. 2019), the reproductive system (De Marzo 1992; De Coninck & Coessens 1981), and the sperm morphology (Dybas & Dybas 1981, 1987).

The taxonomic diversity of Ptiliidae considerably increased in the last years (Polilov 2008b; Grebennikov 2009a; Hall 2016). An entire series of taxonomic contributions was presented by Darby (2013, 2014, 2015a,b, 2016, 2017a,b, 2019) and Darby & Johnson (2011). The number of described genus- and family-group taxa also increased in the last decades (e.g. Sörensson 1997; Hall 1999; Polilov 2008b; Grebennikov 2009b; Darby 2015a,b, 2017a, Sörensson & Delgado 2019). The distribution of 877 valid species among the 100 genera (including a sprinkle of fossils) is, however, highly asymmetrical, with the five most speciose genera accounting for more than half of the diversity (*Acrotrichis*, *Ptenidium*, *Ptinella*, *Ptilium*, *Actidium* with 233, 76, 59, 46 and 33 species, respectively; Newton 2019). In contrast to this, 49 among 100 Ptiliidae genera are monotypic (Newton 2019). This uneven distribution of species suggests future significant changes in (and perhaps additions to, see below) the species- and genus-level taxa of Ptiliidae.

Named species are relatively uniformly distributed over temperate and tropical parts of the globe without any marked geographic bias. Faunas of large areas, however, remain acutely understudied, with the inventory of Australia and South America perhaps the least known. Due to their poorly documented diversity, inadequate taxonomy, and lack of a phylogenetic framework, Ptiliidae have been mainly ignored as a biogeographic tool. However, considering the high vagility of the prevailing winged species, a strong dispersal capacity can be assumed for the majority of the family. Thus, in a survey of the Malagasy fauna (Darby 2013, 2014, 2017a) not a single endemic ptiliid genus was found. The lack of adequate faunal knowledge undermines any general evaluation of biogeographic or diversity pattern. To mention only one example, one of four ptiliid genera originally considered as endemic to New Zealand (Johnson 1982: *Kuschelidium*) was subsequently reported from the Okinawa Islands, Japan (Sawada & Hirowatari 2002). Considering all these uncertainties, it is plausible to expect that the number of Ptiliidae species-group taxa will at least double.

The biology of Ptiliidae is an interesting mixture of idiosyncratic properties and those likely linked with minute size. Adults are either mycophagous (i.e. sporophagous, if feeding on fungal spores, or consuming other fungal materials, such as mycelia) or saprophagous (i.e. feeding on decaying organic material, Yavorskaya et al. 2017). Feeding preferences of larvae are less known, although Jałoszyński (2015) reared those of *Ptenidium pusillum* from eggs to pupae entirely on yeast. Adults and larvae of Ptiliidae are found in environments rich in organic materials, such as forest leaf litter, flood- and agricultural debris (De Marzo 2002), under the bark of relatively fresh dead trees, or in association with ants (Maruyama et al. 2013) or termites (Grebennikov 2008a). Some species have seemingly unique preferences. The eyeless and wingless *Malkinella cavatica* is restricted to caves in the South African Cape Peninsula (Dybas 1960), while *Motschulskium sinuaticolle* inhabits the rocky intertidal zone on the pacific coast of North America (Caterino et al. 2015). The number of larval instars in Ptiliidae is either two (De Marzo 1996; Jałoszyński 2015) or three (Polilov & Beutel 2009). Females lay disproportionally large eggs, about half as long as their bodies (Taylor et al 1982; Polilov 2008a), while the spermatozoa can be even longer, comparable to the adult body length or longer than that (Dybas & Dybas 1981, 1987; De Marzo 1992). Species of *Ptinella*, *Pteryx*, *Ptinellodes* and their close relatives exhibit strong polymorphism with respect to their compound eyes and hind wings (Dybas 1978; Taylor 1981), and some species are parthenogenetic (Dybas 1966).

Seventeen records of Ptiliidae are known from the Cenozoic, and four from the Mesozoic (table 1 in Shockley & Greenwalt 2013; Yamamoto et al. 2018). The oldest fossils, reported from Lebanese amber (Early Cretaceous, about 125 Myr ago, Poinar & Poinar 2008; Peris et al. 2016), remain inadequately known. The only extinct genus, *Kekveus* from Burmese amber (Late Cretaceous, 99 mya), is the second oldest ptiliid fossil. This member of the crown group is only 0.536 mm long, which is small even by ptiliid standards (Yamamoto et al. 2018). Stem group fossils are unknown.

In contrast to the greatly improved morphological knowledge, the phylogeny of the family remains clearly understudied, particularly when compared to other staphylinoid families, such as Hydraenidae (e.g. Beutel et al. 2003; Trizzino et al. 2013; Rudoy et al. 2016; Villastrigo et al. 2019), Leiodidae (e.g. Fresneda et al. 2011; Antunes-Carvalho et al. 2019) or Staphylinidae (e.g. Hansen 1997; McKenna et al. 2015a). The sister group relationship of Ptiliidae with the aquatic Hydraenidae is well-established, whereas the precise affinities with the potentially related terrestrial staphylinoid Leiodidae and Agyrtidae (Beutel & Leschen 2005; Grebennikov & Newton 2012) are still uncertain (McKenna et al. 2015a; Zhang et al. 2018).

Ptiliidae was historically divided into two to four subfamilies and up to eight tribes, while the traditional system includes three subfamilies and eight tribes (Hall 2000, 2016). Only two of the tribes and one subfamily have been given any phylogenetic justification: Nanosellini (Hall 1999), Discheramocephalini (Grebennikov 2009b) and Nossidiinae (Sörensson & Delgado 2019). For one of the subfamilies, Acrotrichinae, apomorphic characters have been outlined, and the subfamily is recognized by most authors (Johnson 2004; Hall 2000, 2016). One of the subgroups, Cephaloplectinae, has been historically treated as a separate family, Limulodidae (Seevers & Dybas 1943). Morphologically highly distinct genera

Fig. 1. Ptiliidae beetles, habitus, SEM. The smallest (**A**: *Scydosella musawasensis*) and one of the largest (**B**: *Sindosium* sp.).

of this group were alternatively described in Ptiliidae (Matthews 1867) or in Staphylinidae (Sharp 1883). The subfamily Ptiliinae, which contains the largest number of genera, has long been recognized as paraphyletic (Hall 2000, 2016). For some representatives of Ptiliidae, sequences of a few genes were used in phylogenetic studies of Staphyliniformia (Caterino et al. 2005; McKenna et al. 2015a; Trizzino et al. 2013) or Coleoptera in general (Hunt et al. 2007; Lawrence et al. 2011; Hendrich et al. 2014; McKenna et al. 2015b; Zhang et al. 2018). However, the taxonomic sampling of Ptiliidae was always too limited to clarify phylogenetic relationships within the family. The first phylogenetic analyses of the family was carried out very recently (Sörensson & Delgado 2019). However, it is restricted to morphological characters of the larvae, with a strong focus on chaetotaxy, and with nine ingroup genera the taxon sampling is quite limited.

The primary aims of our study are (1) to document adult morphological characters of all major ptiliid lineages, mainly using scanning electron microscopy (SEM), (2) to conduct the first family-wide phylogenetic analysis using DNA sequences *and* adult morphological characters, and (3) to propose a phylogeny-based classification of the family.

2. Material and methods

2.1. Material

Thirty terminals representing 29 genera of Ptiliidae were used for assessing morphological characters, together with 4 non-ptiliid staphylinoid outgroup taxa. For the analyses of molecular data, 35 specimens of 30 species of Ptiliidae belonging to 22 genera were sequenced (Table S1). Both approaches were designed to cover all currently recognised subfamilies and tribes. As outgroup taxa we used an ample representation of Hydraenidae, considered to be the sister group of Ptiliidae, and also representatives of the staphylinoid Leiodidae and Staphylinidae. Specimens used for morphological study were fixed in FAE (formaldehyde-acetic acid-ethanol) or in Bouin solution and stored in 70% ethanol. All specimens used for DNA sequencing were preserved in 95–100% ethanol.

2.2. Morphological techniques

Permanent mounts were prepared from specimens after DNA extraction and from additional specimens. All were treated with sodium peroxide solution and hydrogen peroxide, dehydrated, and embedded in Euparal. The mounts were examined under an Olympus BX43 microscope. External morphology was studied using Philips XL 30 ESEM and Jeol JSM-6380 scanning electron microscopes. Before this, specimens were dehydrated in a series of increasing concentration of alcohol and in acetone, dried at the critical point, mounted on the tip of a fine needle fixed on a rotatable specimen holder (Pohl 2010), and sputter-coated with gold.

2.3. DNA extraction and sequencing

We extracted DNA non-destructively, using a standard Phenol-Chloroform extraction or with commercial kits ("DNeasy Tissue Kit", Qiagen GmbH, Hilden, Germany). Voucher specimens and DNA extractions are deposited in the collections of the Institut de Biología Evolutiva, Barcelona (IBE) and Lomonosov Moscow State University, Moscow (MSU). We obtained fragments from 7 different genes (five mitochondrial and two nuclear) in 5 different amplification reactions (see Table S2 for the primers used): (1) 3′ end of Cytochrome Oxidase Subunit 1 (COI-3′); (2) 5′ end of 16S rRNA plus tRNA transfer of Leucine plus 3′ end of NADH subunit 1 (16S); (3) an internal fragment of 12S rRNA (12S); and internal fragments of the nuclear (4) small ribosomal unit, 18S RNA (18S) and (5) large ribosomal unit 28S RNA (28S). In some specimens, due to difficulties with amplification, we used internal primers for the COI-3′ sequence, obtaining two fragments of 400 bp each (Table S2). PCR products were purified by standard ethanol precipitation and sent to external facilities for sequencing. DNA se-

quences were assembled and edited using Geneious 6 software (Biomatters Ltd, Auckland, New Zealand). Ambiguous calls in the nuclear genes were coded as "N"s. New sequences (118) were deposited in GenBank with accession numbers given in Table S1.

2.4. Phylogenetic analyses

A total of 68 morphological characters were entered in a matrix created with Winclada (Nixon 1999) and analysed using parsimony with NONA (ratchet, 1000 replicates) (Goloboff 1995) and TNT (traditional search, 1000 replicates) (Goloboff et al. 2008). In the first analysis all morphological characters were assigned equal weight and all were treated as unordered. The implied weighting option in TNT (Goloboff et al. 2008) was used in the second morphology-based analysis (K = 3.000). Bremer support values were calculated with NONA. Character evolution tracing and enforced topologies were done using Mesquite (Maddison & Maddison 2018). Trees were rooted between Ptiliidae + Hydraenidae and the rest of the families, following recent molecular (e.g. Hunt et al. 2007; McKenna et al. 2015a) and morphological phylogenies (Hansen 1997; Lawrence et al. 2011).

For molecular characters, edited sequences were aligned with MAFFT v.6 using the G-INS algorithm and default values for other parameters (Katoh & Toh 2008). We analysed the aligned matrix using a fast Maximum Likelihood (ML) heuristic algorithm in RAxML-HPC2 (Stamatakis et al. 2008) in the CIPRES Science Gateway (Miller et al. 2010), using a partition by gene (pooling 16S+tRNA+NAD1 in a single partition) with a GTR+G evolutionary model independently estimated for each partition. Node support was assessed with 100 pseudoreplicas with a rapid bootstrapping algorithm (Stamatakis et al. 2008).

We also analysed the molecular data using Bayesian methods implied in BEAST 1.7.4 (Drummond et al. 2012). We used a Yule speciation model and a relaxed molecular clock, with the same partitions as in the ML analysis. Given the amount of missing data (Table S1), we opted to test the robustness of the analyses to model assumptions rather than the goodness of fit of the model to the data (Nascimento et al. 2017). We thus compared three different model combinations: (A) all partitions with the most complex GTR+G model; (B) mitochondrial partitions with a GTR+G, and nuclear with a HKY+G, model; and (C) all partitions with a HKY+G model. We established a prior tree root height of 200 Ma, and an age of the ingroup (i.e. the clade Hydraenidae + Ptiliidae) of 150 Ma, following the estimations of Hunt et al. (2007) and McKenna et al. (2015b), both with a normal distribution with a standard deviation of 1 Ma. Note that these are only tentative calibrations with an exploratory purpose. A precisely dated phylogeny is out of the scope of our study, and in any case the obtained divergence dates are irrelevant for the estimation of the topologies and do not affect our results and conclusions. We used flat priors for the rates of all genes, and default priors for the rest of the parameters. The analyses ran for 100 million generations (saving trees every 5,000) and convergence was assessed with the Effective Sample Size (ESS) values, as measured with Tracer v1.6.

We analysed the combined morphological and molecular data matrixes for 26 terminals with both morphological and molecular data using Bayesian probabilities with MrBayes 3.2 (Ronquist & Huelsenbeck 2003), using the Mkv model (Lewis 2001) with a single partition for the morphological data, and a partition by genes for the molecular data.

In all Bayesian analyses two separate runs were conducted, each with one cold and three heated chains, checking for adequate mixing with the statistics provided by the program. To assess convergence and establishing a burn-in fraction, we initially set the analyses to run for an overestimated 25×10^6 generations, sampled every 1,000. We then assessed convergence by visual examination of a plot of the standard deviation of the split frequencies between the two simultaneous runs, establishing the burn-in when it reached stable values at ca. 0.01. Once the burn-in was fixed, we let the analyses run until the effective sample size (ESS) reached values above 200 as estimated in Tracer v1.6, considered to be sufficient for a good sampling of the post-burn-in tree space (Rambaut et al. 2014). The resulting trees were combined in a majority rule consensus topology with posterior probability (pp) of nodes calculated using the Sumt command in MrBayes.

3. Results

3.1. List of morphological characters

Due to fragmentary knowledge of the immature stages of the taxa under consideration, our choice of morphological characters is restricted to adults of Ptiliidae. It includes characters used in earlier publications on particular groups (Hall 1999; Grebennikov 2009b), features traditionally used in taxonomic or phylogenetic studies on Ptiliidae and related groups of beetles (e.g. Seevers & Dybas 1943; Perkins 1980; Hansen 1997), data from a comprehensive morphology-based study on the phylogeny of Coleoptera (Lawrence et al. 2011), and also new characters based on recent observations (Table S3).

1 Orientation of head: **(0)** not distinctly deflexed; **(1)** strongly deflexed, frons and postclypeus in ventral position. — *Limulodes* and *Cephaloplectus* and other cephaloplectines are characterized by a strongly deflexed head, which appears thus largely hidden below the pronotum in dorsal view (Figs. 2C,D, 3A). This is associated with their myrmecophile mode of life according to Park (1933), Seevers & Dybas (1943) and Wilson et al. (1954). The head is prognathous in the remaining Ptiliidae, like in the vast majority of Coleoptera (e.g. Lawrence et al. 2011) (Fig. 2).

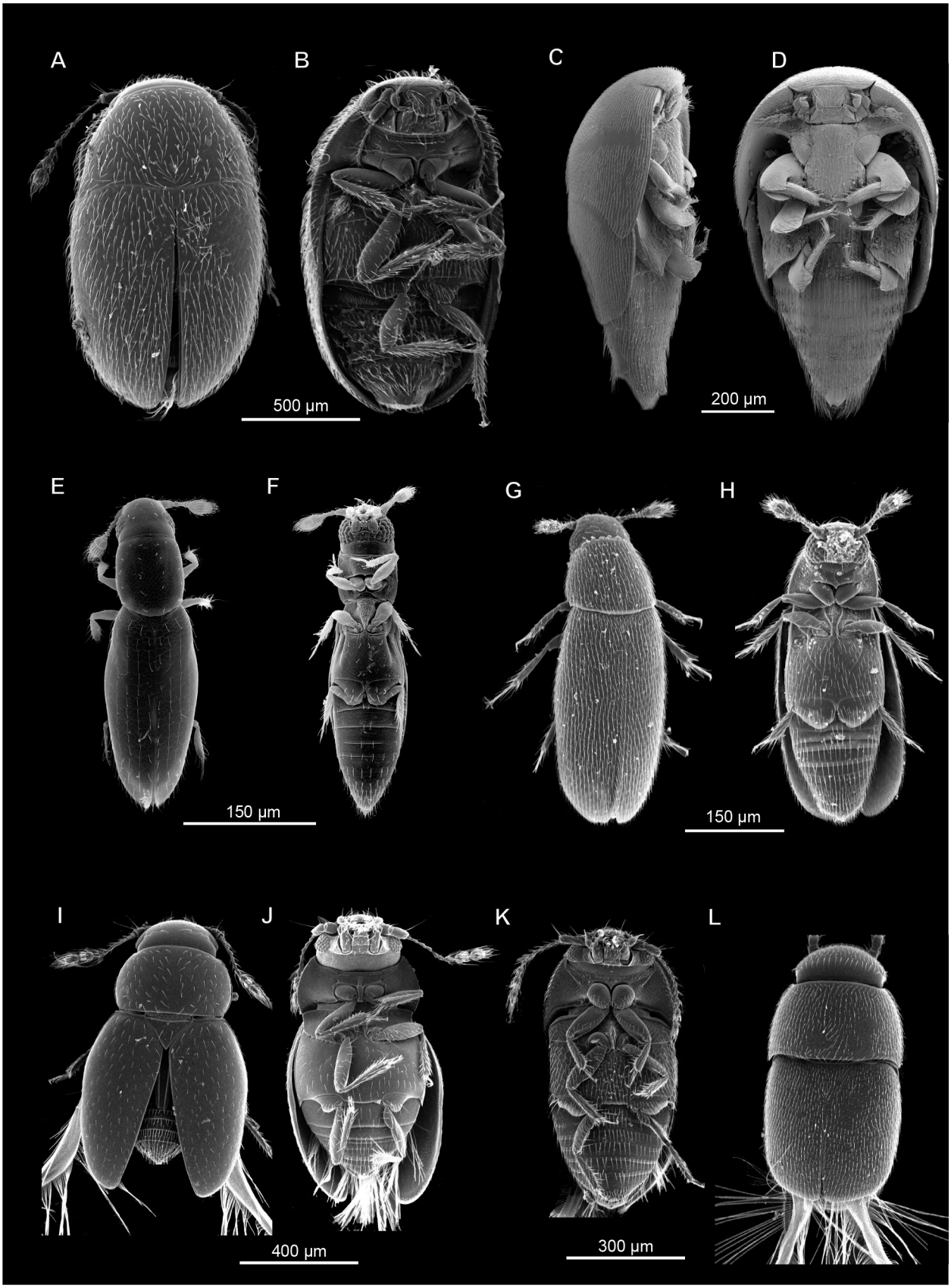

Fig. 2. Ptiliidae beetles, habitus, SEM. **A**,**B**: *Nossidium pilosellum*; **C**,**D**: *Limulodes parki*; **E**,**F**: *Scydosella musawasensis*; **G**,**H**: *Primorskiella anodonta*; **I**,**J**: *Ptenidium formicetorum*; **K,L**: *Acrotrichis montandoni*; A,E,G,I,K: dorsal view; B,D,F,H,J,L: ventral view; C: lateral view.

2 Epistomal ridge: **(0)** present; **(1)** absent. — The ridge delimiting frons and clypeus is present in Hydraenidae and Agyrtidae. It is usually also preserved in Leiodidae (Hansen 1997), but absent in *Catops* (Antunes et al. 2017). The frontoclypeal transverse strengthening ridge is absent in all ptiliids (e.g. Yavorskaya et al. 2018). The reduction occurs frequently in other groups of Coleoptera (e.g. Lawrence et al. 2011).

3 Gular sutures: **(0)** present; **(1)** absent. — The gular sutures are absent in Ptiliidae (e.g. Yavorskaya et al. 2017). The sutures and corresponding internal ridges are almost generally present in Coleoptera (e.g. Lawrence et al. 2011).

4 Transverse ventral genal bulge: **(0)** absent; **(1)** present. — This structural modification is present in Hydraenidae (Jäch et al. 2000; Beutel et al. 2003: figs. 1b, 6b).

5 Compound eyes: **(0)** present in all specimens; **(1)** dimorphic; **(2)** absent in all specimens. — Most ptiliids have compound eyes, but there are dimorphic species with some eyeless individuals (e.g. species of *Pteryx* and *Ptinella*). Species of *Limulodes* and other Cephaloplectinae always lack compound eyes (Seevers & Dybas 1943). Compound eyes are generally present in Hydraenidae and Agyrtidae (Jäch et al. 2016), and also preserved in most epigean species of Leiodidae (e.g. Antunes-Carvalho et al. 2017).

6 Antennal grooves: **(0)** absent; **(1)** present, shallow; **(2)** present, distinct. — Species of *Limulodes* and other Cephaloplectinae store their folded or basally curved antennae in furrows on the ventral surface of the head (Fig. 3A; Seevers & Dybas 1943: fig. 15). Shallow ventral antennal grooves are present in Hydraenidae (Beutel et al. 2003).

7 Number of antennomeres: **(0)** 11; **(1)** 10; **(2)** 9; **(3)** 8. — Most ptiliids have 11 antennomeres, but *Primorskiella*, *Cylindrosella*, *Scydosella* and some other nanosellines (Sörensson 1997; Hall 1999) have only 9, a number also found in *Limulodes*. Eight or 9 are present in *Paralimulodes* (Seevers & Dybas 1943; Wilson et al. 1954).

8 Insertion of flagellomere 1: **(0)** flagellomere 1 not retracted into pedicellus; **(1)** flagellomere 1 distinctly retracted into pedicellus and proximal flagellum distinctly narrower than the pedicellus; **(2)** flagellomere 1 distinctly retracted into pedicellus, flagellomere 1 and pedicellus forming a continuous cylindrical structure. — A retracted base of flagellomere 1 and a proximal flagellum distinctly narrower than the pedicellus is a characteristic feature of Ptiliidae (Hall 1999: figs. 49–59; Yavorskaya et al. 2017; Yamamoto et al. 2018: fig. 3A). Flagellomere 1 is as wide as the pedicellus in *Cephaloplectus* (Seevers & Dybas 1943: fig. 15) and both form a continuous cylindrical structure. The antennal morphology varies greatly in Hydraenidae (Jäch et al. 2016: fig. 11.1.8), but the base of flagellomere 1 is never retracted (Jäch et al. 2016: fig. 11.1.8).

9 Antennal club: **(0)** absent, antennae filiform or gradually widening distally; **(1)** 5-segmented; **(2)** 3-segmented **(3)** entire flagellum club-shaped, with short cylindrical flagellomeres. — Three-segmented clubs are almost always present in Ptiliidae (e.g. Sörensson 1997: fig. 10). A club is missing in *Cephaloplectus*, where the entire flagellum appears club-shaped, with broad and short flagellomeres (Seevers & Dybas 1943: fig. 15). A five-segmented and densely setose club is usually present in Hydraenidae (Beutel et al. 2003; Jäch et al. 2016). The antennae are filiform or gradually widening distally in most members of Leiodidae and in Agyrtidae (Hansen 1997; Newton 2016).

10 Labral-mandibular locking mechanism: **(0)** absent; **(1)** present. — A lateral labral groove forms a locking device with a lateral process of the mandible in Ptiliidae and Hydraenidae, arresting the labrum in a folded position (Yavorskaya et al. 2017). This mechanism is absent in other groups of Staphylinoidea (Newton 2016; Antunes-Carvalho et al. 2017; Yavorskaya et al. 2017).

11 Shape of mandible: **(0)** apical part of mandibles distinct and prominent; **(1)** distal part shortened, not prominent; **(2)** distal part reduced. — The apical part of the mandible is prominent in Agyrtidae and Leiodidae (Hansen 1997; Newton 2016; Antunes-Carvalho et al. 2017) like in most groups of Coleoptera (Lawrence et al. 2011). It is distinctly shortened but still recognizable in Hydraenidae (Beutel et al. 2003; Jäch et al. 2016) and most Ptiliidae (Hansen 1997; Yavorskaya et al. 2017), and absent in *Primorskiella*, *Cylindrosella*, *Scydosella*, and other nanosellines (Hall 1999; Yavorskaya et al. 2017).

12 Number of segments of galea: **(1)** two-segmented, with distinct suture; **(2)** one-segmented. — The galea is distinctly two-segmented in most ptiliids like in other groups of Staphylinoidea (e.g. Lawrence et al. 2011; Newton et al. 2016). The separating suture is obliterated in Nanosellini and Cephaloplectinae.

13 Shape of apical maxillary palpomere: **(0)** not aciculate; **(1)** aciculate. — An aciculate (Seevers & Dybas 1943) or awl-shaped apical maxillary palpomere is generally present in Ptiliidae (e.g. Yavorskaya et al. 2017).

14 Base of mentum: **(0)** separated from submentum by suture; **(1)** fused with submentum. — The mentum is separated from the submentum by a distinct suture in most ptiliids, like in Hydraenidae, Agyrtidae, Leiodidae and most other groups of Coleoptera (Lawrence et al. 2011). The suture is obsolete or absent in *Oligella* and *Acrotrichis*.

15 Shape of mentum: **(0)** wider than long, greatest width at base; **(1)** approximately as long as wide, or slightly longer than wide, with sides subparallel; **(2)** approximately as long as wide, dilated medially; **(3)** longer than wide, dilated in middle region. — The mentum is almost square in most ptiliids, with subparallel sides. It is longer than wide and dilated in its middle region in Nanosellini. The mentum is transverse and widest at the base in Agyrtidae and

Leiodidae (Newton 2016; Antunes-Carvalho et al. 2017). It is slightly longer than wide and subparallel or slightly converging anteriorly in Hydraenidae (Beutel et al. 2003).

16 Lateral premental lobes: **(0)** distinctly developed; **(1)** vestigial. — Lateral premental lobes, also referred to as bilobed ligula (Lawrence et al. 2011), are present in most genera of Ptiliidae. They are vestigial in *Primorskiella*, *Cylindrosella*, *Scydosella*, and some other nanosellines (Hall 1999). Membranous lateral lobes are also present in Agyrtidae (Newton et al. 2016a), Hydraenidae (Beutel et al. 2003) and *Catops* (Antunes-Carvalho et al. 2017: fig. 3j,k). The homology of these structures is not fully understood yet.

17 Palpiger: **(0)** separated from prementum; **(1)** fused. — The palpiger is fused with the prementum in all ptiliids and free in Hydraenidae, Agyrtidae and Leiodidae (Beutel et al. 2003; Newton 2016a,b; Antunes-Carvalho et al. 2017).

18 Number of labial palpomeres: **(0)** 3; **(1)** 2; **(2)** 1. — Three-segmented labial palps are present in *Nossidium*, *Motschulskium*, *Sindosium*, *Limulodes*, and *Cephaloplectus*, and also in Hydraenidae, Leiodidae and Agyrtidae (Jäch et al. 2016; Newton 2016a,b). They are two-segmented in most ptiliids, and one-segmented in *Primorskiella*, *Scydosella*, and *Cylindroselloides*.

19 Laminatentorium: **(0)** present; **(1)** absent. — Ptiliids differ from other staphylinoids including Hydraenidae (Beutel et al. 2003) and *Catops* (Antunes-Carvalho et al. 2017) in the complete absence of the laminatentorium, with widely separated subparallel tentorial arms (e.g. Seevers & Dybas 1943; Sörensson 1997; Yavorskaya et al. 2017).

20 Dorsal arms of tentorium: **(0)** present; **(1)** absent. — The dorsal tentorial arms are present in most ptiliids, but missing in all examined Nanosellini (Sörensson 1997; Yavorskaya et al. 2017).

21 Cervical sclerites: **(0)** present; **(1)** absent. Almost generally present in Polyphaga but missing in Ptiliidae (Lawrence et al. 2011).

22 Location of greatest width of pronotum: **(0)** in posterior half; **(1)** in middle region; **(2)** in anterior half. — The shape of the prothorax varies strongly within Ptiliidae. It is widest in the posterior half in *Nossidium* and *Sindosium*, and also in several other genera including *Limulodes* and *Cephaloplectus*. This condition is also found in Agyrtidae and *Catops* (Newton 2016a,b). The anterior half is widest in *Hydraena* and *Ochthebius* (Jäch et al. 2016), and also in *Actidium* and some other genera of Ptiliidae.

23 Posterior pronotal angles: **(0)** not or only moderately produced posteriorly, not closely clinging to elytral humeri; **(1)** strongly prolonged and closely clinging to elytral humeri. — Distinctly prolonged and in close contact to elytral humeri in *Limulodes* and *Cephaloplectus* (Seevers & Dybas 1953: figs. 1–14, 34).

24 Microsculpture of dorsal surface of pronotum and elytra: **(0)** smooth or punctate; **(1)** distinctly reticulate or scaly. — All studied ptiliids except for *Nossidium*, *Sindosium*, *Ptenidium*, *Dacrysoma* and *Cissidium* have a reticulate or scaly microsculpture on the dorsal surface (Fig. 3C–E).

25 Microsculpture of ventral surface of thoracic segments: **(0)** smooth or punctate; **(1)** distinctly reticulate or scaly; **(2)** dense vestiture of short hairs forming plastron. — The ventral body surface displays a distinct microsculpture in most genera of Ptiliidae. A ventral plastron is present in Hydraenidae.

26 Sculpture of pronotum: **(0)** smooth or finely sculptured; **(1)** with large rounded impressions; **(2)** with deep longitudinal impressions; **(3)** with large and deep impressions of irregular shape. — A distinct relief is missing on the pronotal surface of most ptiliids, but rounded foveae or longitudinal impressions (e.g. Darby 2013: figs. 19, 20) occur in some genera. Deep impressions of irregular shape occur in *Ochthebius* (Perkins 1980; Jäch et al. 2016).

27 Pubescence of pronotum and elytra: **(0)** homogeneous; **(1)** with two types of hairs. — *Cephaloplectus* is characterized by a specific dual pubescence with short recumbent hairs and long erect ones.

28 Hypomeral antennal pocket: **(0)** absent; **(1)** present. — Present in Hydraenidae with few exceptions (Beutel et al. 2003; Jäch et al. 2016).

29 Prothoracic ectodermal glands along lateral margins of prothorax: **(0)** absent; **(1)** present. — Glands associated with pores on the lateral margins of the prothorax are present in some representatives of Nanosellini including *Mikado* and *Porophila* (Hall 1999).

30 Prosternal process between procoxae: **(0)** present, moderately wide, at least partly separating procoxae; **(1)** present but narrow; **(2)** extremely short or absent, procoxae contiguous; **(3)** elongate and broad, extending beyond mesoventrite. — The prosternal process is absent or very narrow in most ptiliids, with more or less contiguous procoxae. Only *Nossidium* and *Ptenidium* have a wide prosternal process as it is also present in some Hydraenidae (Jäch et al. 2016). *Limulodes* and *Cephaloplectus* are characterized by a prosternal process distinctly widening in the middle region of the procoxal cavities. Their broad and strongly convex hind margin reaches the metaventrite posteriorly (Fig. 3B; Seevers & Dybas 1943: figs. 1, 2).

31 Extension of elytra: **(0)** covering entire dorsum of abdomen; **(1)** apical 2–3 abdominal tergites remaining uncovered.

32 Cuticular folds on inner elytral surface: **(0)** absent; **(1)** present. — A field of cuticular folds on the inner elytral surface of *Primorskiella*, *Cylindrosella*, *Scydosella* and some other nanosellines (Sörensson 1997; Hall 1999) (Fig. 3I) corresponds with cuticular ridges on the metapleuron. Based on the morphological similarity with stridulatory organs occurring in some other groups of Coleoptera, the same function is assumed for these structures.

33 Horizontally oriented deep fossa on each side of mesoventral keel: **(0)** absent; **(1)** present. — Present in Discheramocephalini (Grebennikov 2009b) and also in some other ptiliids such as *Sindosium* or *Millidium*.

34 Mesopleuron: **(0)** separated from ventrite; **(1)** partly fused with ventrite; **(2)** completely fused. — The suture separating the mesanepisternum from the mesoventrite is usually missing in Ptiliidae. Exceptions are *Nossidium*, *Motschulskium*, and *Sindosium*, where it is incompletely reduced like in Hydraenidae.

35 Shape of meso- and metaventral processes between metacoxae: **(0)** mesoventral process ending near middle region of mesocoxae with distinct suture; **(1)** mesoventral process ending at posterior margin of mesocoxae with distinct suture; **(2)** mesoventral process projecting beyond posterior mesocoxal margin; **(3)** boundary between mesoventral process and metaventrite indiscernible, mesocoxae separated by narrow process; **(4)** boundary between mesoventral process and metaventrite discernible, with mesocoxae separated by wide process; **(5)** metaventral process wider than metacoxae, anteriorly directed, fused with mesoventral process. — The mesoventral process reaches the posterior margin of the mesocoxae in most ptiliids. The process ends near the middle region of the coxae in Hydraenidae.

36 Length of metaventrite: **(0)** longer than diameter of mesocoxae; **(1)** similar to diameter of mesocoxae. — The metaventrites of *Limulodes* and *Cephaloplectus* and other representatives of Cephaloplectinae are very short (Seevers & Dybas 1943), in contrast to other groups of Ptiliidae.

37 Longitudinal impression along anterior margin of metaventrite: **(0)** absent; **(1)** present. — Present in basal representatives of Ptiliidae, reaching the mesocoxae anteriorly (Fig. 3G).

38 Metaventral lines: **(0)** absent; **(1)** present, reaching anterolateral angle of metaventrite; **(2)** present, reaching lateral margins of mesocoxae anteriorly. — Present in all Nanosellini with the exception of *Mikado* (Hall 1999). Also present in *Ptilium*, *Micridium*, and *Oligella*, but with different position (Fig. 3L,M).

39 Shape of metendosternite: **(0)** common stem short, arms long; **(1)** common stem widened and flattened, arms long; **(2)** common stem represented by thin wide transverse element, arms widely separated, long; **(3)** common stem short, arms long but thin and weakly sclerotized; **(4)** metendosternite compact, anterior arms short. — The shape and size of the metendosternite varies considerably within Ptiliidae and also in related groups (Jäch et al. 2016; Newton 2016a,b).

40 Apical muscular disc of arm of metendosternite: **(0)** present; **(1)** absent. — Most ptiliids have a distinct rounded muscular disc at the top of the arms of the metendosternite. It is absent in *Primorskiella*, *Cylindrosella*, *Scydosella* and all other genera of Nanosellini (Hall 1999).

41 Length of alacrista: **(0)** not reaching beyond hind margin of metapostnotum; **(1)** distinctly reaching beyond hind margin of metapostnotum; **(2)** reaching beyond abdominal tergite III. — The alacristae are distinctly elongated in Hydraenidae and Ptiliidae (e.g. Yavorskaya et al. 2019). It is shorter in the basal series of genera and Cephaloplectinae than in the majority of Ptiliidae (Fig. 3H; Sörensson 1997: fig. 17 [*Baranowskiella ehnstromi*]).

42 Single spur on each side of anterior region of metascutellum: **(0)** absent; **(1)** present. — Generally present in Acrotrichinae (Fig. 3H).

43 Shape of metacoxae: **(0)** wider than long, contiguous or very narrowly separated; **(1)** small, distinctly separated; **(2)** enlarged, widening towards medial margin, contiguous, with wide metacoxal plates covering metafemora; **(3)** flattened, extending towards lateral margin, with cavity for retracted legs below them. — Most ptiliids have small and distinctly separated metacoxae. However, they are wider than long and contiguous or almost contiguous in representatives of the basal genera, like in Hydraenidae. The metacoxae are extended in Nanosellini and Cephaloplectinae.

44 Shape of mesotrochanter: **(0)** unmodified; **(1)** narrowed, elongated. — *Scydosella* and *Scydoselloides* are characterized by an elongated and uniquely shaped mesotrochanter (Hall 1999).

45 Shape of femora: **(0)** not broadened and flattened; **(1)** distinctly broadened and flattened. — Distinctly broadened in *Limulodes* and other cephaloplectines, forming a lamina partly covering the tibia (Seevers & Dybas 1943: figs. 39, 40, 59–62).

46 Number of tarsomeres: **(0)** five; **(1)** three. — Only three tarsomeres are present in Ptiliidae versus five in the outgroup taxa (Hansen 1997).

47 Insertion of basal tarsomere: **(0)** not retracted into tibial apex; **(1)** retracted into tibial apex. — The basal tarsomere is retracted into the tibial apex to a certain degree in Ptiliidae (Sörensson 1997: fig. 32; Hansen 1997; Yavorskaya et al. 2019). The tarsus is unusually slender compared to the tibia and tarsomere 1 is often not or only scarcely visible.

48 Shape of apical tarsomeres: **(0)** cylindrical; **(1)** dilated and flattened. — The apical tarsomeres of most ptiliids are elongated and cylindrical as in Hydraenidae, Agyrtidae and Leiodidae (Jäch et al. 2016; Newton 2016a,b). *Cephaloplectus* is characterized by a dilated and flattened apical tarsomeres. The pretarsi bear additional prehensile structures (Fig. 3F).

49 Size of pretarsal claws: **(0)** equal in size; **(1)** subequal. — The paired claws are of equal size in most ptiliids. One of the claws is somewhat reduced in the smallest representatives of the family.

50 Wings: **(0)** present; **(1)** dimorphic, present in some individual and absent in others; **(2)** absent. — Wings are usually present in ptiliids, but species of genera of Ptiliini include both winged and wingless individ-

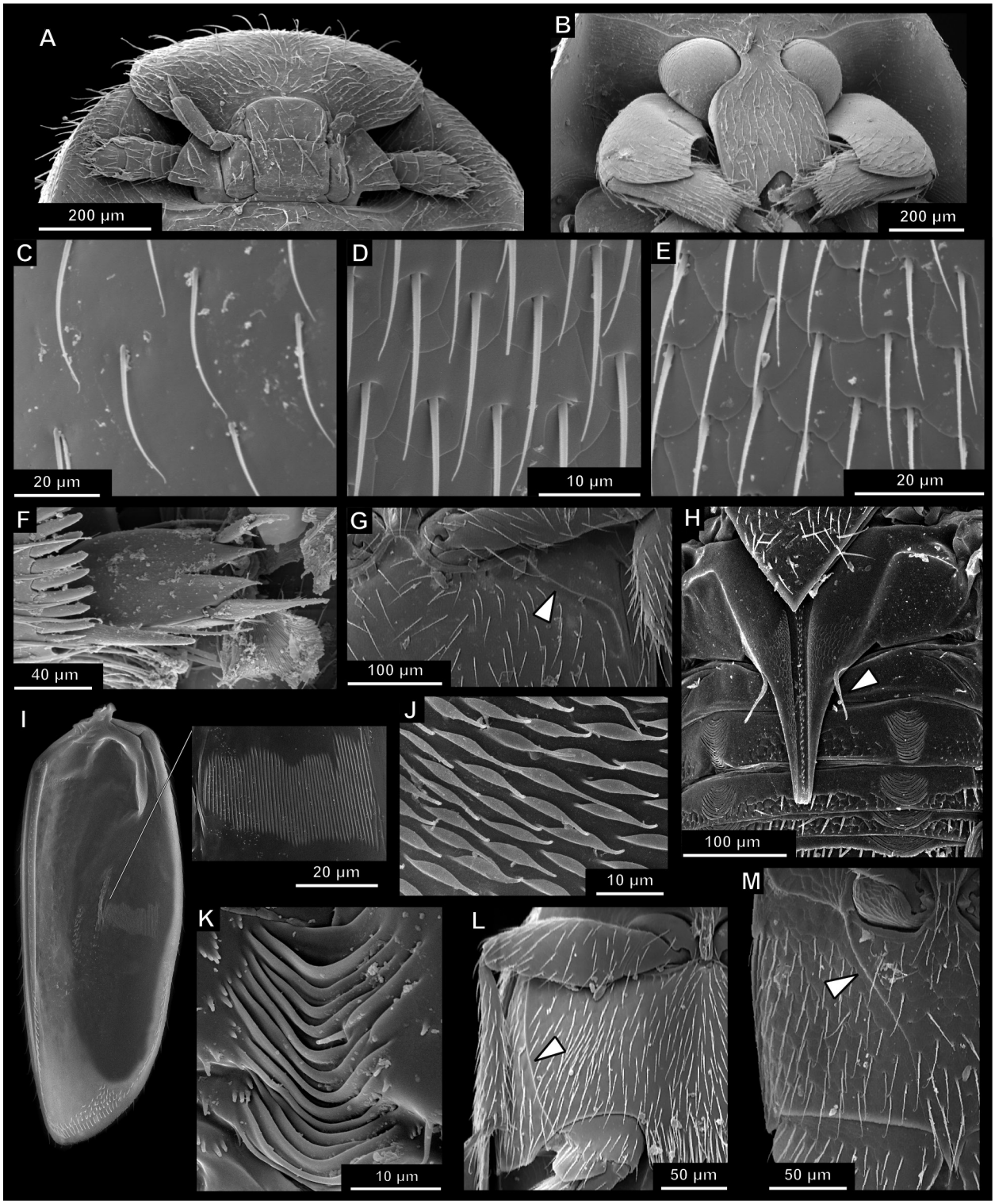

Fig. 3. Ptiliidae beetles, body parts, SEM. **A**: head of *Cephaloplectus* sp., ventral view. **B**: prosternal process of *Cephaloplectus* sp. **C**–**E**: areas of elytral surface of *Ptenidium pusillum* (C), *Ptilium myrmecophilum* (D) and *Nephanes titan* (E). **F**: protarsus of *Cephaloplectus* sp. **G**: impression along anterior margin of metaventrite (arrow) of *Sindosium* sp. **H**: alacrista and long spur at its base (arrow) of *Acrotrichis grandicollis*. **I**: stridulatory organ on internal surface of elytron of *Primorskiella anodonta.* **J**,**K**: WFP of *Motschulskium sinuatocolle* (J) and *Porophila cedri* (K). **L**,**M**: metaventral lines of *Ptilium myrmecophilum* (L) and *Porophila cedri* (M) (arrow).

uals, which also differ in the degree of eye development and pigmentation. *Limulodes* and other cephaloplectines (Seevers & Dybas 1943) are wingless, and also *Rioneta* and some other representatives of the family (Grebennikov 2008b).

51 Wing base: **(0)** membranous wing blade with veins; **(1)** petiole without membranous wing blade (Fig. 4). — The wing base of ptiliids is generally characterized by a petiole formed by one or several veins (Polilov et al. 2019b).

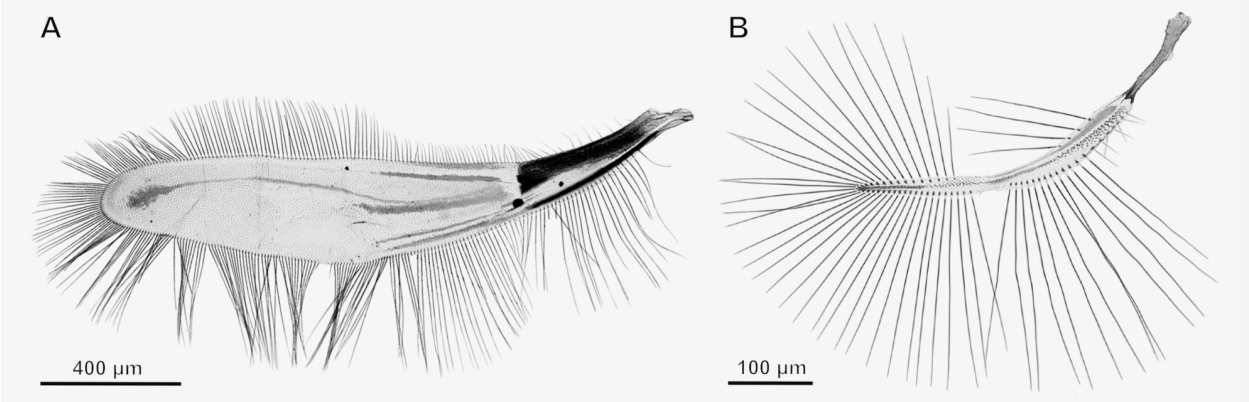

Fig. 4. Hind wings of Ptiliidae, dorsal view, anterior at top. **A**: *Motschulskium sinuatocolle.* **B***: Primorskiella anodonta*.

52 Arrangement of folded wings below elytra: **(0)** overlapping; **(1)** parallel arrangement without overlap below elytra. — Like in most groups of beetles, the folded hind wings overlap in their resting position in the genera considered as basal by Polilov et al. (2019b: fig. 7A). In the remaining Ptiliidae they are folded symmetrically below the elytra without overlapping, involving four or five (*Ptenidium*) bending lines (Polilov et al. 2019b: fig. 7B,F).

53 Ratio of width of wing blade and length of setae along its margin: **(0)** wing blade much wider than length of setae; **(1)** wing blade width similar to length of setae (Fig. 4A); **(2)** wing blade less than half as wide as length of setae (Fig. 4B). — All ptiliids are characterized by a "feather-like" shape of the wing: the wing blade is narrowed and most of the flapping surface of the wing is formed by marginal setae (Fig. 4A,B). A relatively wide wing blade (Fig. 4A) is preserved in *Nossidium* and other genera of the basal series (Polilov et al. 2019b).

54 Vein cubitus anterior (CuA): **(0)** present; **(1)** absent. — Always absent in Ptiliidae, in contrast to alate species of Hydraenidae, Agyrtidae and Leiodidae (Hansen 1997: figs. 148–150; Darby 2015b: fig. 5).

55 Number of veins in petiole or wing base: **(0)** three or more; **(1)** two; **(2)** one. — The petiole consists of one vein in the majority of ptiliids. Two are present in representatives of the basal genera *Nossidium*, *Motschulskium*, and *Sindosium* (Darby 2019: fig. 2I). Three or more are also present in Hydraenidae, Agyrtidae and Leiodidae (Hansen 1997: figs. 148–150).

56 Number of veins in wing blade: **(0)** four or more; **(1)** three; **(2)** two. — Number generally reduced in Ptiliidae (e.g. Sörensson 1997; Darby 2019: fig. 2I; Polilov et al. 2019b).

57 Number of setae along margin of wing: **(0)** > 200; **(1)** 60–200; **(1)** < 60. — Highest number in basal genera. Fewer than 60 in Nanosellini.

58 Wing folding patches (WFPs) on abdominal tergites: **(0)** absent; **(1)** present on tergites II–VII; **(2)** present on tergites II–VI; **(3)** present on tergites II–V. — Cuticular structures on the abdominal tergites (Fig. 3J,K) are involved in folding the wings in all alate species of Ptiliidae. The number of tergites with WFPs varies within the family.

59 WFPs: **(0)** absent; **(1)** represented by denticles; **(2)** represented by cuticular folds. — The WFPs are formed by cuticular denticles in *Motschulskium*, *Nossidium* and *Sindosium*, but by cuticular folds in the other groups (Fig. 3J,K).

60 Shape of pterothorax and abdomen: **(0)** subparallel and posteriorly rounded; **(1)** distinctly triangular, tapering towards abdominal apex. — A triangular shape of the pterothorax and abdomen is characteristic for *Limulodes* and other Cephaloplectinae (Seevers & Dybas 1943: figs. 1, 2).

61 Position of abdominal segments VIII and IX: **(0)** retracted; **(1)** everted. — Almost always retracted in Coleoptera, but everted in Ptiliidae and Hydraenidae (e.g. Hansen 1997).

62 Shape of seventh visible sternite: **(0)** undivided; **(1)** divided into two lobes. — Divided into two lobes in *Limulodes* and other representatives of Cephaloplectinae (Seevers & Dybas 1943).

63 Hind margin of tergite X (pygidium): **(0)** without teeth or with few small teeth; **(1)** with 1–3 distinct apical teeth; **(2)** with specific flattened central tooth (sometimes bifurcated); **(3)** with two widely separated teeth and third tooth between them apically (the latter can be absent). — The unique presence of a flattened central tooth on tergite X of Nanosellini (partim) was pointed out by Sörensson (1997).

64 Spermathecal sperm pump: **(0)** absent; **(1)** present. — A spermathecal pump is a unique feature of Ptiliidae and Hydraenidae according to Hansen (1997) (see also Perkins 1980).

65 Shape of spermatheca: **(0)** strongly curved; **(1)** spherical; **(2)** funnel-shaped; **(3)** horseshoe-shaped, often asymmetrical; **(4)** ring-shaped; **(5)** poorly sclerotized, irregularly shaped; **(6)** simple helical; **(7)** complex helical. — The shape of the spermatheca varies strongly within Ptiliidae, from simple and spherical (e.g. Darby 2016: figs. 27–32 [*Ptenidium* spp.]) to complex helically twisted (e.g. Darby & Johnson 2011: figs. 36–47 [*Smicrus* spp.]).

66 Position of aedeagus: **(0)** shifted towards lateral body region; **(1)** placed along midline. — Like in Hydraenidae, an asymmetric aedeagus is found in most representatives of Ptiliidae, shifted towards the lateral body region. In contrast, the aedeagus of Acrotrichinae is placed along the median body axis.

67 Parameres of aedeagus: **(0)** present; **(1)** strongly reduced; **(2)** absent. — The parameres are absent in most ptiliids but preserved in the basal series of genera. They are vestigial but still recognizable in *Necrophilus* (Newton 2016a).

68 Shape of aedeagus: **(0)** elongated and round in cross-section; **(1)** shortened and oval in cross-section; **(2)** shortened and flattened. — The aedeagus is elongated in most ptiliids and either rounded or oval in cross-section. *Acrotrichis* and related genera are characterized by a shortened and flattened aedeagus.

Additional characters not included in the analyses:

69 Ocelli: **(0)** present; **(1)** absent. — Ocelli are always absent in Ptiliidae like in most groups of Staphylinoidea and Coleoptera (Leschen & Beutel 2004; Lawrence et al. 2011; Newton 2016a,b). They are present in some genera of Hydraenidae (Beutel et al. 2003; Jäch et al. 2016).

70 Notosternal suture of prothorax: **(0)** present; **(1)** absent. — The prothoracic notopleural suture is often reduced in Ptiliidae (e.g. Sörensson 1997), but distinct in *Nephanes* and some other genera (Yavorskaya et al. 2019). The documentation is insufficient presently.

71 Process of lateral part of prophragma: **(0)** absent; **(1)** present. — This feature was illustrated for *Baranowskiella* by Sörensson (1997: fig. 12). Presently it is not sufficiently documented in several lineages of Ptiliidae and outgroup taxa.

72 Mycangia associated with mesocoxal cavities: **(0)** absent; **(1)** present. — The presence of mycangia was suggested as a feature typical for Nanosellini (Sörensson 1997: fig. 18), even though in at least one case their aperture is in times smaller than the diameter of the host fungus spores (Grebennikov & Leschen 2010). A precise documentation is required for a reliable phylogenetic interpretation.

73 Abdominal glands: **(0)** absent; **(1)** present. — A pair of widely separated glands is present in the posterior abdominal segments of *Pteryx* and *Ptinella* and other representatives of Ptinellini. They are associated with a transverse row of cuticular pores (Hall 2003: figs. 17–23). This specific condition is a potential autapomorphy of Ptinellini (= Pterycini; Dybas 1966; Hall 2003). However, similar glands also occur in other genera of Ptiliidae (Hall 2003: figs. 24–26). A precise documentation (e.g. histological sections, SEM images) among genera of Ptiliidae is still lacking.

Several characters of the ventral side of the thorax were used by Darby (2012) in a key for Ptiliidae of Britain and Ireland. The features are obviously useful for diagnostic purposes, but difficult to score for representatives of the family on a world-wide scale, apparently quite variable, partly even at the species level (e.g. Darby 2017b: figs. 8–13, mesoventrite of species of *Cissidium*), and partly overlapping with the characters analysed in this study. Therefore, they were not included here.

3.2. Results of the analysis of the morphological data

The analysis with NONA (ratchet, 1000 replicates) yielded 37 minimum length trees with 150 steps (consistency index [CI]: 0.75, retention index [RI]: 0.87). Only 12 minimum length trees with the same number of steps were obtained with TNT (traditional search, 100 replicates). The strict consensus trees are congruent. All characters were equally weighted in the first runs (Fig. 5). The clades with their unambiguously optimized apomorphies are listed below (reversals and homoplasies are in italics).

Hydraenidae + **Ptiliidae** (branch support value [= Bremer support, Bs]: 5): 10.1. Labral-mandibular locking mechanism present; 11.1. Mandible with distal part shortened and not prominent; *15.1. Mentum approximately as long as wide or slightly longer than wide, with sides subparallel*; *41.1. Alacrista distinctly reaching beyond hind margin of metapostnotum*; 61.1. Abdominal segments VIII and IX everted; 64.1. Spermathecal sperm pump present.

Ptiliidae (Bs: 10): 3.1. Gular sutures absent; 8.1. Insertion of flagellomere retracted into pedicellus; 13.1. Apical maxillary palpomere aciculate; 17.1. Palpiger fused with prementum; 19.1. Laminatentorium absent; 21.1. Cervical sclerites absent; 37.1. Longitudinal impression along anterior margin of metaventrite present (groundplan); 46.1. Three tarsomeres; 47.1. Insertion of basal tarsomere retracted into tibial apex; 51.1. Wing base transformed into petiole without membranous wing blade; 54.1. Vein CuA absent; 65.1. Spermatheca spherical (groundplan).

Ptiliidae excl. *Sindosium*, *Nossidium* and *Motschulskium* (Bs: 5): 18.1. Labial palp 2-segmented; 34.2. Mesopleuron completely fused with ventrite; 35.1. Mesoventral process ending at posterior margin of mesocoxae with distinct suture; 39.2. Metendosternite with common stem represented by thin wide transverse element, arms widely separated, long; 41.2. Alacrista reaching beyond abdominal tergite III; 43.1. Metacoxae small, distinctly separated; 52.1. Parallel arrangement of folded wings without overlap below elytra; 56.1. Two veins in wing blade; 57.1. 60–200 setae along wing margin; 67.2. Parameres of aedeagus absent.

Ptiliola + ***Ptiliolum*** (Bs: 1): *63.1. Hind margin of tergite X (= pygidium) with 1–3 distinct apical teeth*.

Actidium + ***Oligella*** + ***Micridium*** + ***Ptilium*** (Bs: 1): 15.2. Mentum approximately as long as wide, dilated medially; 68.1. Aedeagus shortened and oval in cross-section.

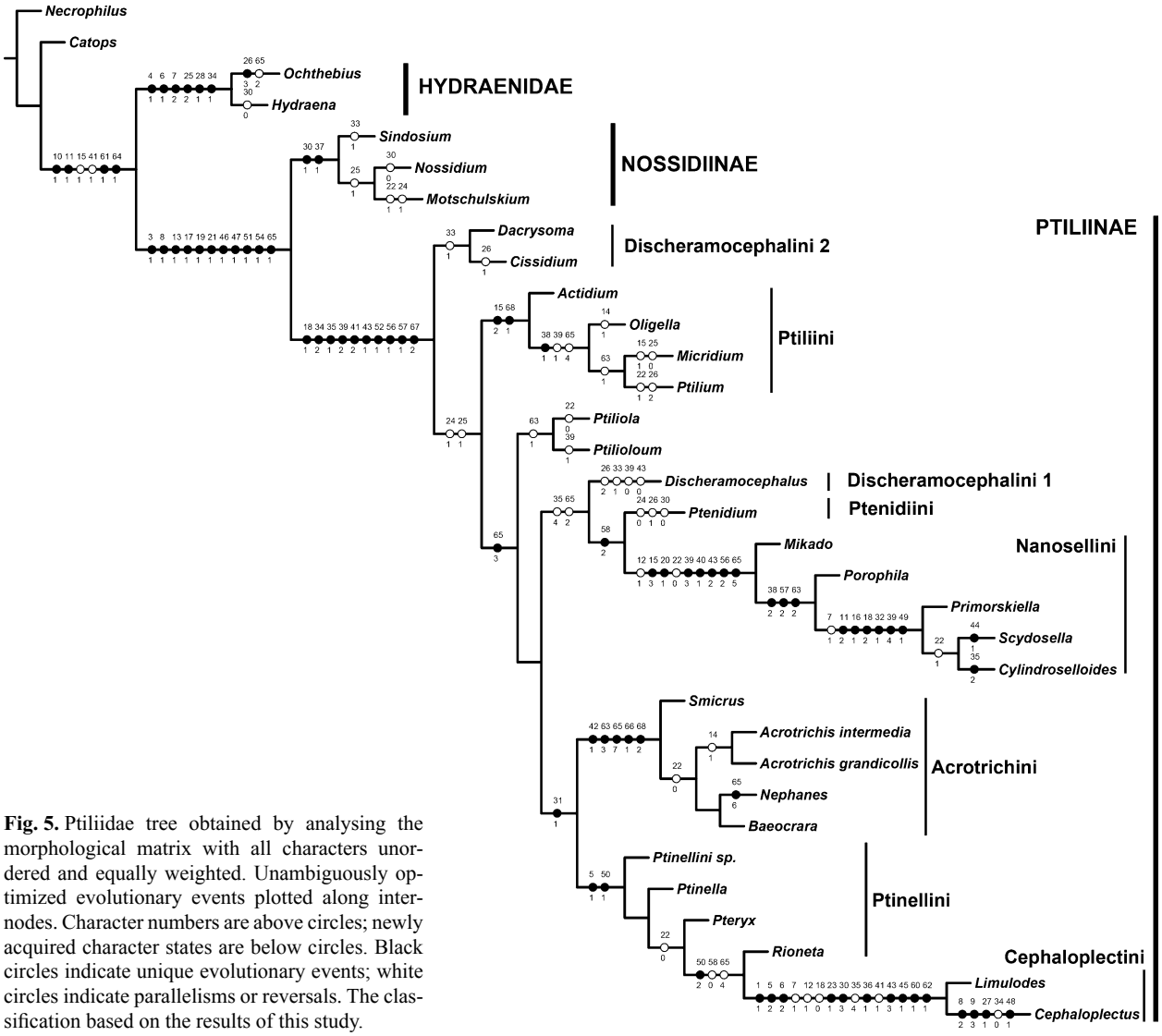

Fig. 5. Ptiliidae tree obtained by analysing the morphological matrix with all characters unordered and equally weighted. Unambiguously optimized evolutionary events plotted along internodes. Character numbers are above circles; newly acquired character states are below circles. Black circles indicate unique evolutionary events; white circles indicate parallelisms or reversals. The classification based on the results of this study.

Oligella + ***Micridium*** + ***Ptilium*** (Bs: 1): 38.1. Metaventral lines present, reaching anterolateral angle of metaventrite; *39.1. Metendosternite with common stem widened and flattened, arms long*; *65.4. Spermatheca ring-shaped*.

Micridium + ***Ptilium*** (Bs: 1): *63.1. Hind margin of tergite X with 1–3 distinct apical teeth*.

Nanosellini (Bs: 6): *12.1. Galea one-segmented*; 15.3. Mentum longer than wide, dilated in middle region; 20.1. Dorsal tentorial arms absent; *22.0. Pronotum widest in posterior half*; 39.3. Metendosternite with common stem short, arms long but thin and weakly sclerotized; 40.1. Apical muscular disc of arm of metendosternite absent; 43.2. Metacoxae flattened, extending towards lateral margin, with cavity for retracting legs below them; 56.2. Two veins in wing blade; 65.5. Spermatheca poorly sclerotized, irregularly shaped.

Nanosellini excl. ***Mikado*** (Bs: 2): 38.2. Metaventral lines reaching lateral margins of mesocoxae anteriorly; 57.2. Fewer than 60 setae along wing margin; 63.2. Pygidium with specific flattened central tooth.

Primorskiella + ***Scydosella*** + ***Cylindroselloides*** (Bs: 7): *7.1. Antenna 10-segmented*; 11.2. Distal part of mandible reduced; 16.1. Lateral premental lobes vestigial; 18.2. Labial palp one-segmented; 32.1. Cuticular folds on inner elytral surface present; 39.4. Metendosternite compact, anterior arms short; 49.1. Pretarsal claws subequal.

Scydosella + ***Cylindroselloides*** (Bs: 1): *22.1. Pronotum widest in middle region*.

Smicrus + ***Nephanes*** + ***Baeocrara*** + ***Acrotrichis*** (Bs: 4): 42.1. Single spine or spur anteriorly on each side of metascutellum present; 63.3. Pygidium with two widely separated teeth and third tooth between them apically; 65.7. Spermatheca complex helical; 66.1. Aedeagus placed along midline; 68.2. Aedeagus shortened and flattened.

Acrotrichis (Bs: 1): *14.1. Base of mentum fused with head capsule*.

Pteryx + ***Ptinella*** + **Ptinellini gen.** + ***Rioneta*** + ***Limulodes*** + ***Cephaloplectus*** (Bs: 1): 5.1. Compound eyes dimorphic; 50.1. Wings dimorphic, present in some individual and absent in others.

Rioneta + ***Limulodes*** + ***Cephaloplectus*** (Bs: 2): 50.2. Wings absent; *58.0. WFPs absent*; *65.4. Spermatheca ring-shaped*.

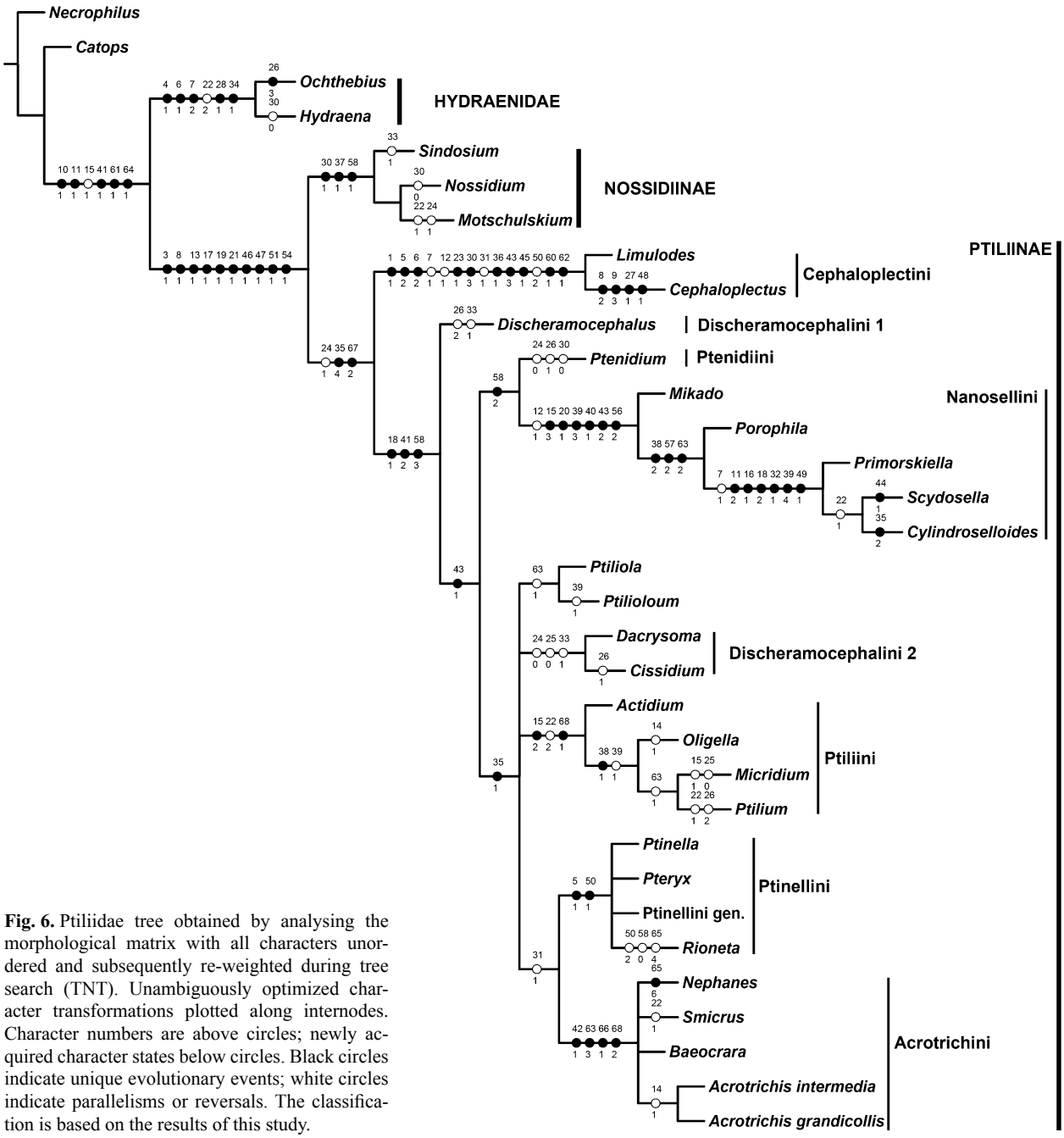

Fig. 6. Ptiliidae tree obtained by analysing the morphological matrix with all characters unordered and subsequently re-weighted during tree search (TNT). Unambiguously optimized character transformations plotted along internodes. Character numbers are above circles; newly acquired character states below circles. Black circles indicate unique evolutionary events; white circles indicate parallelisms or reversals. The classification is based on the results of this study.

Limulodes + ***Cephaloplectus*** (Bs: 10): 1.1. Head deflexed; 5.2. Eyes absent; 6.2. Antennal grooves present as distinct furrows; *7.1. Antennae 10-segmented*; *12.1. Galea one-segmented*; *18.0. Labial palp three-segmented* (reversal); 23.1. Posterior pronotal angles prolonged and closely clinging to elytral humeri; 30.3. Prosternal process elongate and broad, extending over mesoventrite; *35.4. Boundary between mesoventral process and metaventrite discernible, with mesocoxae separated by wide process*; 36.1. Length of metaventrite similar to diameter of mesocoxae; *41.1. Alacrista reaching beyond hind margin of metapostnotum* (reversal?); 43.3. Metacoxae flattened, extending towards lateral margin, with space for storing legs below them; 45.1. Femora distinctly broadened and flattened; 60.1. Pterothorax and abdomen distinctly triangular, tapering towards abdominal apex; 62.1. Abdominal sternite VII divided into two lobes.

Alternative branches and character interpretations were obtained after using the implied weighting option (Fig. 6):

Ptiliidae excl. *Sindosium*, *Nossidium* and *Motschulskium*: *24.1. Microsculpture of dorsal surface of pronotum and elytra reticulate or scaly*; 35.4. boundary between mesoventral process and metaventrite discernible, with mesocoxae separated by wide process; 67.2. Parameres of aedeagus absent.

Genera *Sindosium*, *Nossidium* and *Motschulskium*: 30.1. Prosternal process present, narrow; 37.1. Longitudinal impression along anterior margin of metaventrite

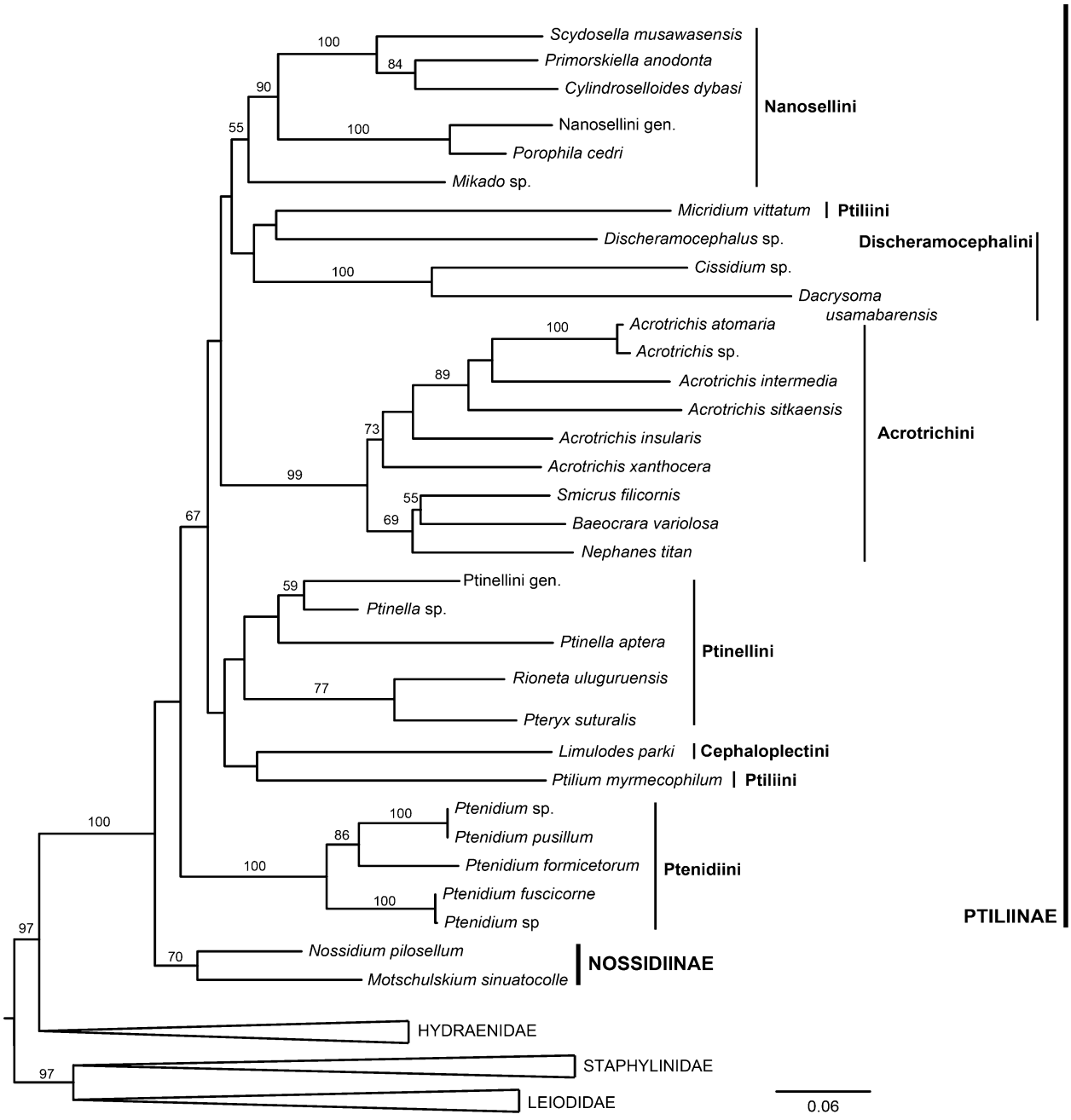

Fig. 7. Maximum Likelihood inference phylogram of Ptiliidae obtained with RAxML with the molecular data only, with outgroup taxa and two redundant terminals (AN360, AN424) collapsed for readability (see Fig. S8 for a tree with all taxa). Digits at internodes are bootstrap values > 50%. The classification is based on the results of this study.

present; 58.1. Wing folding patches present on tergites II–VII.

Ptiliidae excl. (*Sindosium*, *Nossidium* and *Motschulskium*) and (*Limulodes* + *Cephaloplectus*): 18.1. Labial palps two-segmented; 41.2. Alacristae reaching beyond abdominal tergite III; 58.3. Wing folding patches present on tergites II–V.

Limulodes* + *Cephaloplectus: 1.1. Head deflexed; 5.2. Eyes absent; 6.2. Antennal grooves present as distinct furrows; *7.1. Antennae 10-segmented*; *12.1. Galea one-segmented*; 23.1. Posterior pronotal angles prolonged and closely applied to elytral humeri; 30.3. Prosternal process elongate and broad, extending over mesoventrite; *31.1. Elytra shortened*; 36.1. Length of metaventrite similar to diameter of mesocoxae; 43.3. Metacoxae flattened, extending towards lateral margin, with cavity for retracting legs below them; 45.1. Femora distinctly broadened and flattened; *50.2. Wings absent*; 60.1. Pterothorax and abdomen distinctly triangular, tapering towards abdominal apex; 62.1. Abdominal sternite VII divided into two lobes.

Ptiliidae excl. *Sindosium*, *Nossidium* and *Motschulskium*, Cephaloplectinae and *Discheramocephalus*: 43.1. Metacoxae small, widely separated.

Ptiliidae excl. (*Sindosium*, *Nossidium* and *Motschulskium*), Cephaloplectinae, *Discheramocephalus*, and

(*Ptenidium* + Nanosellini): 35.1. Mesoventral process ending at posterior margin of mesocoxae with distinct suture.
Dacrysoma* + *Cissidium: 24.0. Microsculpture of dorsal surface of pronotum and elytra smooth or punctate; 25.0. Microsculpture of ventral surface of thoracic segments smooth or punctate; *33.1. Horizontally oriented deep fossa on each side of mesoventral keel present*.
Smicrus* + *Nephanes* + *Baeocrara* + *Acrotrichis* + *Pteryx* + *Ptinella* + Ptinellini gen. + *Rioneta: *33.1. Horizontally oriented deep fossa on each side of mesoventral keel present*.

3.3. Results of the analyses of DNA data

The RAxML analysis (Figs. 7, S8) recovered the monophyly of Hydraenidae + Ptiliidae and of Ptiliidae with very strong support. In contrast, the monophyly of Hydraenidae was poorly supported (bootstrap < 50%). The internal topology of Ptiliidae was in general poorly supported, with the exception of some clades, which were also recovered in other analyses (see below). *Nossidium* and *Motschulskium* were placed in a clade as sister to the rest of Ptiliidae, but with poor support. Nanosellini was recovered as monophyletic, with *Mikado* as sister to the rest of the species, which formed a clade with strong support (Bs: 90%). We recovered a strongly supported clade (Bs: 99%) including *Acrotrichis*, *Smicrus*, *Nephanes* and *Baeocrara*. *Limulodes* was deeply nested within Ptiliidae, although with low support (Figs. 7, S8).

Of the three BEAST analyses, the ones with the most complex models (A, B) had a very poor convergence of the prior and posterior probabilities. In contrast, the analysis with the simplest models (C) had a good convergence (Fig. S9). Despite of these differences, the topology resulting from all BEAST analyses was almost identical, with the exception of *Smicrus*: nested within *Acrotrichis* in A and B, and as sister to *Nephanes* + *Baeocrara* in C, in both cases with low support (data not shown). Support values of the three trees were also very similar.

The Bayesian and likelihood trees had some important differences, although generally in nodes with low support in at least one of the analyses. In the Bayesian tree (Fig. S9) *Nossidium* + *Motschulskium* formed a clade with *Ptenidium* sister to the rest of Ptiliidae, instead of a paraphyletic grade as in the likelihood tree (Fig. 7). *Limulodes* was also deeply nested within Ptiliidae and sister to *Ptilium*, as in the likelihood tree, but in the Bayesian analysis *Limulodes* + *Ptilium* were sister to the clade formed by *Acrotrichis* + *Smicrus* + *Nephanes* + *Baeocrara*, with moderate support (pp: 0.77) (Fig. S9), in contrast to the likelihood tree (Fig. 7). Nanosellini was again recovered as monophyletic, with *Mikado* as sister to the rest of the studied species, although in this case with strong support (pp: 0.98).

The estimated age of the crown Ptiliidae was 131 Ma (95% HPD 122 – 138 Ma), in the lower Cretaceous, and largely overlapping with that of the crown Hydraenidae, 123 Ma (95% HPD 103 – 140 Ma) (Figs. 8, S9). In both cases these ages are fully congruent with the age of the oldest known fossils belonging to their respective crown groups, from Burmese amber (ca. 99 Ma, Yamamoto et al. 2017, 2018; Villastrigo et al. 2019). Separation between the tribes as recognised here ocurred mostly during the Upper Cretaceous (Figs. 8, S9).

3.4. Results of the combined analysis

The analysis of the combined morphological and molecular data (Fig. 9) resulted in a tree with lower resolution than the ones with molecular data only, with a topology similar to that of the morphological and maximum likelihood trees. The strong support for the monophyly of Ptiliidae was maintained (pp: 1; Fig. 9), as well as a first split of the family in *Nossidium* + *Mostchulskium* (there were no molecular data for *Sindosium*) and the rest of the studied species (pp: 0.99), and a second separating *Ptenidum* (recovered with the molecular data only). Some of the main clades identified in the morphological and molecular analyses were also recovered in the combined Bayesian analysis: Nanosellini (excluding *Mikado*, in an unresolved position) (pp: 1) and the clade *Acrotrichis* + *Smicrus* + *Nephanes* + *Baeocrara* (pp: 1). *Limulodes* was deeply nested within Ptiliidae, and there was no support for tribe Discheramocephalini, as happened in all previous analyses (Fig. 9).

4. Discussion

4.1. Sister group and monophyly of Ptiliidae

The placement of Ptiliidae as sister group of the aquatic Hydraenidae (e.g. Hansen 1997; McKenna et al. 2015a) is very well supported in the analyses of molecular and morphological data sets presented here. Unambiguous morphological synapomorphies are a weakly developed apical part of the mandible (Hansen 1997), a specific labro-mandibular locking mechanism (Yavorskaya et al. 2017), distinctly elongated metanotal alacristae (Yavorskaya et al. 2019), everted abdominal segments VIII and IX, and a spermathecal sperm pump, a unique feature according to Hansen (1997). Monophyly of Ptiliidae was also corroborated in all analyses. They are supported by an entire series of unambiguous apomorphies, including the lack of gular sutures, a three-segmented antennal club, an awl-shaped apical maxillary palpomere (e.g. Yavorskaya et al. 2017), a palpiger fused with the prementum, the complete absence of the laminatentorium, widely separated tentorial arms (e.g. Yavorskaya et al. 2017), the absence of laterocervicalia (Hansen 1997), three-segmented tarsi with the basal tarsomere at least partly retracted into the tibia, a petiole of the wing without wing blade, and the absence of the longitudinal vein

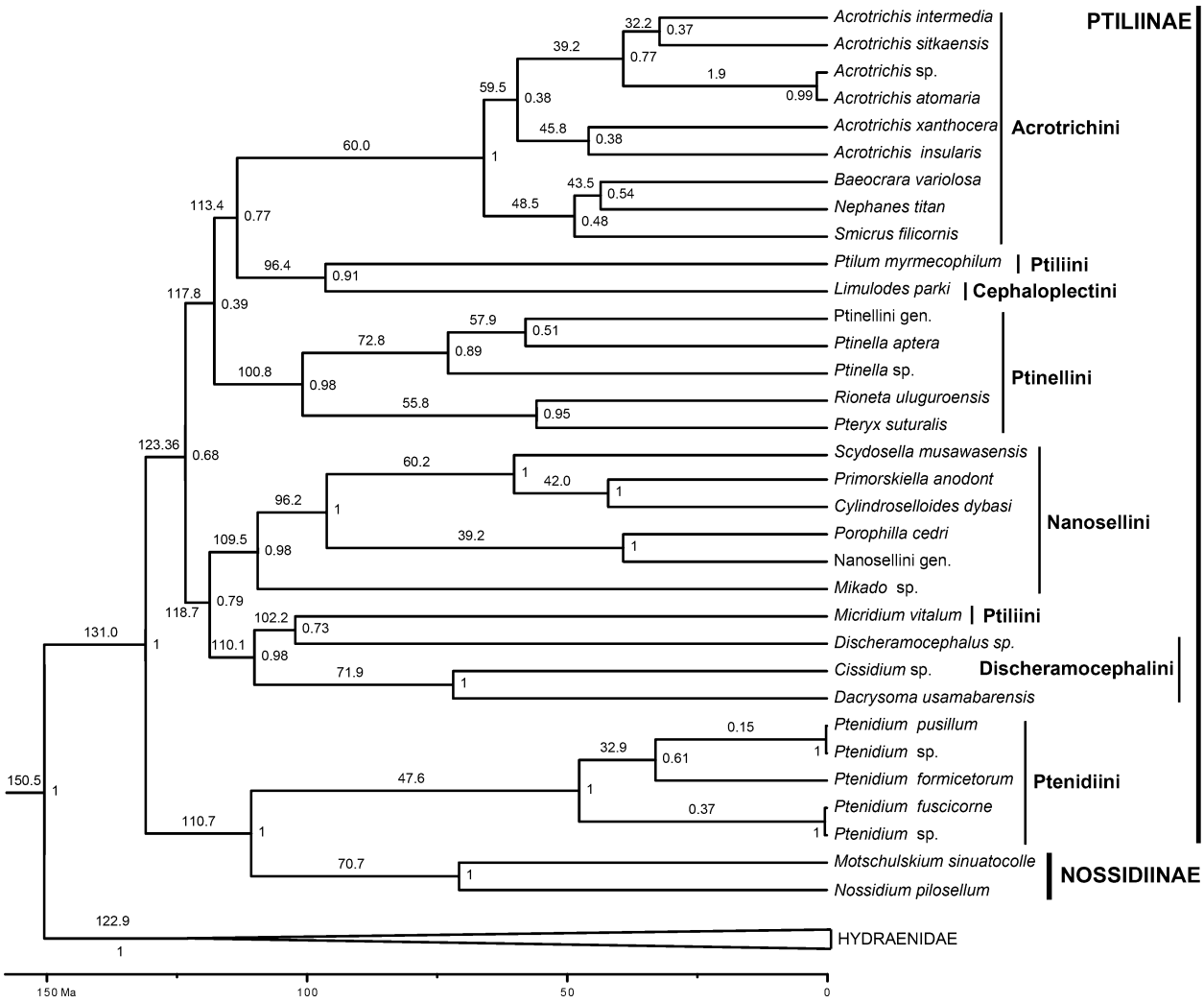

Fig. 8. Majority rule consensus tree of the Bayesian analysis (obtained with BEAST) of the molecular characters, with the simplest models (analysis C, see Methods) with outgroups and two redundant terminals collapsed for readability (see Fig. S9 for a tree with all taxa). Numbers in nodes are posterior probabilities > 0.5; numbers in branches estimated age of divergence (Ma). The classification is based on the results of this study.

CuA. In the recent study of Sörensson & Delgado (2019) Ptiliidae were nested within Hydraenidae in the phylogenetic tree, rather than as sister group of this family. This is probably the result of the limited number of analysed characters: the study is only based on larval features, and has a strong focus on chaetotaxy, a character system prone to homoplasy (e.g. Dressler et al. 2011).

4.2. Phylogeny of Ptiliidae

Ptiliidae excluding *Sindosium*, *Nossidium* and *Motschulskium* appear solid and were also retrieved by Sörensson & Delgado (2019) based on larval features. The group is unambiguously supported by morphological features, and also in our analyses of molecular and combined data. The retrieved apomorphies differ distinctly before and after applying the implied weighting option, depending on the placement of Cephaloplectinae (see 3.2.). The loss of the parameres is an unambiguous apomorphy under both scenarios. A scaly or reticulate pronotal and elytral surface are additional potential apomorphies. The wing characters are ambiguous, as Cephaloplectinae are completely wingless.

Sindosium, *Nossidium* and *Motschulskium* were supported as a clade when all characters were equally weighted. The presence of longitudinal impressions along the anterior margin of the metaventrite is a potential apomorphy, and possibly also a narrow prosternal process wing folding patches on tergites II–VII. Their monophyly was supported after implied weighting of morphological data and also in the molecular and combined analyses (although there was no molecular data for *Sindosium*). These genera have retained many plesiomorphic characters, including the presence of two veins in the peduncle, at least four veins in the wing blade, a wide wing blade with relatively short setae along the margin, WFP consisting of teeth, a metendosternite with a short common stem, and the presence of parameres of the aedeagus. *Nossidium*, a member of this group of genera, differs from all other Ptiliidae with known immature stages (Sörensson & Delgado 2019): the larvae have

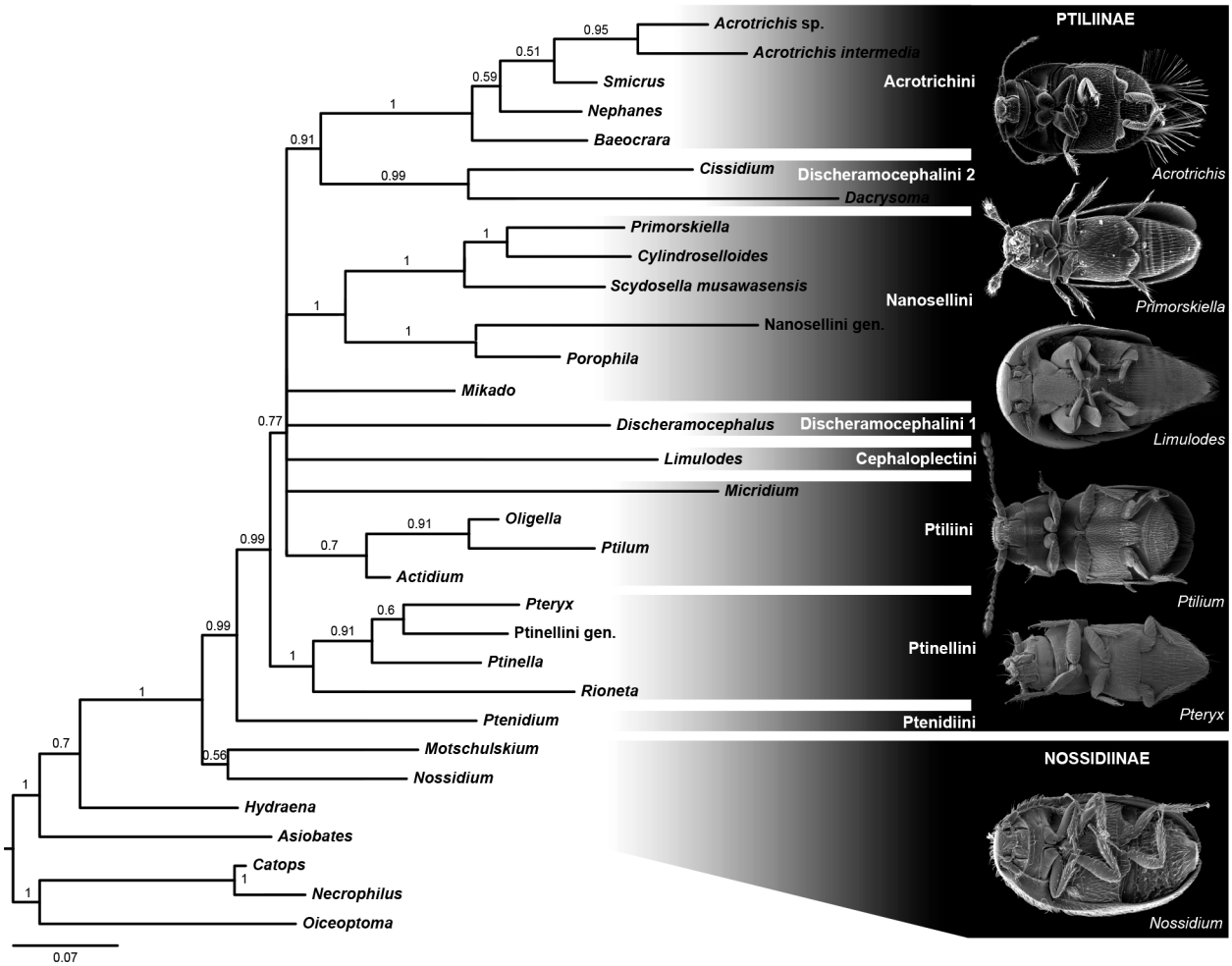

Fig. 9. Majority rule consensus tree of the Bayesian analysis (obtained with MrBayes) of the combined morphological and molecular characters. Digits at internodes are posterior probabilities > 0.5. The classification is based on the results of this study.

an elongate tarsungulus, a partially reduced, distinctly shortened clypeus, and also differ in their chaetotaxy pattern.

Considering the character evolution, a placement of *Limulodes* + *Cephaloplectus* as the next divergence within Ptiliidae appears likely, as also suggested by Sörensson & Delgado (2019) based on larval features. However, among the three potential apomorphies of Ptiliidae excl. *Nossidium* and *Limulodes* (Sörensson & Delgado 2019: fig. 77) two are likely plesiomorphies (labrum even or slightly emarginate, distal mandibular part slender) and one is a simple reduction (distal part of lacinia reduced, non-fimbriate, frayed or not). In our analysis a placement of *Limulodes* + *Cephaloplectus* as second branch in Ptiliidae was supported after implied weighting of the morphological data. Apomorphies of Ptiliidae excluding *Sindosium*, *Nossidium* and *Motschulskium*, and also *Limulodes* and *Cephaloplectus* include the presence of only two instead of three labial palpomeres, and alacristae posteriorly reaching beyond abdominal tergite III. An alternative placement of *Limulodes* + *Cephaloplectus* deeply nested in the family is likely an artefact, caused by the absence of wings shared with *Rioneta*, even though the analyses of the molecular data also placed *Limulodes* deeply within Ptiliinae (Fig. 7). It is conceivable that the placement of this specialized group of myrmecophiles is impeded by accumulated apomorphies on the phenotypic and genotypic levels.

Synapomorphies of *Limulodes* and *Cephaloplectus* include a strongly deflexed head, compound eyes always completely reduced, specific cephalic antennal furrows, prolonged posterior pronotal angles interacting with the elytral humeri, a broad prosternal process reaching the metathorax posteriorly, a very short metaventrite, enlarged metacoxal plates entirely covering the femora, broadened and flattened femora partly covering the tibia, and a triangular shape of the abdomen. Additional potential apomorphies are the far-reaching reduction of the prothoracic sutures, a locking device enhancing the connection between the elytra and the mesonotum, and the entirely membranous metanotum (Seevers & Dybas 1943). It was pointed out by Seevers & Dybas (1943) that these features are linked with myrmecophilous habits. Larvae of *Limulodes* differ from immature stages of other genera of Ptiliidae in their chaetotaxy (Sörensson & Delgado 2019). The unusual pattern is probably linked with morphological adaptations to a myrmecophilous lifestyle.

Nodes in the backbone of Ptiliidae excluding the basal genera and *Limulodes* + *Cephaloplectus* are generally not strongly supported, with several branches forming a polytomy. The analysis of morphological data places *Discheramocephalus* as sister to all remaining genera, the latter supported by small and widely separated metacoxae, and wing folding patches with denticles on tergites II–V, with secondary variation within the group. A placement of *Ptenidium* + monophyletic Nanosellini as sister to all following groups is also weakly supported. However, Nanosellini, with *Mikado* as sister to the rest of taxa, was confirmed in the analyses of morphological and molecular data, although in the combined analysis the position of *Mikado* was uncertain (with low support). Apomorphies of these extremely small beetles include an elongated mentum dilated in the middle region, lack of dorsal tentorial arms, enlarged metacoxal plates widening towards the mesal margin, long but weakly sclerotized metafurcal arms, and a wing blade with only two veins. The subelytral stridulatory organ was presented as an additional evolutionary novelty of Nanosellini by Sörensson (1997). The presence of cavities called "mycangia" by Sörensson (1997; but see Grebennikov & Leschen 2010) associated with the mesocoxae is another potentially derived feature of this tribe. However, a more precise documentation of this character is required for a reliable phylogenetic interpretation.

A sister-group relationship between *Dacrysoma* and *Cissidium* is supported by morphology with implied weighting and molecular and combined data. Potential synapomorphies are a secondarily smooth dorsal surface, and a horizontally oriented fossa of the keel of the mesoventrite.

Ptiliinae are not confirmed as a clade. *Ptiliola* + *Ptiliolum* are placed in a polytomy with other ptiliid lineages in the morphological trees. However, *Actidium*, *Oligella* and *Micridium* + *Ptilium* form a reasonably well supported monophylum when using morphological data, characterized by a specific shape of the mentum, a pronotum widest in the anterior region, and a shortened and moderately flattened penis.

A clade supported in all analyses comprises *Smicrus*, *Nephanes*, *Baeocrara* and the large genus *Acrotrichis*. Unambiguous synapomorphies are the presence of a single metascutellar spine, a funnel-shaped spermatheca, and an aedeagus placed along the mid-line of the body. Derived larval characters shared by *Nephanes* and *Acrotrichis* also suggest that both genera belong to a monophyletic unit (Sörensson & Delgado 2019).

A clade comprising the wingless *Rioneta*, *Ptinella* and *Pteryx* is sister to this clade in the morphology-based analysis with implied weighting. *Ptinella* and *Pteryx* also share apomorphic larval features (Sörensson & Delgado 2019).

4.3. Subfamilies and tribes of Ptiliidae

The traditionally used taxonomic system of Ptiliidae divides the family into three subfamilies: Ptiliinae, Cephaloplectinae, and Acrotrichinae (Johnson 2004; Hall 2016). The monophyly of the last two groups is fully supported by our morphological, molecular (except that of Cephaloplectinae, which could not be tested due to lack of molecular data for *Cephaloplectus*), and combined analyses. The subfamily Ptiliinae as currently understood, the monophyly of which has been questioned (Hall 2000), turned out to be paraphyletic in all our analyses. Recently, based on the analysis of the morphology of the larvae, a new subfamily Nossidiinae was proposed, and for the subfamily Acrotrichinae a rank of a tribe was suggested (Sörensson & Delgado 2019).

Our analyses of morphological and molecular data recovers Nossidiinae as a clade. This group was proposed by Dybas (1976) and discussed by different authors (Hall 2000; Darby 2015b; Sörensson 2015), but formally it was described only very recently by Sörensson & Delgado (2019). This subfamily, which combines genera with the maximum of preserved plesiomorphic features in Ptiliidae, is the clearly the sister group of all the other genera.

All our tests confirm that the subfamily Acrotrichinae should be downgraded as a tribe Acrotrichini within a newly defined subfamily Ptiliinae **stat. rev.** comprising Ptiliidae excl. Nossidiinae. This is also in agreement with a recent evaluation of larval characters (Sörensson & Delgado 2019).

The former subfamily Cephaloplectinae (previously considered a separate family, Limulodidae) is downgraded to tribe Cephaloplectini **stat. n.** within Ptiliinae **stat. rev.**

The clade Nanosellini, originally ranked as subfamily (Barber 1924) and later treated as a tribe (Hall 2000), is retained with the latter rank in subfamily Ptiliinae.

The group Pterycina (Hall 2003) is herein recognized as Ptinellini. Larval morphological characters are conform with the separate status of this tribe (Sörensson & Delgado 2019).

The clade that includes *Ptilium*, *Actidium*, *Oligella*, and *Micridium* is now defined as tribe Ptiliini. The tribe Actidiini based on larval morphology (Sörensson & Delgado 2019) is in conflict with this concept. However, the evaluation of larval characters did not include representatives of *Ptilium*, *Oligella*, *Micridium*, and other Ptiliini. The phylogenetic and taxonomic positions of the genera *Ptiliola* and *Ptiliolum*, formerly included in a tribe Ptiliini, remain unclear. Thus, we conclude that the tribe Ptiliini as currently understood is likely paraphyletic and its taxonomy requires further study.

As the tribe Ptenidiini was represented only by its type genus in our analyses, its monophyly could not be tested. Larval morphological characters (absence of urogomphi and abdominal segment X with 7 setae on each side) tentatively support its taxonomic status (Sörensson & Delgado 2019).

The tribe Discheramocephalini, defined on the basis of a single character (the fossa on each side of the mesoventral keel, also found in *Sindosium* or *Millidium*), was paraphyletic in all our analyses, but is provisionally retained due to insufficient evidence of an alternative arrangement.

We list below our proposed synoptic classification of Ptiliidae (all other family-group synonymies, authorities and years follow Bouchard 2011):

Ptiliidae Motschulsky, 1845 / Erichson, 1845
- Nossidiinae Sörensson & Delgado, 2019
- Ptiliinae Motschulsky, 1845 / Erichson, 1845 **stat. rev.**
  - Acrotrichini Reitter, 1909
  - Cephaloplectini Sharp, 1883 **stat. n.**
  - Discheramocephalini Grebennikov, 2009
  - Nanosellini Barber, 1924
  - Ptenidiini Flach, 1889
  - Ptiliini Motschulsky, 1845 / Erichson, 1845 (incl. Actidiini Portevin, 1929)
  - Ptinellini Reitter, 1906

4.4. Some aspects of the evolution of Ptiliidae

Modifications of the mouthparts and of other head structures played an important role in the early evolution of Ptiliidae and Hydraenidae, likely linked with a shared trend towards body size reduction. A characteristic feature found in adults of both families is the partial internalisation of the mouthparts. The configuration is apparently suitable for saprophagy (Hydraenidae, Ptiliidae *partim*) and for feeding on fungal spores (*Nossidium*) (Yavorskaya et al. 2017). The locking mechanism between the mandible and labrum is a highly unusual feature linked with the semi-entognathous mouthparts. This enables the beetles to seal the preoral space hermetically. Seevers & Dybas (1943) noted a close contact between the labrum and mentum and largely hidden paired mouthparts as a characteristic of Cephaloplectini. However, this condition is found in Ptiliidae including the Nossidiinae (Yavorskaya et al. 2017). Within Ptiliidae, a switch to microsporophagy took place in the extremely miniaturized Nanosellini. Surprisingly, an exceptionally complicated hypopharyngeal and epipharyngeal apparatus occurs in these extremely small beetles (Yavorskaya et al. 2017). Whether the reduced apical part of the mandibles is linked with feeding on very small particles or just with a high degree of miniaturisation is debatable.

As pointed out by Polilov et al. (2019), modifications of the hind wings played a major role in the evolution of Ptiliidae and was likely linked with body size reduction. Fringes of setae are present in the related Hydraenidae and Leiodidae (Hansen 1997), but distinct modifications take place within Ptiliidae (Polilov et al. 2019). A far-reaching reduction of the wing blade and wing venation is a major character transformation in the family. However, despite the very small size and the strongly modified wings, the beetles do not drift passively in the air. They fly very rapidly and are capable of active manoeuvres, with a unique mechanism not described in any other groups of beetles (Yavorskaya et al. 2019; Polilov et al. 2019b).

The elytral opening mechanism of Ptiliidae is not fully clarified yet (Yavorskaya et al. 2019). The metathoracic locking mechanisms with distinctly elongated alacristae is already present in Hydraenidae. Within Ptiliidae, the length of the alacristae is even increased (e.g. Sörensson 1997). It is likely that the elytra snap open passively when the locking mechanism is released by lowering the metathorax and abdomen. Wing folding patches with a specific distribution on the abdominal tergites and varying structural properties are characteristic for the family. A very unusual derived feature is the parallel arrangement of the hind wings below the elytra, an apomorphy of Ptiliidae excluding Nossidiinae and possibly the secondarily wingless Cephaloplectini.

A tendency to fuse sclerites was already present in the groundplan of the clade Hydraenidae + Ptiliidae. The mesoventrite and mesanepisternum are largely or completely fused. The sutures of the prothorax of Ptiliidae are also usually absent, even though this condition likely does not belong to the groundplan of the family (Yavorskaya et al. 2019). The functional background of two similar derived features of Ptiliidae remains unclear: the retracted position of the articulation of the slender proximal flagellomere and tarsomeres.

Detailed anatomical data on the genital apparatus of Ptiliidae are still sparse. The greatly reduced number of eggs, one developing and deposited at a time (Dybas 1966; Polilov 2015), is likely a derived condition linked with body size reduction. A remarkable feature shared with Hydraenidae is the spermathecal pump, arguably a unique characteristic in the entire Coleoptera (Hansen 1997; Lawrence et al. 2011). The shape of the spermatheca varies greatly within both families (e.g. Jäch et al. 2016; Darby 2019). The functional background of this character system and its diverse modifications is still largely unclear.

5. Conclusions

Our morphological, molecular, and combined analyses suggest the following well-supported clades: (1) Ptiliidae + Hydraenidae; (2) Ptiliidae; (3) Ptiliidae excluding *Nossidium*, *Motschulskium* and *Sindosium*, which are the sister group (Nossidiinae) of the remaining Ptiliidae (Ptiliinae); (4) the wingless myrmecophiles Cephaloplectini; (5) Acrotrichini; (6) Ptinellini; and (7) the extremely small Nanosellini. We propose the following classification of the family Ptiliidae: Nossidiinae + Ptiliinae (Acrotrichini + Cephaloplectini + Discheramocephalini + Nanosellini + Ptenidiini + Ptiliini + Ptinellini). Most of these tribes are supported by morphological and molecular data, but further studies taking into account more representatives of Ptiliidae are needed to stabilize the family classification and to clarify the composition Discheramocephalini and Ptiliini. Miniaturization is an extremely important factor of the evolution of Ptiliidae, manifested most distinctly in far-reaching transformations of the

wing apparatus, but also the fusion of skeletal elements, and transformations of mouthparts and other structures.

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Authors' contributions

A.A.P. and R.G.B. designed the study. M.I.Y. and A.A.P. performed the study of morphology. I.R. and A.C. performed the DNA extraction and sequencing. R.G.B., I.R., A.A.P. analyzed the data. A.A.P., R.G.B., V.V.G. and I.R. drafted the work. All authors approved the final draft of the manuscript.

Electronic Supplement File

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File 1: polilov&al-ptiliidaephylogeny-asp2019-electronicsupplement-1.pdf — **Table S1.** Studied specimens, localities and GenBank accession numbers. — **Table S2.** Primers used for DNA amplification. — **Table S3.** Data matrix of 68 morphological characters scored for 33 terminals and used for the phylogenetic analysis of Ptiliidae beetles. — **Figs. S1 – S7.** SEM habitus images of Ptiliidae. — **Fig. S8.** Maximum Likelihood inference phylogram of Ptiliidae obtained with RAxML with the molecular data only. Digits at internodes are bootstrap values when > 50%. — **Fig. S9.** Majority rule consensus tree of the Bayesian analysis (obtained with BEAST) of the molecular characters, with the simplest models (analysis C, see Methods). Numbers in nodes are posterior probabilities when > 0.5.