

Wing morphology in featherwing beetles (Coleoptera: Ptiliidae): Features associated with miniaturization and functional scaling analysis

Alexey A. Polilov ^{a, b, **}, Natalia I. Reshetnikova ^a, Pyotr N. Petrov ^{a, *}, Sergey E. Farisenkov ^a

^a Department of Entomology, Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

^b Joint Russian-Vietnamese Tropical Research and Technological Center, Hanoi, Viet Nam

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ABSTRACT

The wings of Ptiliidae, the coleopteran family containing the smallest free-living insects, are analyzed in detail for the first time. A reconstruction of the evolutionary sequence of changes associated with miniaturization is proposed. The wings of several species are described using light microscopy and scanning electron microscopy. The morphology and scaling are analyzed in comparison with larger representatives of related groups. The wings of all studied ptiliids show some degree of ptiloptery (feather-like shape, typical for extremely small insects). In larger ptiliids the wing contains at least five veins, has a wide blade, and bears a marginal fringe of 200–300 setae; in the smallest species it has three veins or fewer, a narrow blade, and about 40 setae along the margin. The setae are brush-like; peculiar outgrowths, denser towards the apex, increase the effective diameter of the setae. Morphometric analysis shows that the geometry of the wings and their elements strongly differs from those of other staphyliniform beetles, suggesting that the aerodynamics of the feather-like wings may also differ distinctly from the usual pattern.

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1. Introduction

The vast majority of Insecta, the most speciose class of animals (Zhang, 2013), is represented by winged species. Winged insects (including wingless descendants of winged ancestors) form the monophyletic group Pterygota, which probably evolved in the Late Silurian to Early Devonian and is known from reliably identified fossils from the Early Carboniferous (Misof et al., 2014). The unprecedented evolutionary success of pterygotes can be largely explained by the benefits of active flight. This is also true of Coleoptera (beetles), which includes more known species than any other insect order, or, indeed, any other order of organisms (Zhang, 2013). Most beetles are winged and nearly all (at least in one sex) have elytra (wing cases), the modified forewings that cover and protect parts of the body. The wings are used in flight and folded under the elytra at rest (Beutel and McKenna, 2016). The smallest beetles usually have functional wings, and the elytra are always present.

* Corresponding author. Leninskie gory 1–12, Moscow 119234, Russia.

** Corresponding author. Leninskie gory 1–12, Moscow 119234, Russia.

E-mail addresses: polilov@gmail.com (A.A. Polilov), tinmonument@gmail.com (P.N. Petrov).

Many lineages of hexapods have evolved at least some microscopic forms, but none of the free-living (non-parasitoid) insects are known to include such small forms as beetles of the family Ptiliidae (Polilov, 2016). Miniaturization in all hexapods is associated with morphological and physiological changes, many of them emerging independently in different lineages. Some of these changes are known to affect the wing apparatus. Most flying microinsects are characterized by ptiloptery: strongly narrowed wing blade fringed with long setae along the margin (Rohdendorf, 1949). Ptiliids are no exception: the wings of all winged species show at least some degree of ptiloptery, i.e., consist of narrow wing blades bearing a fringe of relatively long setae. We have shown recently (personal observation) that ptiliids are not passive fliers, as previously suggested: even some of the smallest species of the family have active flight of a peculiar kind, and their flight is apparently highly maneuverable.

Although rather brief descriptions of wings are available for some species of Ptiliidae (Forbes, 1926; Dybas, 1966: figs 8, 9; Naomi, 1989; Hansen, 1997; Fedorenko, 2009; Polilov, 2016), the wings of a wide range of ptiliids have never been studied in detail or analyzed broadly.

Most species of the superfamily Staphylinoidea have well-developed wings, but reductions occur (Gusarov, 2003). The

shape of the wing varies between families; two types of wing shape can be recognized: the staphylinid type, characteristic for Staphylinidae (Naomi, 1989), and the agyrtid type, characteristic for Ptiliidae, Hydraenidae, Agyrtidae, and Leiodidae (Fedorenko, 2009). To analyze the evolutionary changes that accompanied miniaturization in the subfamily Staphylinioidea, it is therefore useful to compare ptiliid wings to those of both types found in larger staphylinoid beetles, including moderately small species of Hydraenidae, the sister group of Ptiliidae, and also larger species of Staphylinidae.

The purpose of this study was to fill the currently existing gaps in our knowledge of ptiliid wings and to reconstruct evolutionary changes in the wings that accompanied miniaturization in this family.

2. Material and methods

2.1. Material

We studied the adults of 11 species of the family Ptiliidae: *Nossidium pilosellum* (Marsham, 1802); *Motschulskium sinuatocolle* Matthews, 1872; *Sindosium* sp.; *Ptenidium pusillum* (Gyllenhal, 1808); *Porophila mystacea* Polilov, 2008; *Primorskiella anodonta* Polilov, 2008; *Scydosella musawasensis* Hall, 1999; *Nephanes titan* (Newman, 1834); *Acrotrichis grandicollis* (Mannerheim, 1844); *Acrotrichis intermedia* (Gillmeister, 1845), and *Acrotrichis sericans* (Heer, 1841); and two species of Hydraenidae: *Limnebius atomus* (Duftschmid, 1805) and *Hydraena riparia* (Kugelann, 1794). All specimens were fixed in alcoholic Bouin or 70% ethanol.

2.2. Light microscopy

The external morphology of the wings was studied using Olympus BX43 and Motic SMZ168 light microscopes. Prior to examination, the wings were first kept in 70% ethanol and then in a mixture of ethanol, glycerol, and distilled water (5: 5: 1 by volume), in which they were heated for an hour for straightening of bends of the wing blade and setae. Then the samples were placed onto a glass slide and covered with a cover slip.

2.3. Scanning electron microscopy

The fixed material was dissected prior to SEM examination: either the elytra or the elytra and wings were removed and then dried. Most of the material was dried at the critical point with a Hitachi HCP-2. To obtain spread exemplars, wings were immersed in a droplet of distilled water on a glass slide glued to the microscope stage with varnish. After the evaporation of the water, adhesion glued the wing to the glass. The mounted samples were sputter-coated using a Giko IB-3 unit and examined under a SEM. The micrographs were made using a SEM JSM-6380 at 20 kV with a distance of 8–25 mm.

2.4. Morphometry

The materials were measured in AutoCAD using images taken with light microscopes and SEM. The photographs were processed using Adobe Photoshop. Wing length, area of wing with and without marginal setae, area occupied by setae, length of setae, and distance between tips of setae were measured using light microscopic photographs. Wing length was measured as the distance between the base of the wing and the point of the wing contour most distant from the base. Aspect ratio was calculated as the ratio of wing length squared and the wing area with setae or without setae, i.e., sum of wing blade area and petiole area. The total wing

surface (area of wing with setae) was measured as the area within the outline marked by the apices of all marginal setae. Body length and diameters of setae were measured using SEM images. We measured both the diameters of the stems of the setae and diameters of the setae including the lengths of the outgrowths in middle regions of the setae. The arithmetic average value of the stem diameter and diameter including outgrowths was taken as the effective diameter of the setae. The distances between apices of the setae in the apical part of the wing were also measured. To calculate the gap between the setae, we subtracted the effective diameter from this distance.

2.5. Statistics

The descriptive statistics and allometric analysis (SMATR – SMA) were performed using R software.

3. Results

3.1. Examined groups of Ptiliidae

The studied genera of Ptiliidae fall into three categories, and our results described below are structured accordingly: the genera that can be interpreted, both according to recent studies that partially outline the phylogeny of the family (Hall, 1999; McKenna et al., 2015; Yavorskaya et al., 2017) and according to our unpublished analyses of molecular data, as early splits in Ptiliidae (tentatively termed “early splits” or “basal genera” in this study) and are likely to have preserved some ancestral characters of the family (*Nossidium*, *Syndosium*, and *Motschulskium*); “typical” ptiliids of relatively large size (c. 0.6–1.0 mm: *Acrotrichis*, *Nephanes*, *Ptenidium*, and others), referred to as “intermediate” in this study; and extremely small (even for ptiliids) representatives of the tribe Nanosellini (*Paratuposa*, *Scydosella*, and *Primorskiella*).

3.2. Wing shape

The wings of all studied species of Ptiliidae show at least some degree of ptiloptery: the wing blade is narrow and fringed with rather long setae (Fig. 1A–F, 2B–D). Ptiliid wings consist of two distinctive parts: the strongly sclerotized basal part termed the petiole (Polilov, 2016), which in cross-section is more or less oval and moderately flattened dorsoventrally, and the strongly flattened wing blade, which bears rather long setae, similar in length to the width of the wing blade (in the early splits) or (in other winged ptiliids) much longer (Fig. 1A–F, 2B–D). The petiole is less than half as long as the wing blade, always narrower, usually more or less straight, but sometimes slightly curved anteriorly, especially in *Nossidium*. In the early splits (Fig. 1A, B, 2C) the wing blade is rather broadly oval, with the anterior margin almost straight, while in the other ptiliids the wing blade is much narrower and saber-shaped (visibly curved anteriorly). The widest point of the wing is around the middle region in the early splits (Fig. 1A–F, 2B–D) but closer to the base than to the apex, and very close to the base of the wing blade in the other genera (Fig. 1C–F). The apex of the wing blade is broadly rounded in *Nossidium* and the other early splits, but more angulate in other ptiliid genera.

3.3. Setal fringe

The wings of all species bear a setal fringe along the margin anteriorly, in the apical region, and posteriorly. In *Nossidium* and *Motschulskium* (Figs. 1A, 2B), the setae are present on the anterior margin of the petiole, along the margin of the wing blade, and on the posterior margin of the petiole; in *Sindosium* (Fig. 1B), the

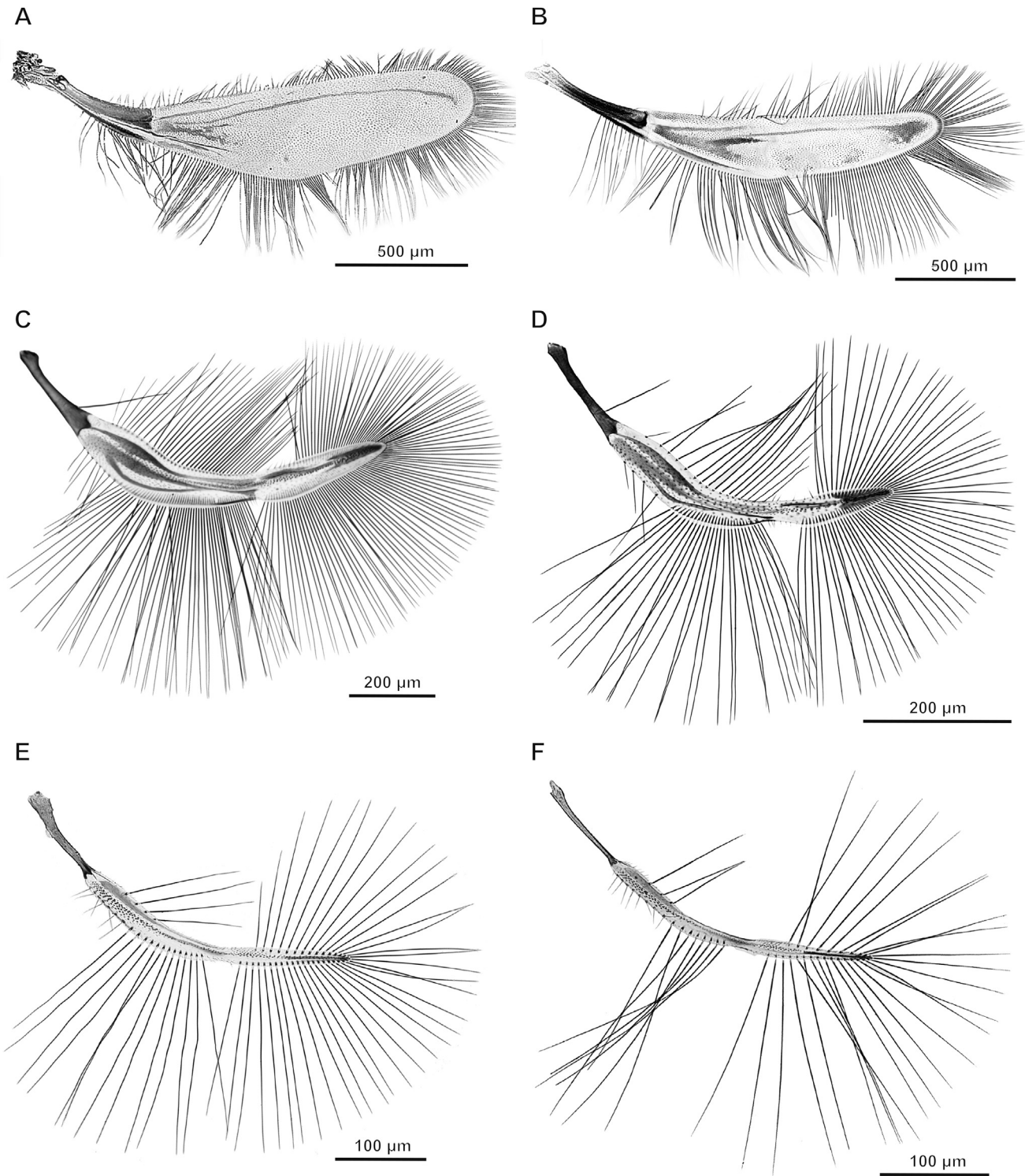


Fig. 1. Wings of Ptiliidae (Coleoptera). (A) *Nossidium*, (B) *Sindosium*, (C) *Acrotrichis sericans*, (D) *Nephanes titan*, (E) *Paratuposa* sp., (F) *Scydosella*.

anterior margin of the petiole is free of setae, but the posterior margin of the petiole bears some setae in the distal half; in the other studied species, the petiole is free of setae on both the anterior and posterior margins, except for a single semierect seta subdistally on the anterior margin of the petiole in *Acrotrichis*, *Nephanes*, and *Ptenidium*. In the three “early split” genera, the distribution of the setae in the fringe is more or less regular, without any distinct gaps; in the other studied ptiliids (Fig. 1C–F, 2C, D), the

fringe is clearly divided into three zones with clearly visible gaps between them; zone 1 on the proximal portion of the anterior margin of the wing blade, zone 2 on the distal portion of the wing blade (reaching closer to the middle region posteriorly than anteriorly), and zone 3 on the proximal portion of the posterior margin of the wing blade (the numbers of the zones are proposed here for convenience of future descriptions and discussions). The number of setae in the fringe varies between genera within a broad range:

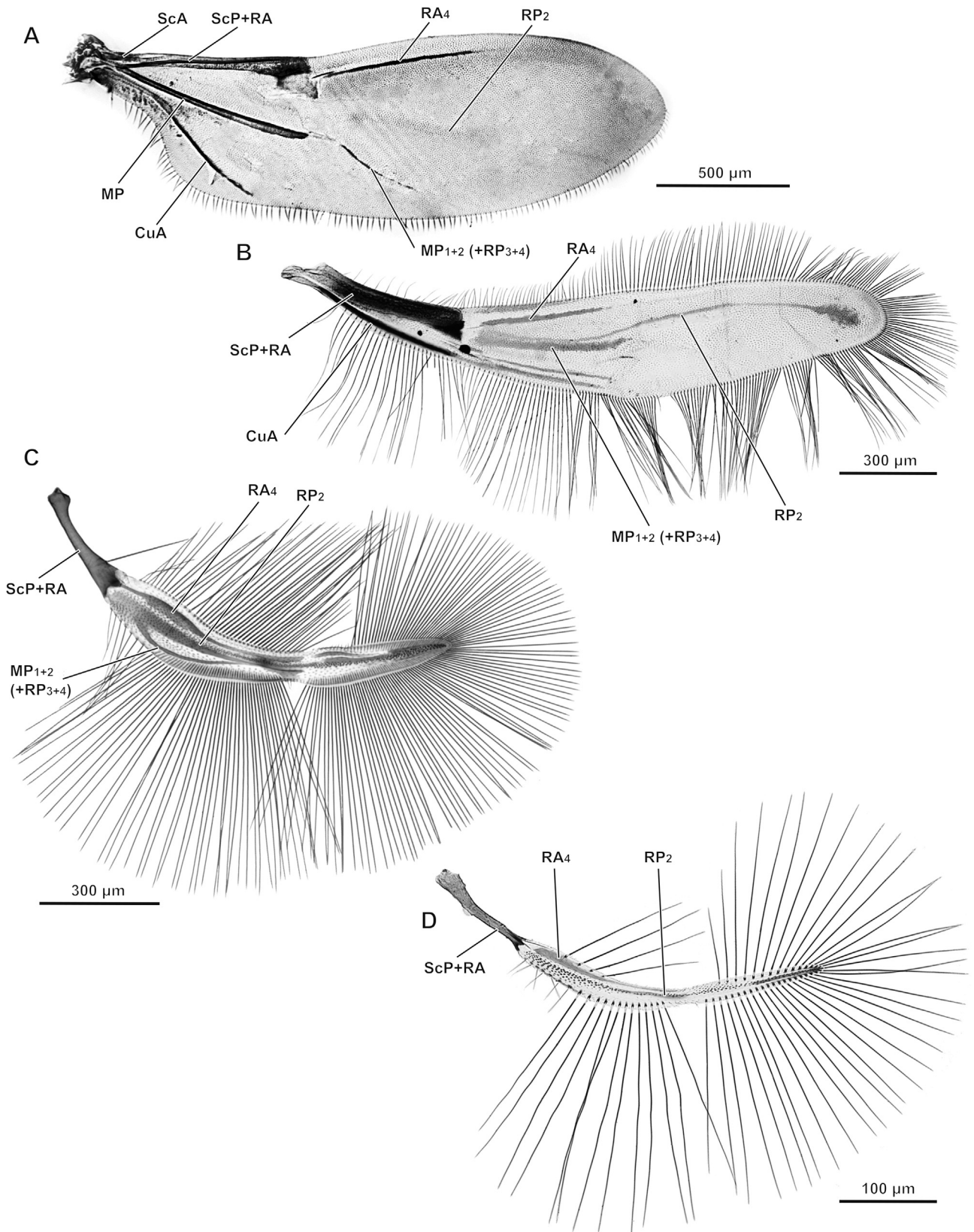


Fig. 2. Hypothetical series reflecting evolutionary changes of venation in Hydraenidae (A) and Ptiliidae (B–D). (A) *Hydraena*, (B) *Motschulskium*, (C) *Acrotrichis grandicollis*, (D) *Primorskiella anodonta*.

from a few dozens in the smaller Nanosellini to nearly 300 in *Nossidium*; the total number of setae and the numbers of setae in each of the three zones decreases with body size, especially in zone 1, down to only three setae in this zone in *Scydosella* (Fig. 1F). In the early splits, especially in *Nossidium*, the setae on the anterior margin are much shorter than those along the posterior margin, with the setae at the apex of intermediate lengths; in the other studied genera the length of the setae of zones 2 and 3 are similar, but the setae of zone 1 are distinctly shorter. The length of the setae relative to the width of the wing blade strongly increases with decreasing body size: from similar to or shorter than the width of the wing blade in the early splits (Fig. 1A, B, 2C) to a few times as long as the width of the wing blade in intermediate ptiliids (Fig. 1C, D, 2CF), to nearly ten times as long in some Nanosellini, especially *Scydosella*, which also has the smallest number of setae in the fringe of all studied ptiliid species.

3.4. Wing venation

The number of veins apparently depends on the body size and width of the wing blade, varying from five in some of the larger species to three in some of the smaller ones. The base of the wing (petiole) in species of the early splits contains two veins, interpreted here as the anterior ScP + RA and posterior CuA (Fig. 1A, B, 2B, 3A, B). These two veins are strongly sclerotized; ScP + RA occupies more than half the width of the petiole in each of the three “early split” genera; CuA is much narrower, occupying less than a quarter of the width of the petiole; in *Sindosium* it is probably partly fused medially with ScP + RA. In each of the three “early split” genera, there is a fold in the wing membrane running between ScP + RA and CuA in the distal portion of the petiole and continuing into the proximal portion of the wing blade. The wing blade in *Nossidium* contains three more or less distinguishable veins, much less strongly sclerotized than the veins of the petiole: the relatively narrow anterior RA₄, which is almost straight and covers the basal half of the wing blade; the similarly narrow RP₂, which originates somewhat distad the very base of the wing blade and runs in a slightly S-shaped curve into the apical portion of the wing to disappear at some distance from the wing margin; and the rather irregularly shaped MP₁₊₂ (+RP₃₊₄), which is wider than the other two veins of the wing blade but much shorter, and visible only in the basal quarter of the wing blade. The two elongate sclerotized areas in the proximal portion of the wing posteriad the above-mentioned fold are difficult to interpret as any particular veins but may be vestiges of the more distal portion of CuA. The venation of *Sindosium* is similar, but RP₂ originates only in the second quarter of the wing blade and ends in the apical portion of the wing in an extension occupying the medial area of the wing blade in that area; MP₁₊₂ (+RP₃₊₄) is longer than in *Nossidium*, covering almost the entire basal third of the wing and forming a dilated fork distally, with the anterior branch more distinct, darker, evenly tapering, and the posterior branch shorter, blurred, and gradually obliterating. The venation of *Motschulskium* (Fig. 2B) is similar to that of *Sindosium*, but the apical dilation of RP₂ is narrower and shorter, and MP₁₊₂ (+RP₃₊₄) is narrower and does not end in a fork, tapering and disappearing slightly proximad the middle region of the wing. In the other studied genera, the petiole contains only one vein, interpreted here as ScP + RA. In *Acrotrichis* and *Nephanes* (Fig. 1C, D, 2C, 3C), RA₄ is strongly dilated in the basal third of the wing blade, and much narrower in the medial third, running along the anterior wing margin and obliterating in the apical third of the wing blade; RP₂ is also much wider basally than in the early splits, and also narrower in the medial third of the wing blade; MP₁₊₂ (+RP₃₊₄) is more distinct than in the early splits, rather narrow, curved anteriorly in the dilated basal third of

the wing blade and fused with RP₂ in the middle region of the wing blade, where these two veins together approach the posterior wing margin and obliterate shortly before reaching the apical third of the wing blade; the apical third of the wing contains rather indistinct sclerotized areas, one of which occupies most of the apical portion of the wing blade. However, they are interrupted around some of the areas where the wing bends when it is folded, and the interpretation of these sclerotized areas as parts of particular veins is problematic. In the studied genera of the tribe Nanosellini (Fig. 1E, F, 2D, 3D), the wing blade contains only two veins: RA₂, which is also dilated in the basal third and obliterates in the middle third, and the narrow RP₂, which is present in the middle third, obliterates shortly before reaching the apical third, and then probably re-emerges, reaching the wing apex as a narrow and rather strongly sclerotized vein. None of the veins are visibly branching in any of the studied Ptiliidae.

3.5. Internal structure of wings

In cross-sections of the wings (Fig. 4A–C), only the petiole and base of RA₄ contain internal cavities typical of insect veins. All the other veins of the wing blade are only represented by swellings of the cuticle, and even RA₄ contains a cavity only in the basal third. It is also visible in the cross-sections that the wing blade consists of two layers of cuticle closely attached to each other, and the microsculpture of the wing consists of cuticular outgrowths (Fig. 4B–C).

3.6. Microsculpture of wing blade

The microsculpture of the surface of the wing membrane is not uniform (Fig. 5A–I). On the wing blade it consists of similar elements in all studied species. The ventral surface of the wing is covered with unevenly distributed rather short and robust cuticular denticles, which are more densely arranged medially but absent closer to the margins; these denticles cover a large area in the basal third of the wing (Fig. 5A) and another relatively large area of the apical third (Fig. 5C); these two areas are connected by a narrower denticulated area in the medial third of the wing (Fig. 5B), in Nanosellini represented only by a single row of denticles. The denticles are semirecumbent and directed towards the apex of the wing, with slightly varying orientation. The dorsal surface of the wing is covered with rather evenly distributed elongate and slender outgrowths; they are also semirecumbent and mostly directed towards the wing apex.

3.7. Marginal setae

The bases of the setae along the posterior margin are attached to elongate sockets that cover much of the ventral surface of the posterior portion of the wing (Fig. 6F); no such sockets are present on the anterior wing margin (6E). The bases of the setae are attached to the ventral surface of the wing blade and hidden behind the wing margin in dorsal view. The setae are brush-like, bearing peculiar outgrowths (Fig. 6A–D, I–K), which are only missing at the smooth stems. More distally, the outgrowths appear as relatively sparse semirecumbent scales; their density and length increase with distance from the base, reaching the highest density at the apex of the seta. The outgrowths are rather evenly distributed on the surface of the seta, forming no whorls or regular rows; they are more or less semirecumbent and directed towards the apex of the seta. The shape and orientation of the outgrowths and also the ratios of the average outgrowth length and the diameter of the stem of the seta vary among different genera of Ptiliidae. In *Nossidium* (Fig. 6I) and the other early splits, the

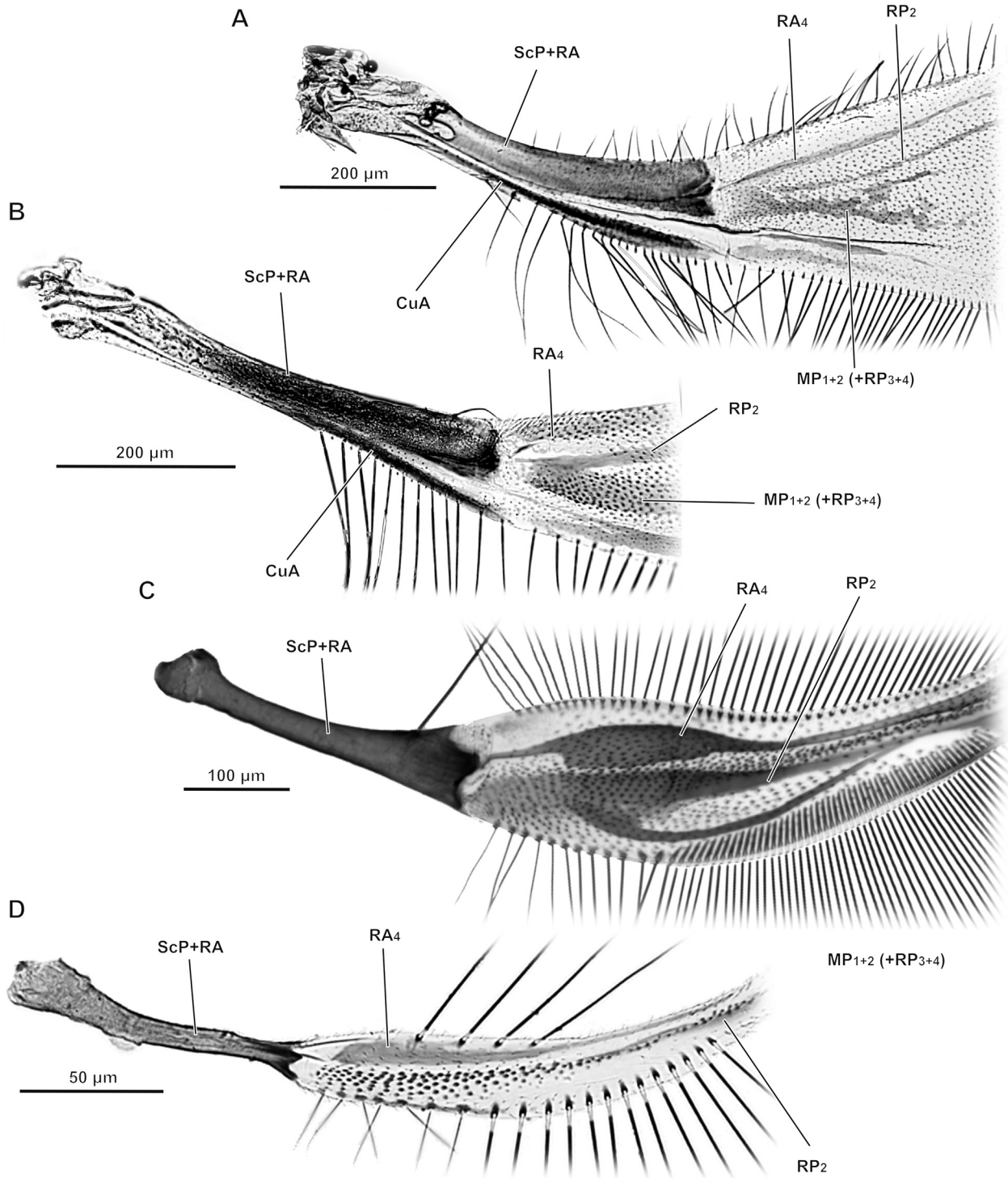


Fig. 3. Bases of wings in Ptiliidae (Coleoptera). (A) *Nossidium*, (B) *Sindosium*, (C) *Acrotrichis intermedia*, (D) *Primorskiella anodonta*.

outgrowths have flattened bases with smoothly curved anterior margins; in *Acrotrichis* (Fig. 6C, D) and the other intermediate ptiliids, they are more densely arranged than in the early splits, almost straight, and more recumbent; in Nanosellini (Fig. 6J, K) they are sparser than in the early splits, narrower and even more recumbent than in intermediate ptiliids, especially in *Scydosella* (Fig. 6K), characterized by small angles between the outgrowths and the stems of the setae.

3.8. Wing folding

In *Nossidium*, the wings are folded by bending along several diagonal lines and asymmetrically overlap at rest, folded below the elytra (Fig. 7A); the folding patterns of the wings in the other two “early split” genera are similar to that of *Nossidium*. In the other genera (Fig. 7B, C, 7F–H) the wings are folded symmetrically, without overlapping below the elytra, by bending along lines

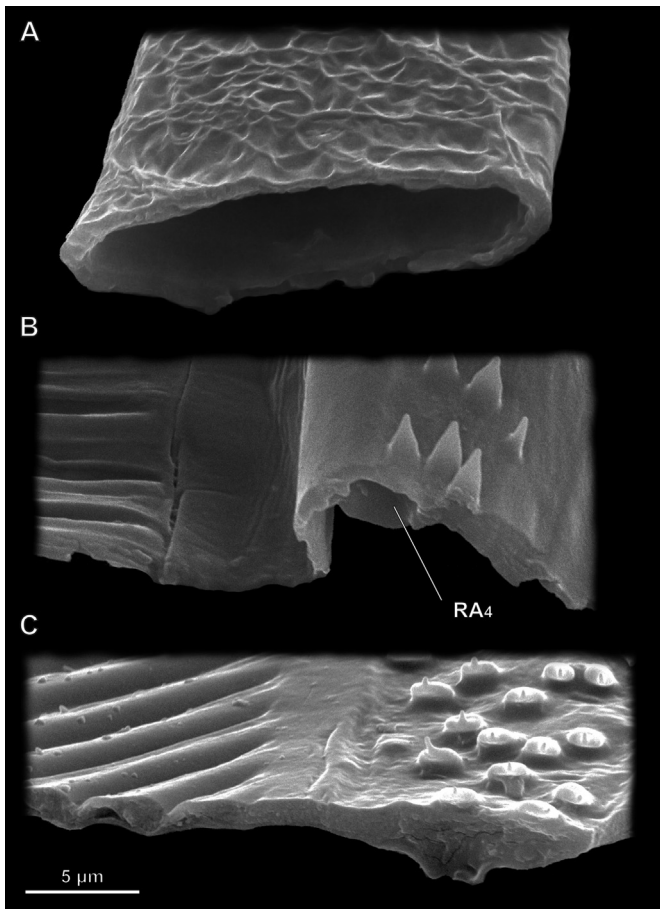


Fig. 4. Cross-sections of wing blade in *Acrotrichis sericans* (Coleoptera, Ptiliidae), SEM micrographs: (A) wing base (petiole), (B) basal part of wing blade, (C) medial part of wing blade.

perpendicular or almost perpendicular to the longitudinal axis of the wing at four points; the setae are also folded by bending (at their bases) and lie more or less parallel to the wing blade in the folded wings (Fig. 7D, E). The wings of *Ptenidium* have one additional, fifth bending line; the posterior angle of the second bend is additionally folded along a diagonal line (Fig. 7C).

3.9. Elytra

The elytra vary in shape and microsculpture (Fig. 8A–H). In the smaller species they are elongate oval, which may reflect the body shape of these beetles, which, in turn, is an adaptation to their habitat; *A. grandicollis*, *A. intermedia*, *A. sericans*, *N. titan* (Fig. 8D), and *P. pusillum* have wider elytra with a pronounced angle near the apex of the scutellum. The epipleura are rather wide in early splits and narrower in the other genera; they are gradually tapering towards the apex in most cases. The elytra of all ptiliids have a well-developed locking mechanism along the medial suture. In *M. sinuatocolle*, *Sindosium* sp., and *N. pilosellum* the longitudinal ribs of the lock are supplemented with protrusions and cavities that fit with corresponding cavities and protrusions on the other elytron.

In *A. grandicollis*, *A. intermedia*, *A. sericans*, and *N. titan* the ventral elytral surface of the elytron medially bears a diagonal series of rounded cuticular protrusions (Fig. 8D, E). In *S. musawasensis* and *Primorskiella anodonta* the ventral surface of the elytron includes an area of straight cuticular folds (Fig. 8H, G), probably used for stridulation. The ventral surface also displays different

areas with spinules, denticles, folds, and other elements of microsculpture, different in different ptiliids, and with unclear functions; they may be involved in the folding of the wing and in keeping it folded (Hammond, 1979).

3.10. Scaling

The relative wing length in Ptiliidae generally increases with body size, in contrast to other Staphylinoida and to Scarabaeoidea, in which it decreases with body size (Fig. 9A).

The number of marginal setae shows a strong and significant correlation with body size: from fewer than 40 in the smallest species to around 300 in the largest (Fig. 1, Table A1).

Decreasing body size in Ptiliidae predictably results in decreasing length and surface area of the wing (Fig. 9B). Comparison with related groups of Coleoptera shows that the values of parameters found in Ptiliidae are typical for staphylinoid beetles.

The aspect ratio of the wing in the studied ptiliids falls within a range between 1.9 and 3.5 and shows no statistically significant dependence on the body length, in contrast to other staphylinoid beetles, in which the aspect ratio of the wing tends to increase with decreasing body size (Fig. 9C).

The ratio of the area occupied by the setae (within the outline marked by the apices of all marginal setae) and the rest of the wing is variable: miniaturization is accompanied by a decreasing size of the area of the petiole and wing blade compared to what is found in larger species. The proportion of the marginal setal region varies from about 59% (e.g., in *Nossidium*) to about 95% (e.g., in *Primorskiella*) of the total wing area (Fig. 9B, D).

The ratio of the average length of the peripheral setae and body length remains more or less constant in Ptiliidae, except for the early splits, in which it is markedly smaller than in most other studied ptiliids (Fig. 9E).

Allometric changes in the morphology of the setae are of special interest. The smaller the body size of a ptiliid, the smaller is the average diameter of the stems of the setae and the diameter of the setae including the lengths of the outgrowths that cover the stem (Fig. 9F). As a result, the effective diameter of the setae decreases in the studied series of ptiliid species from 3.4 to 1.4 µm, while the distances between the setae decrease by half. As a result, the coefficient G/D (gap/diameter) increases by a factor of more than 4. It should be noted that the relative length of the setae also slightly increases (Fig. 9F).

4. Discussion

4.1. Miniaturization and evolution of wings in Staphylinoida

The wings are usually well-developed in the superfamily Staphylinoida, although some species are wingless. The shape of the wings differs among the families. Two types of wing shape can be recognized: the staphylinid type, characteristic of Staphylinidae, and the argyrid type, characteristic of Ptiliidae, Hydraenidae, Agyrtidae, and Leiodidae. Staphylinid type wings are elongate oval, but relatively wide, smooth, without any outgrowths (Naomi, 1989). The argyrid type has some plesiomorphic features, such as the rudimentary RC of the proximal boundary of 2a, while the staphylinid type displays mostly apomorphic characters, such as the absence of RC or boundary of 2a (Fedorenko, 2009). In some staphylinids, however, the wings are secondarily reduced (Gusarov, 2003).

Miniaturization results in considerable modifications of the wing structure: narrowing of the wing blade, elongation of the peripheral setal fringe, reduction of venation. Similar evolutionary changes are found in miniature representatives of many orders and

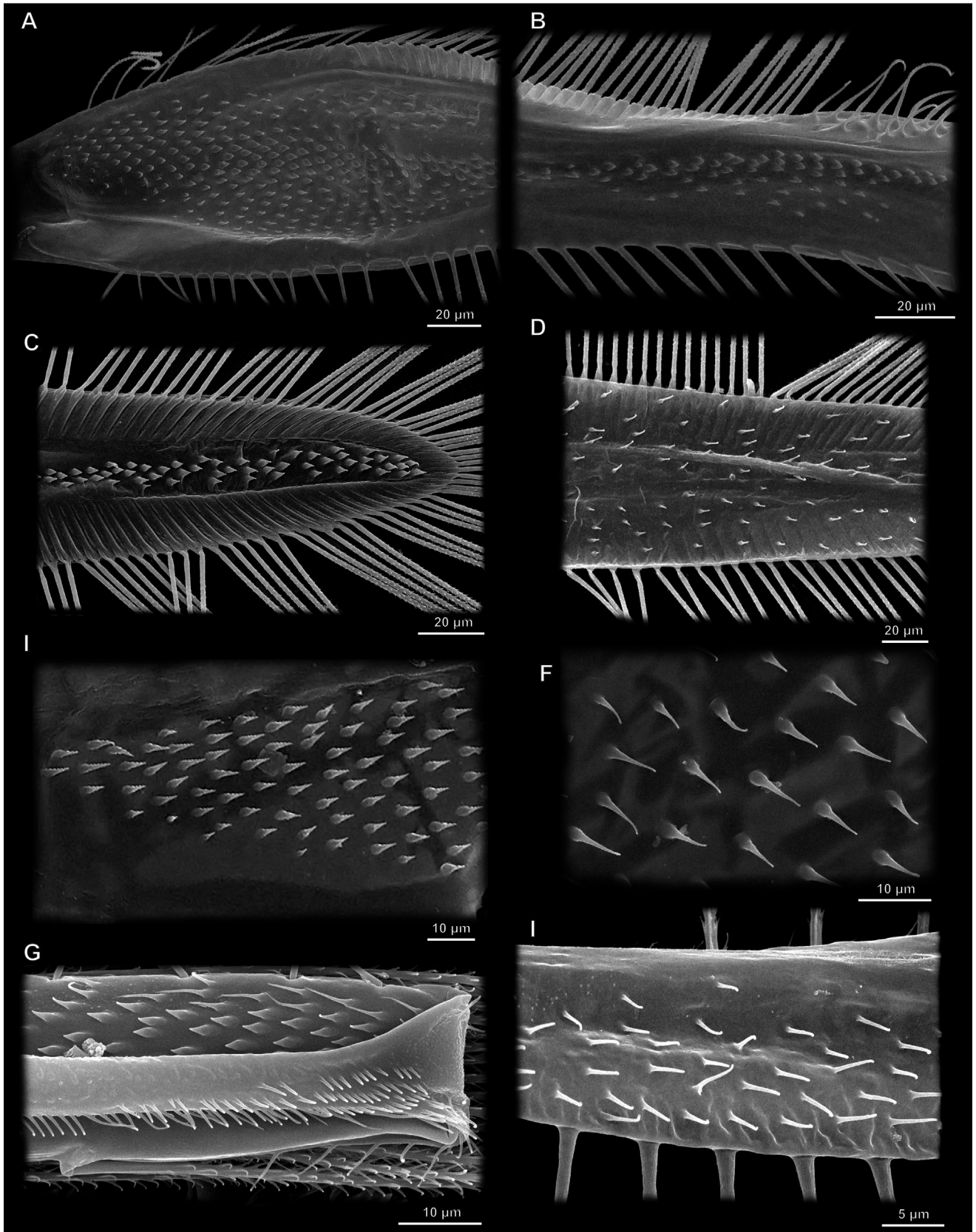


Fig. 5. Microrelief of wing blade surface in Ptiliidae (Coleoptera), SEM micrographs. (A–D) *Acrotrichis sericans*; (A) basal part of wing blade, ventral view; (B) medial part, ventral view; (C) apical part, ventral view; (D) basal part, dorsal view; (I, F) *Sindosium*, basal part of wing blade; (I) ventral view; (F) dorsal view; (G, I) *Primorskiella anodonta*, dorsal view.

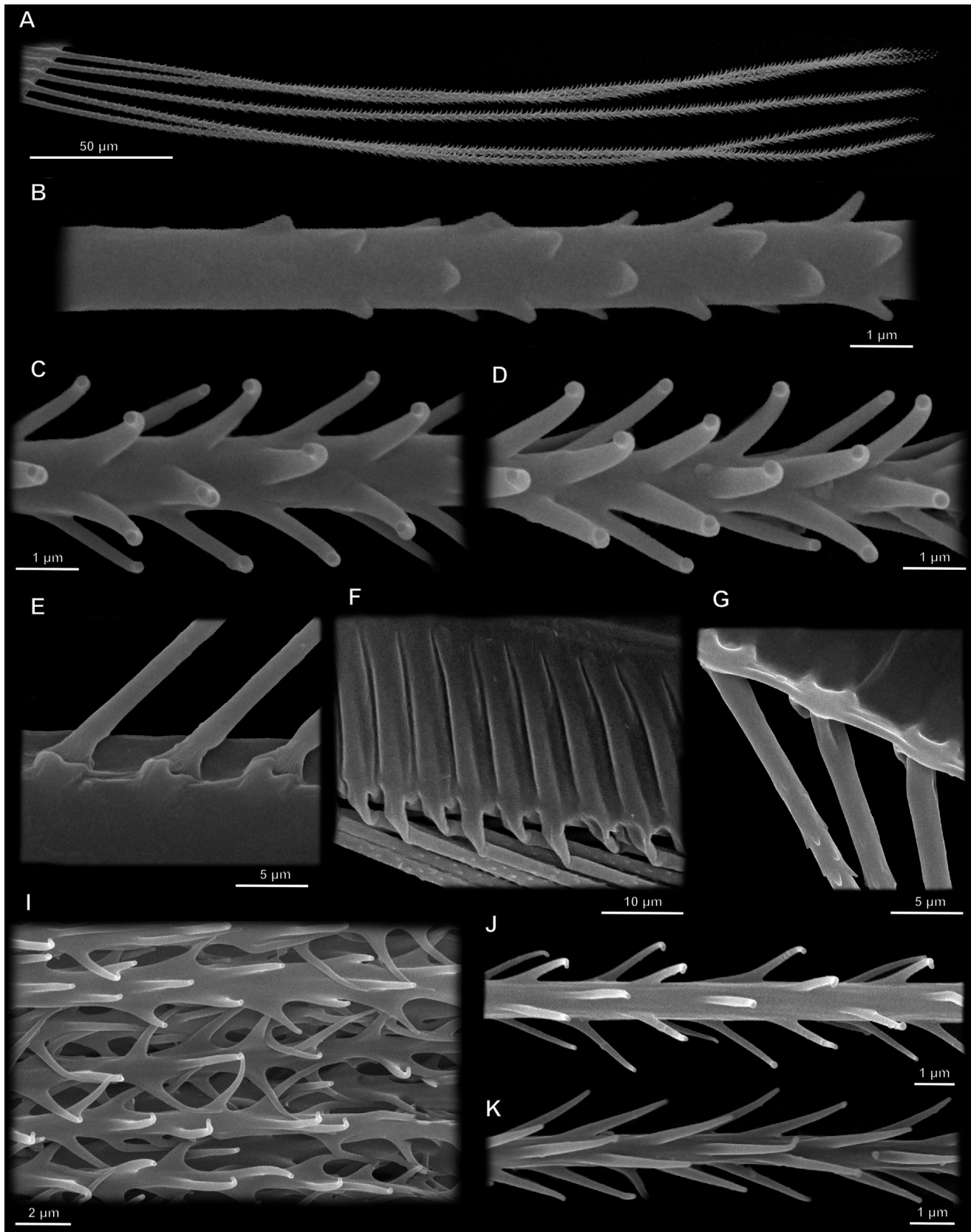


Fig. 6. Setae of Ptiliidae (Coleoptera), SEM micrographs. (A–G) *Acrotrichis sericans*; (B) basal fragment of seta; (C) medial fragment of seta; (D) subapical fragment of seta; (E) base of seta on anterior wing margin, ventral view; (F) base of seta at posterior wing margin, ventral view; (G) base of seta at anterior wing margin, dorsal view; (I) *Nossidium*; (J) *Primorskiella anodonta*; (K) *Scydosella*.



Fig. 7. Wing folding in Ptiliidae (Coleoptera), SEM micrographs. (A) *Nossidium*, ventral view; (B–E) *Ptenidium*; (B) dorsal view; (C–E) ventral view; (F, G) *Primorskiella*, dorsal view, with folded wings (F) and nearly unfolded wings (G); (H) *Acrotrichis sericans*.

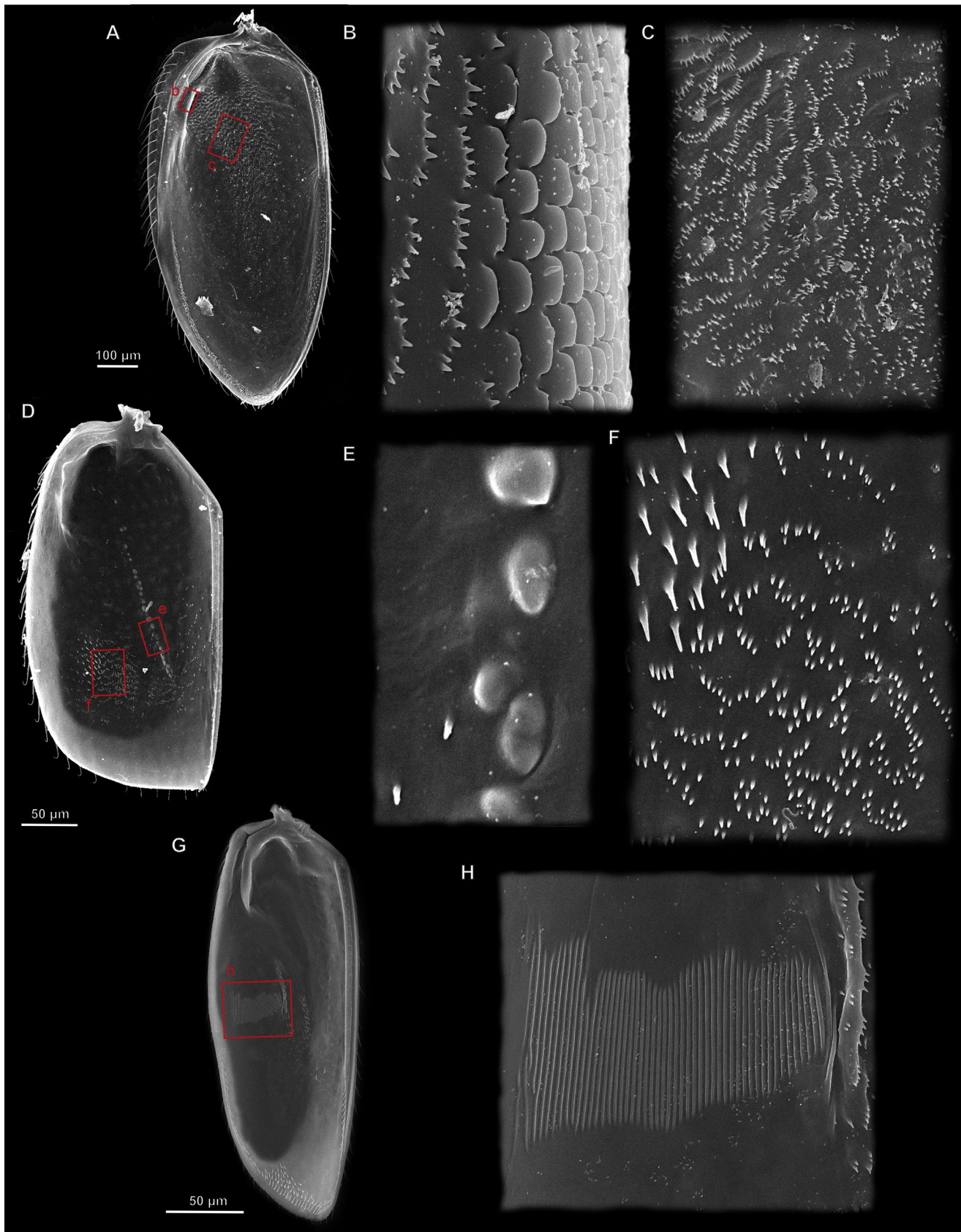


Fig. 8. Ventral surface of elytron in Ptiliidae (Coleoptera), SEM micrographs. (A–C) *Sindosium*, (D–F) *Nephanes*, (G, H) *Primorskiella*.

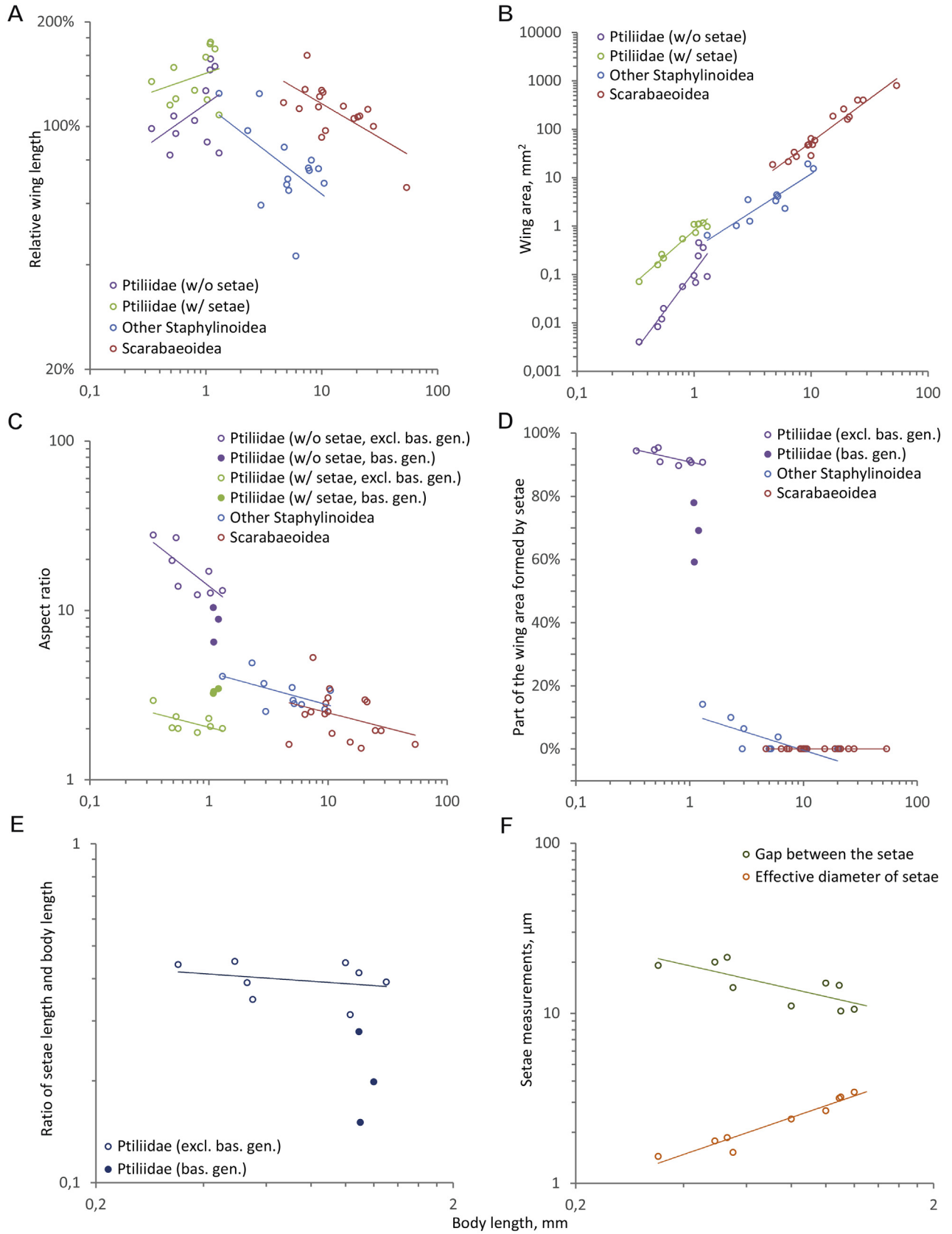


Fig. 9. Regressions of different parameters of coleopteran wings on body size: (A) relative wing length (as proportion of body length); (B) wing area with and without setae; (C) aspect ratio for wing area with and without setae for Ptiliidae excluding basal ptiliid genera (“early splits”), for basal ptiliid genera, for other Staphylinoidae, and for Scarabaeoidea; (D) part of wing area formed by setae for Ptiliidae excluding basal genera, for basal genera, for other Staphylinoidae, and for Scarabaeoidea; (E) ratio of length of setae and body length for Ptiliidae excluding basal genera and for basal genera; (F) gap between setae and effective diameter of setae.

are termed ptiloptery (Rohdendorf, 1949; Polilov, 2015). The degree of ptiloptery can differ considerably between miniature representatives of different insect groups: in most of the smaller dipterans, some of the very small hymenopterans (Trichogrammatidae), and some of the very small beetles (Corylophidae, Sphaeriusidae) the wing blades are relatively wide, while in other miniature insects, e.g., thrips, other hymenopterans (Mymaridae), and other beetles (Ptiliidae), the wing blade is very narrow and the marginal setae are much longer than the width of the wing (Polilov, 2016). It has been shown that in some microinsects the base of the wing forms a petiole, which contains no membranous part and can therefore be distinguished from the membranous wing blade: such structures have been described in Ptiliidae (Coleoptera) and in fore- and hindwings of some Mymaridae and Mymarommatidae and hindwings of some Trichogrammatidae (Hymenoptera). Ptiloptery is a synapomorphy of the ptiliid clade, and further miniaturization, accompanied by changes in the feather-like wings, was the principal trend in some lineages of this clade.

The degree of ptiloptery varies between representatives of Ptiliidae. Species of the early splits (*Nossidium*, *Motschulskium*, and *Sindosium*) have a relatively wide wing membrane and relatively short marginal setae, especially on the anterior margin; the setae occupy 60–80% of the total area of the wing. In other ptiliids the wing blade is strongly narrowed and the relative length of the marginal setae is distinctly increased. The proportion of the total area of the wing occupied by the wing blade decreases with size within this group from 10 to 5% (and therefore the area occupied by the setae, from 90 to 95%). By contrast, in wings of the larger species of Staphylinoidea and Scarabaeoidea the proportion of the area occupied by peripheral setae is close to 0, and only the smallest Staphylinidae and species of Hydraenidae can be close to 10%.

The degree of reduction of the venation also varies within Ptiliidae. Species of the early splits (*Nossidium*, *Motschulskium*, *Sindosium*) have two veins in the petiole (ScP + RA and CuA) and at least three veins in the wing blade (RA₄, RP₂, and MP₁₊₂). In most ptiliids the petiole consists of a single vein and the wing blade contains three veins. In the smallest ptiliids (Nanosellini), only two veins are retained in the wing blade (RA₄ and RP₂). This state is probably a synapomorphy of the tribe Nanosellini.

The homology of the venation in Ptiliidae is difficult to reconstruct, mainly due to the principal reorganization compared to wings of related groups of Coleoptera, but also due to the insufficiently studied venation of different representatives of Staphyliniformia and the almost complete lack of paleontological material. The nomenclature used in this study is based on published data on the venation of Staphyliniformia (Kukalová-Peck and Lawrence, 1993, 2004; Hansen, 1997), and on our hypothetical series of changes in the wing venation with decreasing body size (Fig. 2). A comparison of the nomenclature of the venation used by other authors is given in Table A2. All ptiliids are distinguished from larger representatives of Staphyliniformia (Hansen, 1997; Naomi, 1989; Fedorenko, 2009) by the absence of the anal and cubital zones, reduction of the number of branches in the radial and medial stems, absence of the pterostigma, and absence of ScA. In all Ptiliidae ScP is fused with RA; such a fusion has also been reported in some of the smaller staphylinoids of other families (Naomi, 1989). In the smallest ptiliids, MP₁₊₂ is also missing, and CuA is absent or fused with ScP + RA. A reduced number of veins is a common pattern found in microinsects, with only one vein preserved in some of them (Polilov, 2016).

Sections of ptiliid wings show that only the petiole and the base of RA₄ have a structure typical of insect wing veins (i.e., with a cavity inside). The other veins are only pigmented swellings of the cuticle, raising the question how such wings are unfolded (see below).

4.2. Morphometric parameters

The obtained values of the aspect ratio for ptiliid wings are relatively low. However, since their wings have a peculiar geometry (presence of the petiole, large area occupied by setae), direct comparison of this parameter with values obtained for membranous (non-ptiliid) wings of other beetles would have been inadequate.

The allometric decrease in the proportion of the area occupied by the petiole and wing blade in ptiliids is associated with the disproportionate decrease in the lateral load on the wing during flight with decreasing body size. The drag force that acts on the wing depends on the area, angle of attack, and velocity squared. The area of the wing, as we have shown, changes allometrically. The average wing velocity squared, as a result of decreasing wing length, also decreases at a higher rate than body size.

4.3. The setal fringe of the wings and flight

The division of the setal fringe into three zones in all the studied genera except for the basal three is probably correlated with the folding of the setal fringe at rest.

The marginal setae of ptiliid wings differ from similar setae of all other microinsects in the presence of cuticular outgrowths (Fig. 6) and in the capacity of folding along the wing membrane (Fig. 7). In all studied microinsects except Ptiliidae, the microrelief of the setae is smooth, without outgrowths (Polilov, 2016). Among other microinsects, only thrips and miniature beetles of other families (e.g., Corylophidae) are capable of folding the wing setae, in contrast to the studied microhymenopterans (Mymaridae and Trichogrammatidae), in which this capacity is lacking (Polilov, 2016).

The functioning of feather-like wings of microinsects remains incompletely studied, but studies using scaled models (Sunada et al., 2002; Hansen and Tiselius, 1992) and computational fluid dynamics (Jones et al., 2016; Santhanakrishnan et al., 2014) have provided a theoretical foundation for the main features of the aerodynamics of such wings. One important parameter is the leakiness, which characterizes the capacity of setae to let air pass through them. This parameter depends on several factors: the diameter of the setae and the gap between them, the velocity of the flow, and the Reynolds number (Cheer and Koehl, 1987). Our data on the morphology of ptiliid wings largely agree with the principles revealed in experiments on models.

The relative area of the wing in Ptiliidae is typical of staphylinoid beetles. The elevation and slope of the regression line for Ptiliidae do not differ significantly from those of reference groups, which may reflect the low value of leakiness of their wings. Theoretically, at Reynolds numbers of the order of 10, leakiness is so small that the flow around a feather-like wing is almost not different from that around a membranous wing (Lee and Kim, 2017). Therefore, miniaturization makes the feather-like wing functional, and the smaller the body size, the more beneficial it is to use such wings.

The morphology of feathered wings sets rather strict limits on the maximum body sizes that would make flight beneficial in terms of energy or possible at all, which is also confirmed by the allometric patterns we have obtained.

The decreasing ratio of the gap between setae and their diameter (G/D) at increasing body size in ptiliids is probably required for retaining sufficiently low leakiness. Increasing Reynolds numbers are compensated by changes in the structure of the wing: the number of setae and their density are greater in larger ptiliids. The density of the setae has a limit, which is determined by the limited space available on the margin of the wing blade, and by the minimum diameter that can provide for a sufficient level of elastic deformations.

The setae are usually radially oriented on the wing, especially in the apical portion. As a result of this geometry, the gap between the setae increases proportionally to their length. Therefore, there is some upper limit of effective length of the setae, which makes the leakiness at the external margin of the fringe (near the apices of the setae) sufficiently low. The higher the Reynolds number, the smaller is the effective length of the setae, as shown by the increase in their relative length with decreasing body size in the studied ptiliids.

The limit to the length of the setae can be extended by increasing the effective diameter. This function is performed by the secondary outgrowths, the size of which increases towards the apices of the setae. This highly efficient solution decreases the leakiness of the wing, only slightly increasing its weight and moment of inertia, compared to wings with wider setae without secondary outgrowths.

4.4. Folded wings in Ptiliidae

Ptiliids differ from other microinsects not only in the degree of ptiloptery, but also in the ability to fold their wings and hide them under the protective cover of the elytra. In the early splits (*Nossidium*, *Motschulskium*, and *Sindosium*) the wings are folded asymmetrically, in a manner similar to Staphylinidae (Saito et al., 2014). The other ptiliids fold their wings symmetrically, bending them along four (or five, in *Ptenidium*) lines that are almost perpendicular to the longitudinal axis of the wing. Various elements of the microsculpture of the elytra, together with different elements of the pterothoracic and abdominal dorsum participate in the complex mechanism of folding and unfolding the feather-like wings, remarkably with a single cavity in the petiole, and no cavities within most of the veins in the wing blade, except for a short cavity in the proximal portion of RA₄. Therefore, the folding and unfolding of the wings largely depend on the flexibility and resilience of the cuticle, and can only partly be guided by changes in the pressure of the haemolymph within the veins. This specific mechanism remains largely unclear and requires further study.

5. Conclusions

Ptiloptery is a conspicuous result of convergent evolution found in miniature representatives of different insect orders (Coleoptera, Hymenoptera, and Thysanoptera). All winged species of the family Ptiliidae, which contains the smallest known free-living insects, are ptilopterous. Their peculiar wing apparatus is a distinct apomorphy of the family and important adaptation to minute body size.

The ptiliid wing is strongly simplified (especially in terms of venation) in comparison with wings of larger representatives of related groups and, at the same time, has important novel adaptive features that enable efficient flapping motion in minute insects.

The considerable difference in wing structure in genera that represent “early splits” of ptiliids, compared with all other genera (larger wing blades with shorter peripheral setae, greater number of veins, and several other characters) reflects a major evolutionary transition in the family. The modified wings also provide a useful set of features for identification and also for taxonomic and phylogenetic purposes.

Ptiloptery has provided for the evolutionary success of miniaturization among different groups of insects largely because of the benefits of using a feather-like wing at extremely low values of the Reynolds number. At the same time, ptiloptery is efficient only within a limited range of physical conditions and is probably the morphological factor that prevents microinsects from secondarily evolving larger bodies.

Author contributions

AAP designed the study. AAP and NIR collected the material. NIR made the micrographs. NIR, AAP, and PNP analyzed the morphological aspects of the results. SEF analyzed the aerodynamic aspects of the results. PNP, AAP, and SEF wrote the draft of the manuscript and revised it. All authors have read and approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.asd.2019.01.003>.

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