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The 'twins' and the 'bachelor', new potential synapomorphies inside the Cholevinae (Coleoptera: Leiodidae)

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The fine morphology of the tarsus has recently been shown to provide phylogenetic signal at several supraspecific levels in leiodid beetles, as well as in other insects. We here include another system, namely, the spines at the apical margin of the tarsomeres and associated structures. The tribe Ptomaphagini, with a Holarctic-Neotropical-Oriental distribution, has been characterized by having a comb of equal, flat spines around the apex of the tibiae of all legs, with a row of spines extending along the outer edge of the protibia in some genera (which has also been reported in the Eucatopini). We here recognize (as a new synapomorphy for the tribe) that special, strong spines, forming a loose comb, also appear at the apex of the first three or four tarsomeres of the Ptomaphagini. Special features appear on the apical margin of the mesotarsomeres: a long and thin seta, here called the 'bachelor', appears between two spines at the external-lateral-ventral face of the first tarsomeres, whereas a group of two (three in some cases) special spines (with the apex bent, facing opposite to each other), here called the 'twins', appears on the opposite, internal-lateral-ventral face of the first three (or sometimes two) tarsomeres. We found the 'twins' were also present in the Anemadini: Eunemadina and Nemadina, and the Eucatopini; the 'bachelor seta' seems to also occur in the Anemadini: Nemadina.

ADDITIONAL KEYWORDS: Anemadini – beetles – Eucatopini – Ptomaphagini – tarsal morphology.

INTRODUCTION

The family Leiodidae, placed in the staphylinoid group of beetle families, includes more than 4200 described species (e.g. Newton, 2019) and is characterized (with a few exceptions) by having antenna with an "interrupted" five-segmented club (antennomere 8 being smaller than 7 and 9) and a periarticular gutter on antennomeres 7, 9 and 10 (e.g. Newton, 2016). Recent studies using features of the legs such as the pretarsal structure and the shape and distribution of tenent setae (Antunes-Carvalho & Gnaspini, 2016; Gnaspini *et al.*, 2017a, b) have shown that those are valuable systems for the detection of phylogenetic signals to characterize supraspecific groups. The presence of tenent setae on both pro- and mesotarsomeres of males is considered to be the plesiotypic state in leiodids, and their absence in the mesotarsus occurs several times within the family (Gnaspini *et al.*, 2017b).

The tribe Ptomaphagini (in the subfamily Cholevinae), known from the Holarctic, Neotropical and Oriental regions, is diagnostically characterized by having a comb of equal, flat spines around the apex of the tibiae of all legs, a reduced male genital segment and an aedeagus lacking a basal lamina. Both sexes have slender (i.e. not dilated) meso- and metatarsi (e.g. Jeannel, 1936; Gnaspini, 1993, 1996; Newton, 1998). The Ptomaphagini was previously considered to be close to the Neotropical tribe Eucatopini [e.g. Jeannel (1936), and see discussion in Gnaspini (1994)], because of the presence of the tibial comb of spines (and the lack of a tegmen on the aedeagus), but recent evidence places it closer to the tribes Leptodirini and Sciaphyini, and Eucatopini+Oritocatopini as early branches in the Cholevinae (Antunes-Carvalho & Gnaspini, 2016; Antunes-Carvalho *et al.*, 2019). In fact, even though Jeannel (1936) and followers used the comb

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of spines to unite these two tribes, they recognized combs and/or crowns of spines at the apex of the tibiae in other groups (such as the Paracatopina, Cholevina and some Leptodirini) [see also discussion in Gnaspini *et al.* (2020)].

While studying species of the Neotropical genus *Adelopsis* Portevin, 1907 (Cholevinae: Ptomaphagini) in search of additional characters which would help with species recognition, we detected special setae and spines exclusively on the mesotarsomeres in both males and females. We then increased our analysis to other genera of the Ptomaphagini and to other tribes and subfamilies of the Leiodidae. Since male mesotarsomeres of ptomaphagines lack tenent setae, we decided to determine whether the presence of the special features detected was related to the lack of tenent setae on mesotarsomeres, or if their presence could represent historic/phylogenetic signals. Therefore, we analysed other leiodids that lack tenent setae on their mesotarsomeres, as well as cholevines which do have expanded mesotarsi for comparison (to verify if the presence of tenent setae would impede the occurrence of the special features studied).

We studied the fine morphology of the apical margin of the mesotarsomeres to address these questions, make comparisons and describe new features.

MATERIAL AND METHODS

Methods used here follow the ones previously used in the group (e.g. Antunes-Carvalho & Gnaspini, 2016; Gnaspini *et al.*, 2017a, b). We used material analysed in previous studies (as the ones cited above) and we also used additional specimens (Table 1). For the selection of the taxa analysed, in addition to ptomaphagines for the study of the mesotarsal features, we studied at least one representative of subtribes (in the case of the Cholevinae) or tribes (in the case of non-cholevines) where Gnaspini *et al.* (2017b) recorded the absence of tenent setae on male mesotarsi. We also studied a few taxa with expanded mesotarsi with tenent setae for comparison, namely *Anemadus* Reitter, 1885, *Catops* Paykull, 1798, *Eunemadus* Portevin, 1914, *Paracatops* Portevin, 1907 and *Platycholeus* Horn, 1880. When addressing taxonomic supraspecific names, we followed recent classifications (e.g. Newton, 1998). The dissected legs of additional specimens were mounted on metallic stubs using carbon adhesive pads and sputter-coated with gold. The specimens were examined using a Zeiss DSM 940 scanning electron microscope at Instituto de Biociências, Universidade de São Paulo (IBUSP). Except when noted, the left leg was illustrated.

Considering morphological terminology, when referring to structures placed on the lateral margin/face of a leg article, we add a reference to either 'internal' or 'external' as follows. In the resting position, the dorsal face of the femur (which is frequently flat and with a wide surface) stays parallel and in touch with the ventral surface of the body, and is therefore referred to as the 'internal (or inner) face'. The internal face of the femur can be easily recognized by being mostly glabrous (except for near the margins) and having visible articulation to the trochanter (Fig. 1A). In opposition, the 'ventral face' of the femur (densely covered with setae/trichomes) is referred to as the 'external (or outer) face' (Fig. 1B). The 'ventral face' of the tibia (and tarsus) is the one which bears the articulation to the femur; whereas, consequently, the opposite one is the 'dorsal face' (Fig. 1A, B, 'vf' and 'df', respectively). As a result, the 'internal lateral face' of the tibia (and tarsus) lays parallel to the 'internal (= dorsal) face' of the femur; whereas the 'external lateral face' of the tibia (and tarsus) lays parallel to the 'external (= ventral) face' of the femur. This is summarized in Figure 1C, a hypothetical cross-section of a tarsomere of a left leg.

RESULTS

Crown of spines on tarsomeres

Plesiomorphically, ptomaphagines (and leiodids as a whole) have five tarsomeres on all legs, both in males and females. We recognize that strong, irregularly-sized spines organized in a loose periapical crown appear on the first four tarsomeres of all legs of ptomaphagines, more densely on the ventral and ventrolateral margins (frequently less organized and with a small number of strong spines on the fourth tarsomere) (e.g. Figs 2A, B, 4A, B, stars).

The pattern seen in the Ptomaphagini does not occur in the Eucatopini (Fig. 6), although a few strong spines may appear at the apical margin of the first tarsomeres, mainly as a pair on the ventral margin and/or as a small series on the outer margin (Fig. 6C, stars). That pattern is also lacking in all other leiodids studied (Figs 7, 8C, D, 9B, C, E, 10B, C, E, G, I, 11A, C, E, H, I, L; Supporting Information, Figs S4A, C, S5A, C, E). The apical crown of spines on tarsomeres I–IV is therefore recognized here as an additional synapomorphy of the Ptomaphagini (as summarized in Table 1).

The 'twin spines' on mesotarsomeres

In the Ptomaphagini, the mesotarsomeres of both males and females bear a pair of special spines (here called the 'twin spines'), placed among the apical crown of spines (e.g. Figs 2B, C, 3A, 'tw'). The tips of the 'twin spines' are pointed and displaced to the side – in the pair, the pointed apices are opposite each other (as in Fig. 3A, circle). Their inner faces are close to each

Table 1. List of species of the Leiodidae examined in this study, with a summary of the main morphological characteristics observed on the midleg among the studied taxa. Taxa marked with ** have mesotarsomeres expanded, with tenent setae; the number that follows indicates how many tarsomeres are expanded. Note: the Pseudoliodini studied have four mesotarsomeres; the other taxa have five mesotarsomeres. TW denotes which mesotarsomeres bear 'twin spines'; BS denotes which mesotarsomeres bear 'bachelor setae'; AS denotes if mesotarsomeres have ('Y') [or not ('N')] an apical crown of spines. See text for explanation of characters and character states and for discussion

Systematic assignment		Species	Provenance	TW	BS	AS	Figure no.
Camiarinae	Agyrtodini	*Agyrtolasia calliptera* Szymczakowski†	Australia: NSW: Styx River State Forest; near Wollomombi	-	-	N	Supporting Information, S5A, B
		Chiliopelates pictus (Jeannel)†	Chile: Osorno: Aguas Calientes	-	-	N	Supporting Information, S5C, D
		Dictydiella turneri Jeannel†	South Africa: Western Cape, Dal van Varings.	-	-	N	11A, B
	Camiarini	*Baeosilpha rufescens* Broun†	New Zealand: Wellington Region: Karori Wildlife Sanctuary	-	-	N	11C, D
	Neopelatopini	*Catopsolius laevicollis* Sharp†	C.E. Clarke collection; AMNZ 27281	-	-	N	11E, F
Cholevinae	Anemadini Anemadina	*Anemadus italicus* Zoia **3	Italy: Calabria	-	?	N	8D
	Anemadini Eunemadina	*Dissochaetus vanini* Gnaspini§,†	Brazil: São Paulo: Iporanga	1–4	-	N	7A, B
		Eunemadus chilensis Portevin† **1	Chile: Osorno: near Puaucho	2–3	-	N	7C
		Pseudonemadus (*s.s.*) *cheesmani* (Jeannel)§	Papua New Guinea: Morobe	1–4	-	N	Supporting Information, S3
	Anemadini Nemadina	*Nemadus colonoides* (Kraatz) [female]	Czech Republic: Moravia: Lednice	1–3	>1–2	N	7D, 8A
	Anemadini Paracatopina	*Paracatops alacris* (Broun)† **2	New Zealand: Auckland: Duck Creek Scenic Reserve	-	-	N	8B, C
	Cholevini Catopina	*Catops fuliginosus* Erichson§,† **1	Italy: Friuli Venezia Giulia	-	-	N	9A, B
	Cholevini Cholevina	*Catopsimorphus* (*s.s.*) *orientalis* Aubé§,†	Greece: Peloponnese	-	-	N	9C, D
		Choleva (*s.s.*) *oblonga* Latreille§	Italy: Lombardy	-	-	N	Supporting Information, S4A, B
		Nargus (*s.s.*) *badius* (Sturm)§	Italy: Liguria	-	-	N	Supporting Information, S4C, D
	Eucatopini	*Eucatops (Napocatops) giganteus* Salgado†	Ecuador: Napo: near Baeza	-	-	N	6B, C
		Eucatops sp. 1§	French Guiana: Saint Laurent du Maroni	1–3?4	-	N	6A

Table 1. Continued

Systematic assignment		Species	Provenance	TW	BS	AS	Figure no.
	Leptodirini Bathysciotina	*Bathysciotes khevenhuelleri tergestinus* Müller[§]	Italy: Friuli Venezia Giulia	-	-	N	10A, B
	Leptodirini Leptodirina	*Leptodirus hochenwarti* Schmidt[§]	Italy: Friuli Venezia Giulia	-	-	N	10C, D
	Leptodirini Pholeuina	*Diaprysius serullazi* Peyerimhoff[†]	France: Ardeche: Grotte. des Assiettes	-	-	N	10G, H
	Leptodirini Platycholeina	*Platycholeus opacellus* Fall[†] ** [1]	USA: CA: Amador Creek, Peddler Hill	-	-	N	10E, F
	Oritocatopini	*Afrocatops* sp.[†]	South Africa: Natal; near Estcourt	-	-	N	9E, F
	Ptomaphagini Ptomaphagina	*Adelopsis leo* Gnaspini	Brazil: São Paulo: Guapiara	1–3	1–3	Y	1–3
		Amplexella dimorpha Gnaspini[§,†]	Venezuela: Aragua	1–3	1–3	Y	4A, B
		Parapaulipalpina sp.[§,†]	Venezuela: Bolívar	1–3	1–4	Y	Supporting Information, S1A, B
		Paulipalpina claudicans (Szymczakowski)[§,†]	Brazil: Santa Catarina	1–3	1–4	Y	Supporting Information, S1C, D
		Ptomaphagus (*s.s.*) *divaricatus* Jeannel	Italy: Savogna	?1–2	-	Y	5C, D
		Ptomaphagus (Adelops) brevior Jeannel[†]	USA: OK: Latimer County.	1–2	1	Y	5A, B
		Ptomaphagus (*Adelops*) *meximontanus* Peck[§]	Mexico: Querétaro	1–2	1	Y	-
		Ptomaphagus (*Appadelopsis*) *cumberlandus* (Peck)	USA: Alabama	1–3	1–4	Y	5E; Supporting Information, S2A, B
		Ptomaphagus (*Echinocoleus*) sp. [female]	USA: Florida: Okaloosa County.	1	1–4	Y	Supporting Information, S2C, D
	Ptomaphagini Ptomaphaginina	*Ptomaphaminus chapmani* (Peck)[§,†]	Malaysia: Sarawak	1–4	1–4	Y	4C, D
	Sciaphyini	*Sciaphyes sibiricus* (Reitter)[†]	Russia: Siberia	-	-	N	10I, J
Coloninae	-	*Colon (Mesagyrtes) hirtale* (Broun)[†]	New Zealand: Auckland Region: The Noises Islands; Otata Island.	-	-	N	11G, H

Table 1. Continued

Systematic assignment		Species	Provenance	TW	BS	AS	Figure no.
Leiodinae	Estadiini	*Dietta* sp.[†]	Madagascar: Fianarantsoa: near Ranomafana	-	-	N	11I, J
	Pseudoliodini	*Neohydnobius* sp.[†]	Chile: Valdivia: near Panguipulli	-	-	N	Supporting Information, S5E, F
		Pseudcolenis grandis (Portevin)[†]	Japan: Gifu: near Osaka	-	-	N	11K, L

[§]Specimens used in Antunes-Carvalho & Gnaspini (2016).
[†]Specimens used in Gnaspini *et al.* (2017b).

other, generally touching, sometimes appearing to be fused together, but they are not. The 'twin spines' are articulated to a single elevation on the integument, as if their sockets are fused (Fig. 3A, ellipse), differing from the rest of the periapical spines, which are singly articulated to their own sockets (e.g. Fig. 2A, B, stars). They are obliquely placed towards the ventral face of the tarsomeres (Figs 2C, 3A, 'tw') and not positioned perpendicularly (or almost perpendicularly) to the apical rim of the tarsomere, as occurs with the other periapical spines (e.g. Fig. 2A, B, stars).

The 'twins' are placed at the inner 'corner' of the ventral face (as in Fig. 1C, 'icv'), between a (frequently) long spine (outward) and two (frequently) small spines (inward) (Fig. 2B, C, 'tw'). When analysing different species of *Adelopsis*, we observe that the size of the inner spines may vary, and may be useful for species recognition. The 'twin spines' were not identified on the protarsus or metatarsus (Fig. 3C).

In *Adelopsis*, the 'twins' appear on the first three mesotarsomeres, but not on the fourth (Fig. 3A), and obviously not on the fifth, last tarsomere, which bears the claws. The same occurs in *Amplexella* Gnaspini, 1996 (Fig. 4A), *Parapaulipalpina* Gnaspini, 1996 (Supporting Information, Fig. S1A) and *Paulipalpina* Gnaspini & Peck, 1996 (Supporting Information, Fig. S1C). In *Ptomaphaminus* Perreau, 2000, the 'twins' appear on the first four mesotarsomeres (Fig. 4C).

In *Ptomaphagus* Hellwig, 1795 (subgenera *Adelops* and *Appadelopsis*), the 'twins' are 'triplets', and the one in the middle has its tip bent towards the ventral midline of the mesotarsomere (Fig. 5A, E, 'tw'). In the first subgenus, they appear on the first two mesotarsomeres, but not on the others (Fig. 5A), and in the second subgenus they appear on the first three mesotarsomeres and not on the fourth (Supporting Information, Fig. S2A). In the subgenera *Echinocoleus* and *Ptomaphagus*, it is not possible to be sure if the 'twins' are present (on the first two tarsomeres, and not on the next ones) because the spine tips are blunted (Fig. 5C; Supporting Information, Fig. S2C), but at least on the first mesotarsomere of *Ptomaphagus* (*s.s.*) there is a pair of spines that share a common elevation of the integument and seem to represent the 'twins' (Fig. 5C, '?tw'). In the Eucatopini, the 'twins' either appear placed away from the periapical spines (Fig. 6A – that on the third tarsomere seems a single spine) or are absent (Fig. 6B).

The 'twins' appear in the eunemadinan genera *Dissochaetus* Portevin, 1902 (four basal mesotarsomeres - Fig. 7A) and *Pseudonemadus* Portevin, 1914 [three, maybe four, basal mesotarsomeres (some spines that may represent 'the twins' are missing); Supporting Information, Fig. S3A]. In this group, they are articulated to the top of a tall elevation of the integument that projects from the

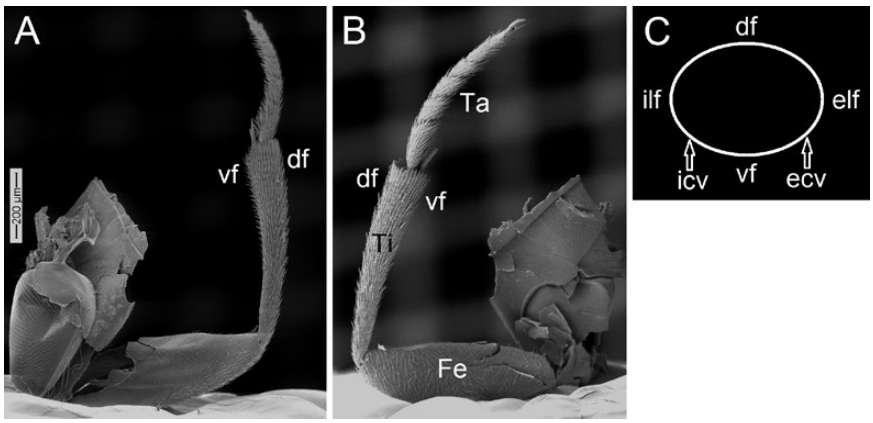

Figure 1. Midleg, Cholevinae: Ptomaphagini - *Adelopsis leo*. A, dorsal/internal view. B, ventral/external view. C, a hypothetical cross-section of a tarsomere of the left leg, showing what we refer to as the dorsal ('df'), internal lateral ('ilf'), ventral ('vf') and external lateral ('elf') faces of the tarsomere, as well as the inner ('icv') and external ('ecv') 'corners' of the ventral face of the tarsomere. Fe = femur; Ta = tarsus; Ti = tibia.

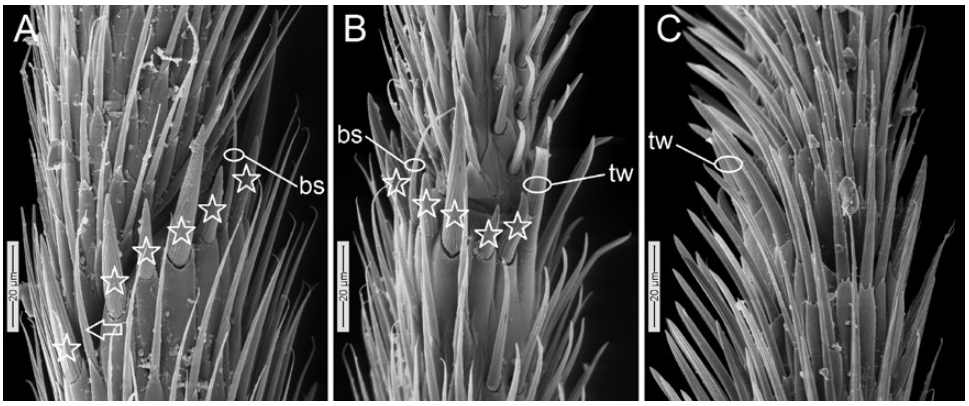

Figure 2. Mesotarsus, Cholevinae: Ptomaphagini - *Adelopsis leo*, articulation between tarsomeres I (below) and II (above). A, lateral-external view. B, ventral view. C, lateral-internal view. bs = 'bachelor seta'; tw = 'twin spines'; arrow = additional slender setae; stars = periapical spines of the apical crown of spines.

apical margin. In *Eunemadus*, which has tenent setae on the expanded first mesotarsomere, this tarsomere does not bear 'twins' (Fig. 7C); however, the next two seem to have 'twins' because we can recognize a pair of spines articulated to a single, projected elevation of the integument (as in the other genera), although their tips are not typically bent to the side (Fig. 7C), and the fourth mesotarsomere also lacks them. The same pattern described for *Dissochaetus* and *Pseudonemadus* was observed in *Nemadus* Thomson, 1867 (Anemadini: Nemadina), with the first three mesotarsomeres bearing 'twin spines' (Fig. 7D).

The 'twin spines' do not appear on the other leiodids examined here – when the first mesotarsomere(s) had tenent setae, we also examined the next one(s) to check for the presence of 'twins' (Figs 8B, 9A, C, E, 10A, C, E, G, I, 11A, C, E, G, I, K; Supporting Information, Figs S4A, C, S5, A, C, E). See summary in Table 1.

THE 'BACHELOR SETA' ON MESOTARSOMERES

In the Ptomaphagini, the mesotarsomeres of both males and females bear a single, slender, elongate seta (here called the 'bachelor seta') placed among the apical crown of spines at the external 'corner' of the ventral face (as in Fig. 1C, 'ecv') between two small spines (Fig. 2A, B, 'bs'). It appears on the first three mesotarsomeres (Figs 3B, 4B, D; Supporting Information, Fig. S1B, D, 'bs'). Other slender setae appear (singly or as a group) on the dorsal and outer-lateral apical margins of the mesotarsomeres (Fig. 2A, arrow); however, the 'bachelor' is specifically

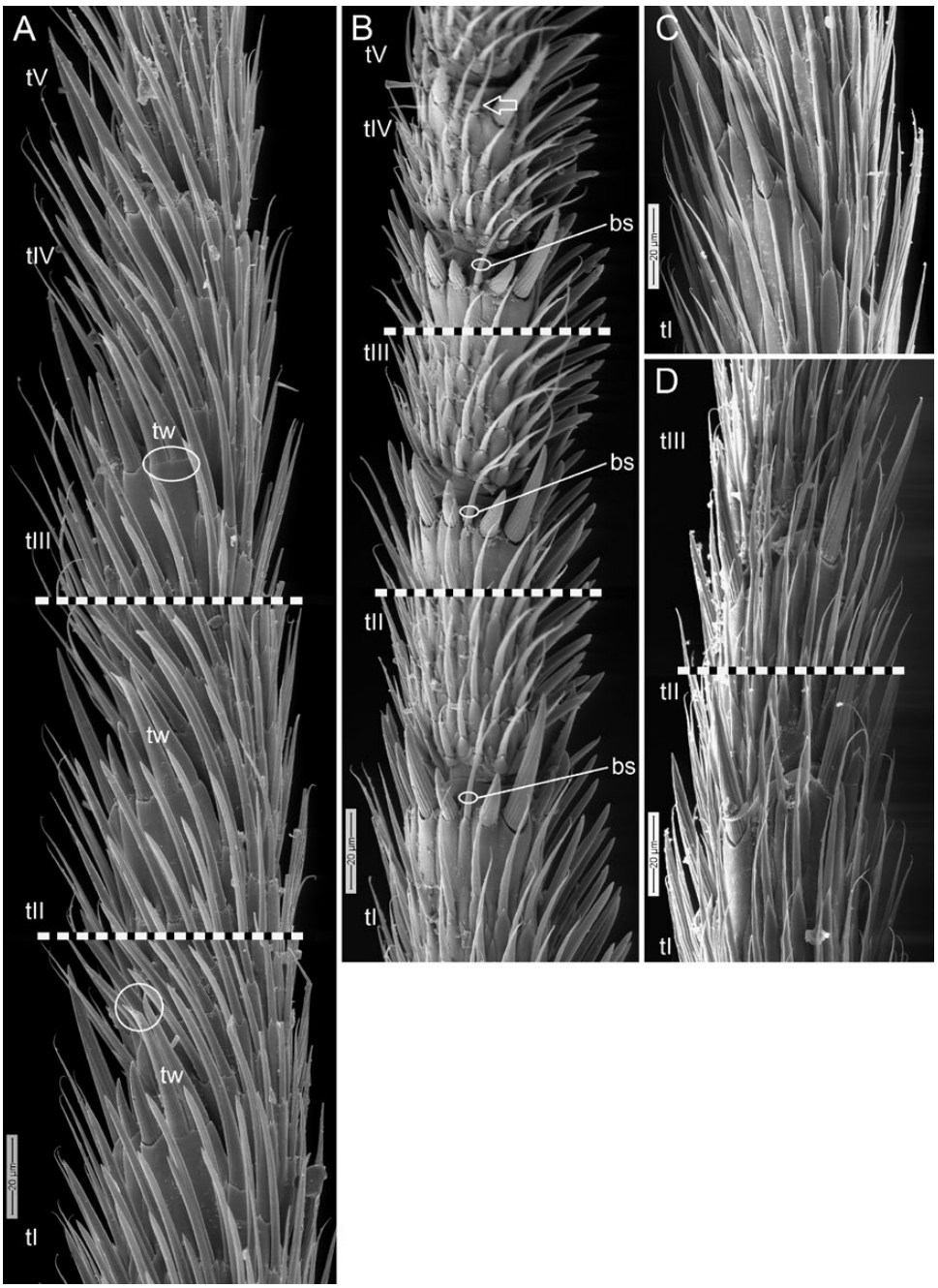

Figure 3. Meso- and metatarsus, Cholevinae: Ptomaphagini - *Adelopsis leo*. A, B, mesotarsus (part). C, D, metarsus (part). A, C, ventro-lateral-internal view. B, D, ventro-lateral-external view. tI-tV = first to fifth tarsomeres; bs = 'bachelor seta'; tw = 'twin spines'; circle = pointy apices of 'twin spines' facing opposite to each other; arrow = pair of setae at the apical margin of mesotarsomere IV; ellipse shows that the 'twin spines' are articulated to a single elevation on the integument.

placed within the apical comb of spines at the external 'corner' of the ventral face (Fig. 2A, B, 'bs'). In the case of the fourth mesotarsomere, the series of apical spines is loose, and a 'bachelor' cannot be clearly recognized. For instance, in *Adelopsis* and *Amplexella*, there is a pair of slender setae where a 'bachelor' would be expected (Figs 3B, 4B, arrow), and in *Parapaulipalpina*, *Paulipalpina*

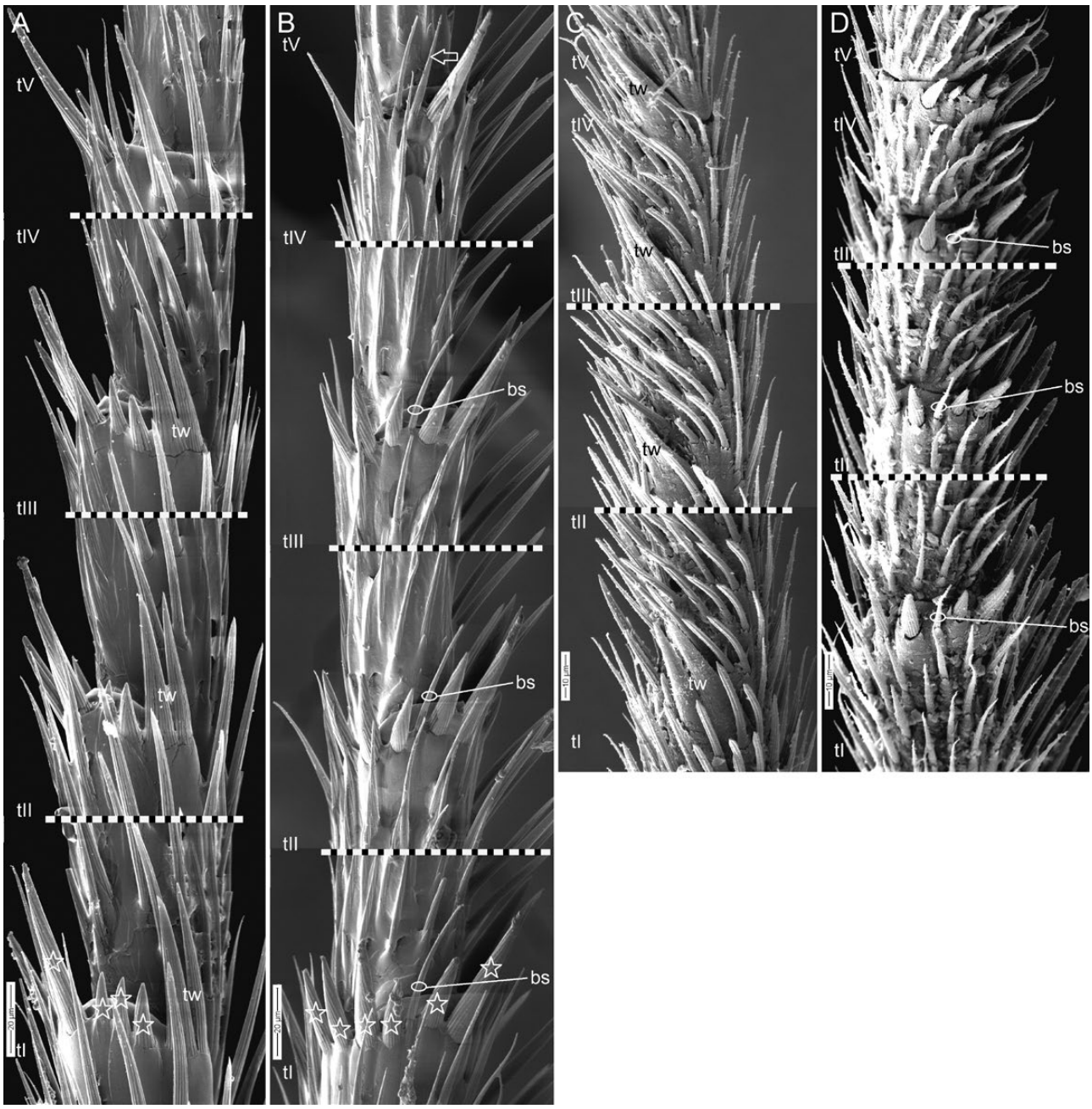

Figure 4. Mesotarsus, Cholevinae: Ptomaphagini. A, B, *Amplexella dimorpha*. C, D, *Ptomaphaminus chapmani*. A, C, ventro-lateral-internal view. B, D, ventro-lateral-external view. tI-tV = first to fifth tarsomeres; bs = 'bachelor seta'; tw = 'twin spines'; arrow = pair of setae at the apical margin of mesotarsomere IV; stars = periapical spines of the apical crown of spines.

and *Ptomaphaminus*, there is a single seta that might represent a 'bachelor' (Fig. 4D; Supporting Information, Fig. S1B, D). In *Ptomaphagus* (*Appadelopsis* and *Echinocoleus*), the 'bachelor' seta appears on all four first mesotarsomeres (Supporting Information, Fig. S2B, D); however, in *Ptomaphagus* (*Adelops*) a 'bachelor' seta appears only on the first mesotarsomere (Fig. 5B) and it was not recognized in *Ptomaphagus* (*s.s.*) (Fig. 5D). A 'bachelor seta' was not identified on the protarsus or metatarsus (Fig. 3D).

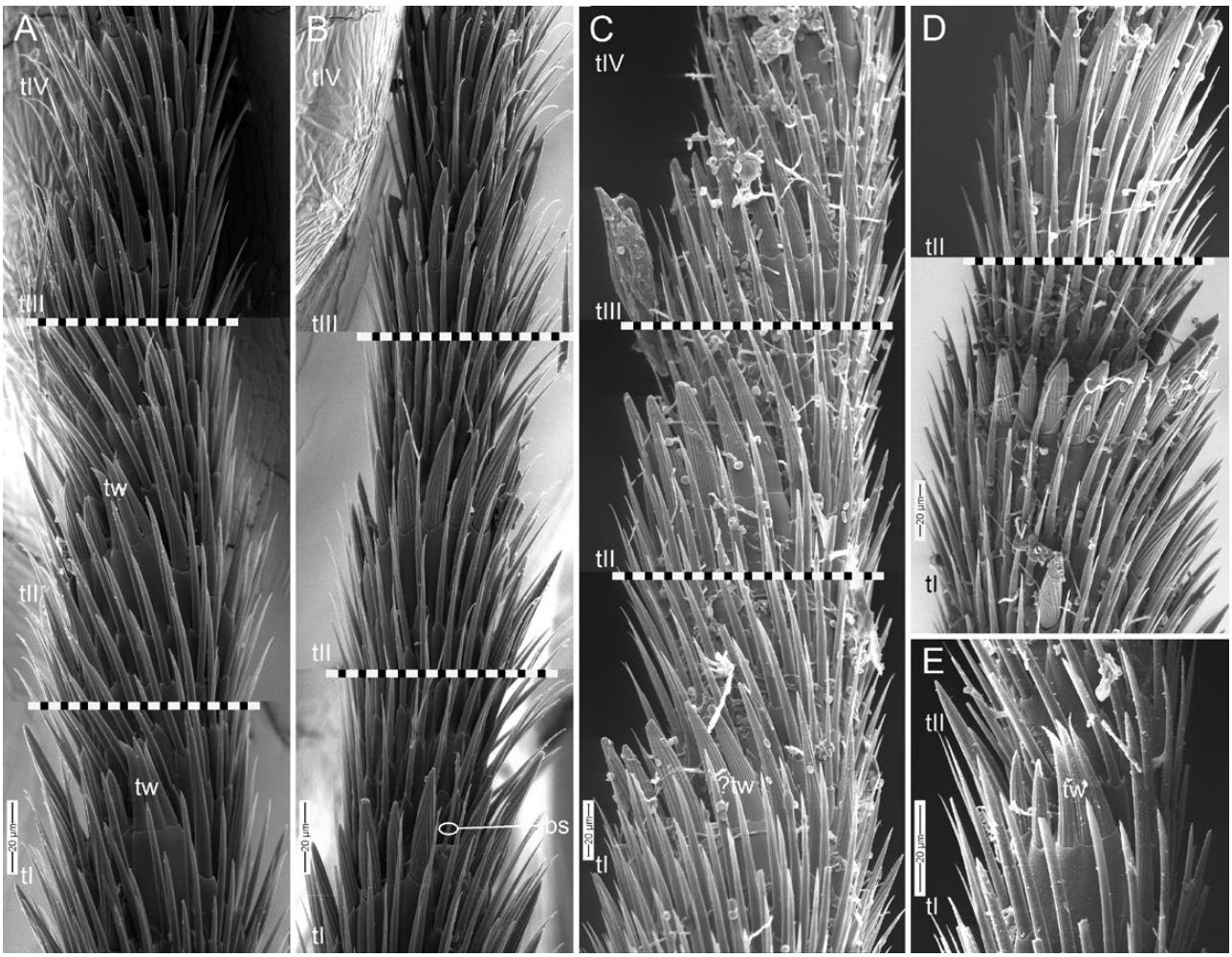

Figure 5. Mesotarsus, Cholevinae: Ptomaphagini. A, B, *Ptomaphagus* (*Adelops*) *brevior*. C, D, *Ptomaphagus* (*s.s.*) *divaricatus*, right leg, mirrored image. E, *Ptomaphagus* (*Appadelopsis*) *cumberlandus*. A, C, E, ventro-lateral-internal view. B, D, ventro-lateral-external view. tI-tIV = first to fourth tarsomeres; bs = 'bachelor seta'; tw = 'twin spines'.

As opposed to the 'twins', which we interpret to be special, modified integumental attachments (with a so far unknown function), the 'bachelor seta' is here interpreted to be a regular seta that remained as such while other setae of the mesotarsomere apical crown transformed into spines (at this point we have no hypothesis for how this took place). In this sense, the seta here called a 'bachelor seta' has the same shape (and probably the same function) as other slender setae that coat the integument, but it is here highlighted with a specific terminology because it has a specific position, placed between apical spines, exactly at the external 'corner' of the ventral face of the tarsomere.

In the Nemadina, a thin seta, similar to the one here defined as a 'bachelor seta', was observed on the apex of the first two tarsomeres (Fig. 8A), and unfortunately the next two tarsomeres were dirty and could not be observed. In turn, 'bachelor setae' were not observed in the Eunemadina (Fig. 7B; Supporting Information, Fig. S3B) or Eucatopini (Fig. 6C).

In the other leiodids here examined, even when setae appear on the apical margin of the mesotarsomeres, they cannot be recognized as 'bachelor setae', because there is no comb of apical spines (see *Crown of spines on tarsomeres*, above). However, in most cases, we may state a 'bachelor seta' does not exist at all, for instance,

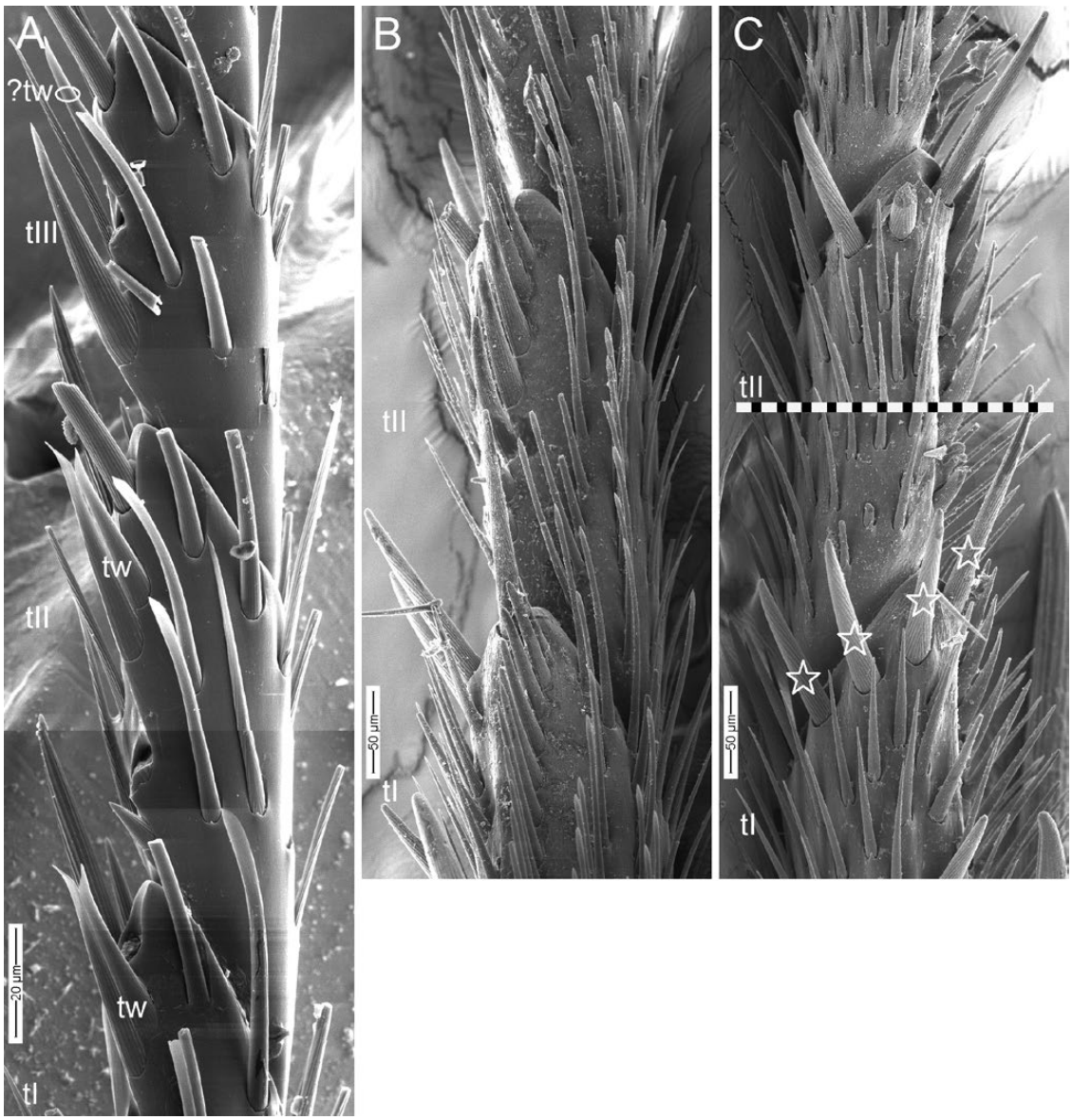

Figure 6. Mesotarsus, Cholevinae: Eucatopini. A, *Eucatops* sp. B, C, *Eucatops (Napocatops) giganteus*. A, B, ventro-lateral-internal view. C, ventro-lateral-external view. tI-tIV = first to fourth tarsomeres; tw = 'twin spines' (the one marked with ? might represent a twin spine, although being a single spine – see text); stars = periapical spines of the apical crown of spines.

when the setae/trichomes on the apical margin is of the same type of those covering the rest of the tarsomeres (Figs 8C, 9B, D, F, 10B, D, F, H, J, 11B, D, F, H, J, L; Supporting Information, Figs S4B, D, S5B, D, F). See summary in Table 1.

DISCUSSION

We here recognize a crown of spines on the apical margin of tarsomeres as a new synapomorphy for the Ptomaphagini. A short, outer loose row of spines also appears in the Eucatopini. Those tribes have been treated as related (e.g. Jeannel, 1936; Newton, 1998), because of the presence of apical combs on the tibiae, among other features (but see Gnaspini *et al.*, 2020). However, recent phylogenetic studies place them apart from each other, with the Eucatopini forming a clade with the Oritocatopini, which, together, are the sister group to the remaining Cholevinae (Antunes-Carvalho *et al.*, 2019).

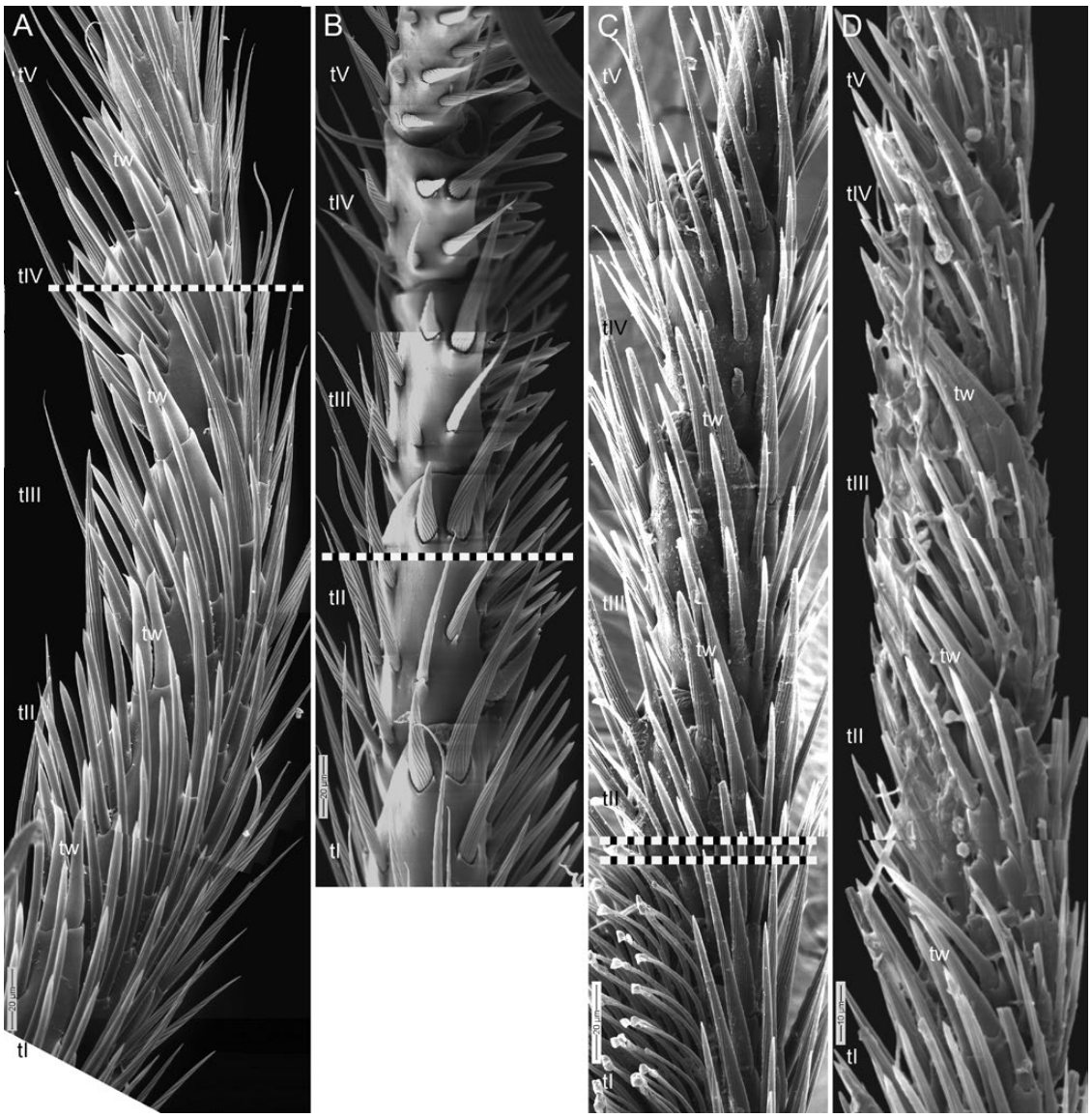

Figure 7. Mesotarsus, Cholevinae: Anemadini. A-C, Eunemadina. A, B, *Dissochaetus vanini*. C, *Eunemadus chilensis*. D, Nemadina – *Nemadus colonoides*, female. A, C, D, ventro-lateral-internal view. B, ventro-lateral-external view. tI-tV = first to fifth tarsomeres; tw = 'twin spines'.

On the other hand, additional resemblances were detected here (see Fig. 12, where our data were plotted onto the 'traditional' and recent phylogenetic frameworks of the Cholevinae). The Ptomaphagini, Eucatopini, Eunemadina and Nemadina have 'twin spines' on the mesotarsomeres. The Nemadina seems to have 'bachelor setae' on the mesotarsomeres, resembling those of the Ptomaphagini. In turn, 'bachelor setae' were not found in the Eucatopini or Eunemadina. Nevertheless, the features shared between these tribes invoke the previously hypothesized close affinity of the Ptomaphagini and Eucatopini (for the first time together with the Eunemadina and Nemadina), and this hypothesis should be tested in a new review of the phylogeny of the Cholevinae.

We should stress that *Eunemadus* (Eunemadina) has the first mesotarsomere expanded, bearing tenent setae, and 'twin spines' were not detected; however, the 'twins' were detected on the next, less expanded mesotarsomere, which, in turn, does not bear tenent

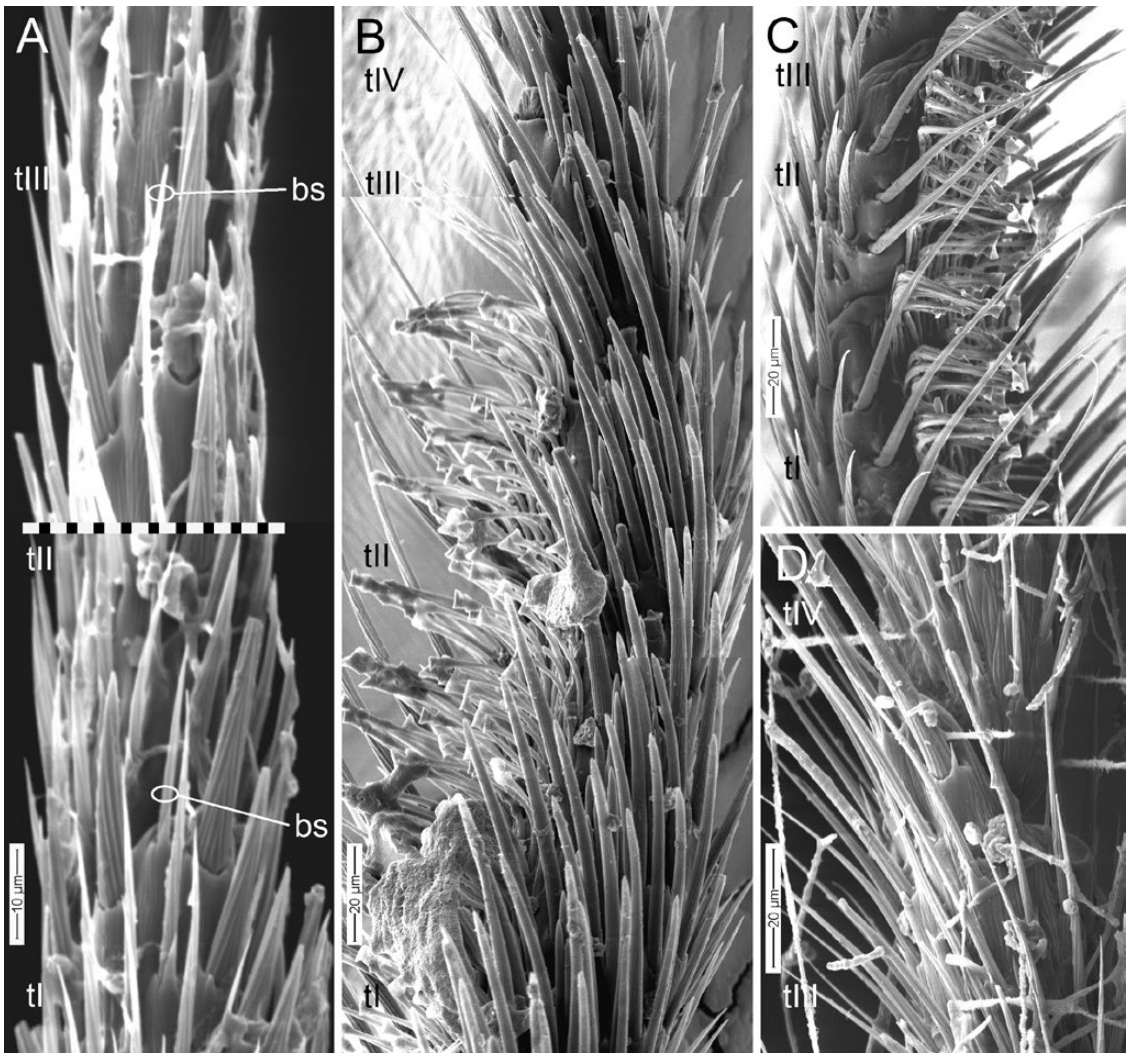

Figure 8. Mesotarsus, Cholevinae: Anemadini. A, Nemadina – *Nemadus colonoides*, female. B, C, Paracatopina – *Paracatops alacris*. D, Anemadina – *Anemadus italicus*. A, C, ventro-lateral-external view. B, D, ventro-lateral-internal view. tI-tIV = first to fourth tarsomeres; bs = 'bachelor seta'.

setae. Therefore, the presence of tenent setae seems to indeed impede the presence of the 'twin spines' even when the mesotarsus has the signal to present 'twin spines', which is probably the case with *Eunemadus*. However, the absence of expanded mesotarsi and of tenent setae does not necessarily imply the presence of 'twin spines', since they were not detected in many other Cholevinae and non-cholevine leiodids with slender mesotarsi.

In addition, the assemblage of subtribes in the Anemadini was tentative (Newton, 1998); they did not form a monophyletic group in the study by Antunes-Carvalho *et al.*, 2019), and the potential affinity of Eunemadina and Nemadina with Ptomaphagini and Eucatopini has never been proposed. The representatives of the other two tribes of Anemadini here studied (Anemadina and Paracatopina) have expanded mesotarsi with tenent setae and lack 'twin spines'. Unfortunately, we could not examine specimens of the remaining subtribe of the Anemadini (Eocatopina).

We recognize that the establishment of homology of integumental structures may sometimes be a difficult task, especially when 'setae' (i.e. elongate integumental structures, being either articulated or not) are analysed, since they may represent different

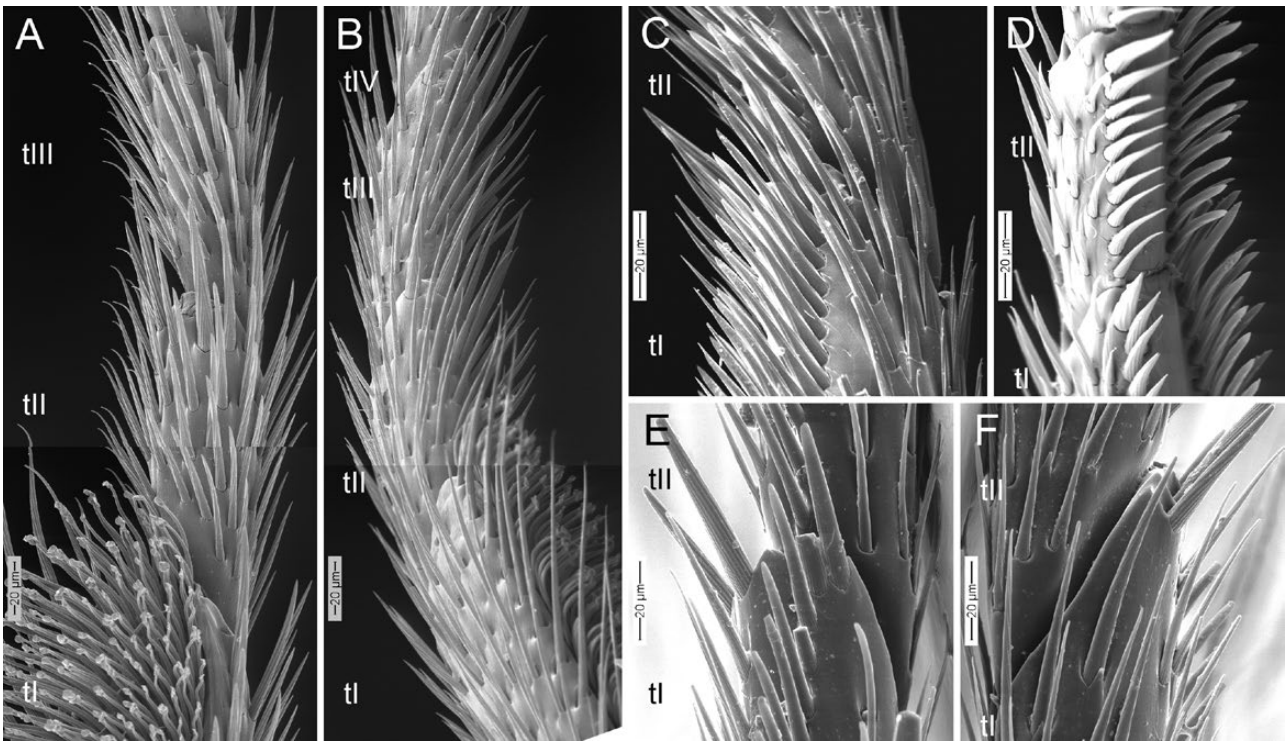

Figure 9. Mesotarsus, Cholevinae: Cholevini: Catopina and Cholevina, and Oritocatopini. A, B, *Catops fuliginosus*. C, D, *Catopsimorphus* (*s.s.*) *orientalis*. E, F, *Afrocatops* sp. A, C, E, ventro-lateral-internal view; B, D, F, ventro-lateral-external view. tI-tIV = first to fourth tarsomeres.

structures with different functions or may represent the same structures although showing different shapes, which may be further addressed with histological and/or transmission electron microscopy studies (see, e.g. Altner & Prillinger, 1980; Snodgrass, 1993; Chapman, 1998). Within the Leiodidae, the use of setae for taxonomic/phylogenetic purposes is uncommon (except in larvae). Part of the difficulty in using setal characters is due to the minute size of these structures, which makes the use of SEM essential. Recent comparative studies using SEM have indeed discovered new setal characters with phylogenetic value (e.g. Antunes-Carvalho & Gnaspini, 2016; Gnaspini *et al.*, 2017a, b; Antunes-Carvalho *et al.*, 2019; Gnaspini *et al.*, 2020). We think that the combined use of shape and position of such structures may help to interpret them (as a hypothesis) to the establishment of primary homology (*sensu* de Pinna, 1991). Therefore, we propose that the armature of the apical margin of the mesotarsomeres has proved to provide a valuable phylogenetic signal, and needs to be further explored and tested.

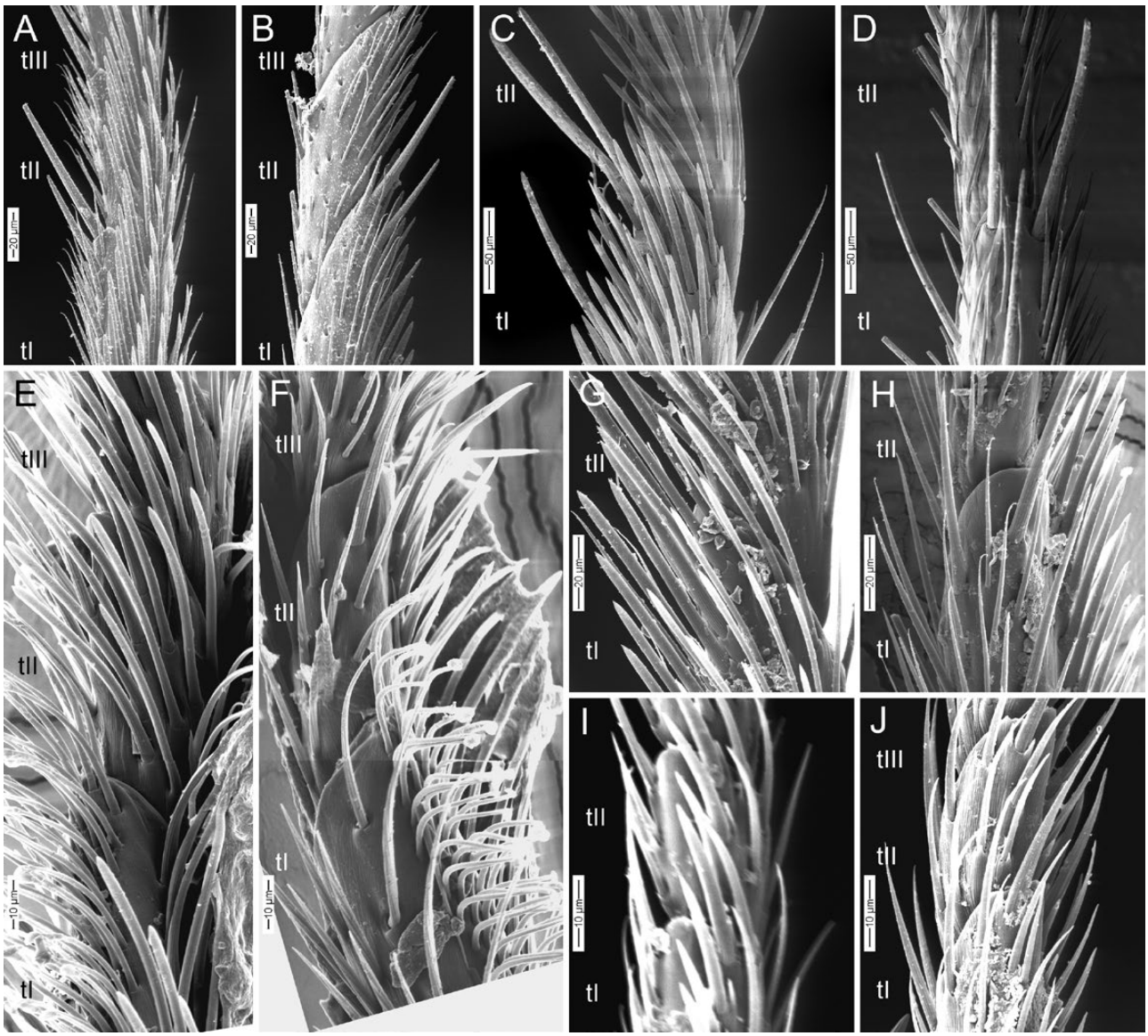

Figure 10. Mesotarsus, Cholevinae. A-H, Leptodirini. A, B, Bathysciotina – *Bathysciotes khevenhuelleri tergestinus*. C, D, Leptodirina – *Leptodirus hochenwarti*. E, F, Platycholeina – *Platycholeus opacellus*. G, H, Pholeuina – *Diaprysius serullazi*. I, J, Sciaphyini – *Sciaphyes sibiricus*. A, C, E, G, I, ventro-lateral-internal view. B, D, F, H, J, ventro-lateral-external view. tI-tIII = first to third tarsomeres.

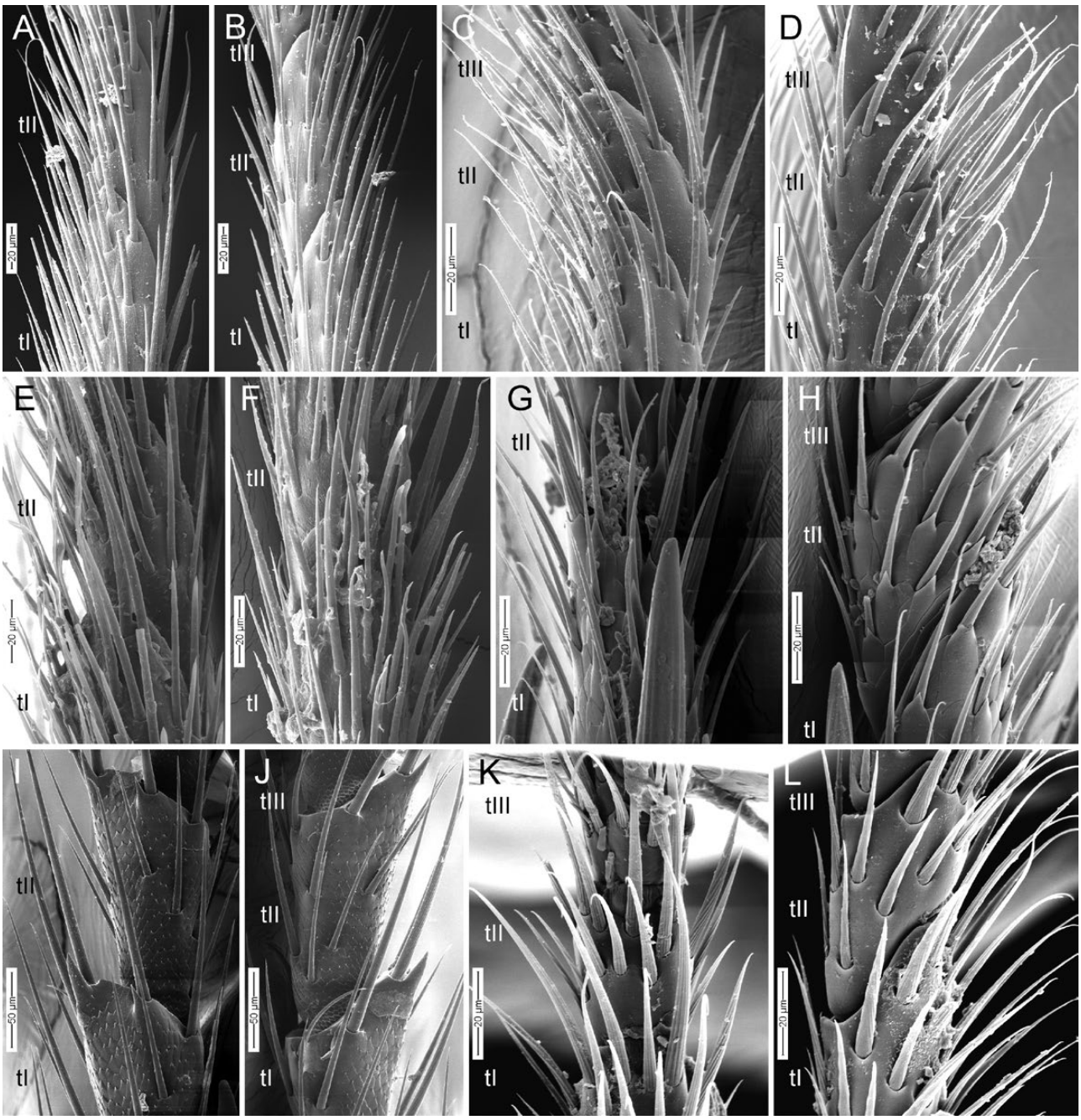

Figure 11. Mesotarsus. A-F, Camiarinae. I-L, Leiodinae. A, B, Agyrtodini – *Dictydiella turneri*. C, D, Camiarini – *Baeosilpha rufescens*. E, F, Neopelatopini – *Catopsolius laevicollis*. G, H, Coloninae – *Colon (Mesagyrtes) hirtale*. I, J, Estadiini – *Dietta* sp. K, L, Pseudoliodini – *Pseudcolenis grandis*. A, C, E, G, I, K, ventro-lateral-internal view; B, D, F, H, J, L, ventro-lateral-external view. tI-tIII = first to third tarsomeres.

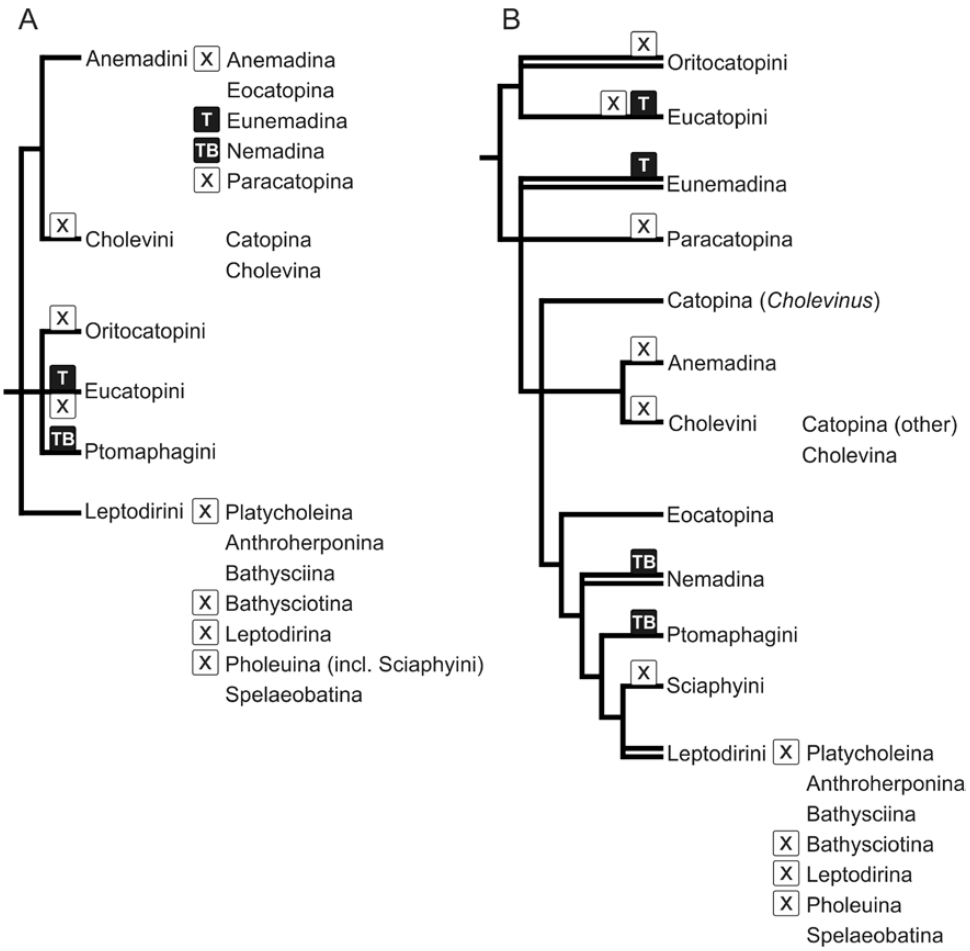

Figure 12. Data from Table 1 mapped on taxonomic diagrams of the subfamily Cholevinae. A, the 'traditional' division of the Cholevinae (based on Newton, 1998). B, phylogenetic analysis of the Cholevinae [based on Antunes-Carvalho *et al.* (2019: fig. 25) – in our figure we represent non-monophyletic taxa with a double line connection]. Taxa without an assigned symbol were not studied here. 'Anemadini' as a taxon is not represented in (B), because it was considered non-monophyletic in that study; however, its subtribes are represented. The black squares denote taxa where the features studied here were observed – 'T' denotes presence of 'twin spines' and 'B' denotes presence of a 'bachelor seta'; the open squares with a 'X' denote taxa where the features studied were not observed in the specimens analysed.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Mesotarsus, right leg (mirrored image), Cholevinae: Ptomaphagini. A, B, *Parapaulipalpina* sp. C, D, *Paulipalpina claudicans*. A, C, ventro-lateral-internal view. B, D, ventro-lateral-external view. tI-tV = first to fifth tarsomeres; bs = 'bachelor seta'; tw = 'twin spines'.

Figure S2. Mesotarsus, Cholevinae: Ptomaphagini. A, B, *Ptomaphagus* (*Appadelopsis*) *cumberlandus*. C, D, *Ptomaphagus* (*Echinocoleus*) sp., female. A, C, ventro-lateral-internal view. B, D, ventro-lateral-external view. tI-tV = first to fifth tarsomeres; bs = 'bachelor seta'; tw = 'twin spines'.

Figure S3. Mesotarsus, Cholevinae: Anemadini: Eunemadina - *Pseudonemadus* (*s.s.*) *cheesmani*. A, ventro-lateral-internal view. B, ventro-lateral-external view. tI-tV = first to fifth tarsomeres; tw = 'twin spines'.

Figure S4. Mesotarsus, Cholevinae: Cholevini: Cholevina. A, B, *Choleva* (*s.s.*) *oblonga*. C, D, *Nargus* (*s.s.*) *badius*. A, C, ventro-lateral-internal view. B, D, ventro-lateral-external view. tI, tII = first and second tarsomeres.

Figure S5. Mesotarsus. A-D, Camiarinae: Agyrtodini. A, B, *Agyrtolasia calliptera*. C, D, *Chiliopelates pictus*. E, F, Leiodinae: Pseudoliodini – *Neohydnobius* sp. A, C, E, ventro-lateral-internal view. B, D, F, ventro-lateral-external view. tI-tIII = first to third tarsomeres.