

To be or not to be a tibial comb: A discussion on the (past) use of tibial armature in tribal/subtribal organization in Cholevinae (Coleoptera: Leiodidae)

Pedro Gnaspini^{1,6}; Stewart Blaine Peck²; Caio Antunes-Carvalho³; Michel Perreau⁴ & Eduardo Coutinho Gomyde^{1,5}

¹ Universidade de São Paulo (USP), Instituto de Biociências (IB-USP), Departamento de Zoologia. São Paulo, SP, Brasil.

² Canadian Museum of Nature, Research and Collections Division, Entomology, Research Associate. Ottawa, ON, Canada.
ORCID: <http://orcid.org/0000-0002-3407-4978>. E-mail: StewartPeck@cunet.carleton.ca

³ Universidade Federal Fluminense (UFF), Instituto de Biologia (EGB), Departamento de Biologia Geral (GBG). Niterói, RJ, Brasil.
ORCID: <http://orcid.org/0000-0002-0124-2509>. E-mail: caioac@id.uff.br

⁴ Université de Paris, IUT Paris Diderot. Paris, France. ORCID: <http://orcid.org/0000-0001-6124-9334>. E-mail: michel.perreau@u-paris.fr

⁵ ORCID: <http://orcid.org/0000-0001-7450-5691>. E-mail: eduardo.c.gomyde@gmail.com

⁶ ORCID: <http://orcid.org/0000-0003-4734-5156>. E-mail: gnaspini@ib.usp.br

Abstract. Detailed studies of microstructure have recently been shown to provide phylogenetic signals at several supraspecific levels within leiodid coleopterans, as well as in other insects. The tribe Ptomaphagini (Leiodidae: Cholevinae), with a Holarctic-Neotropical-Oriental distribution, has been characterized, among other things, by having a comb of equal-sized, flat spines around the apex of the tibiae of all legs, with a row of spines extending along the outer edge of the protibiae in the subtribes Baryodirina and Ptomaphaginina (but not in Ptomaphagina). A pattern similar to the one in Ptomaphaginina also occurs in the Neotropical cholevine tribe Eucatopini, and this has been used to indicate a phylogenetic relationship between the two tribes (but recent phylogenetic studies have not supported such a close relationship). We here review and revise the presence and structure of periapical (here called an 'apical crown') and marginal (here called an 'external comb') combs of spines on tibiae in Ptomaphagini, using other cholevines (with and without apical tibial combs) for comparison. We find a phylogenetic signal in an apical crown of tibial spines not interrupted at the outer spur, which seems to be an additional synapomorphy of Ptomaphagini, differing from the pattern in Eucatopini and remaining cholevines with an apical comb of spines, in which the comb is interrupted. We highlight differences not previously noticed between the apical protibial armature of Ptomaphaginina and Eucatopini.

Key-Words. Coleoptera; Cholevinae; Ptomaphagini; Eucatopini; Tibial morphology.

INTRODUCTION

The family Leiodidae, placed in the staphylinoid group of beetle families, includes more than 4,200 described species (*e.g.*, Newton, 2019), and is characterized (with a few exceptions) by having antenna with an "interrupted" 5-segmented club, antennomere 8 being smaller than 7 and 9, and a periarticular gutter on antennomeres 7, 9 and 10 (*e.g.*, Newton, 2016). One character in particular that is present in many leiodids is a comb of flattened spines that form a ring around the apex of the tibiae, or is present along the lateral margin of the tibiae. In this paper we examine these structures in detail in one group, Ptomaphagini, and evaluate its significance as a phylogenetic character.

The tribe Ptomaphagini of Cholevinae (the most diverse subfamily in Leiodidae), with a Holarctic-Neotropical-Oriental distribution, is diagnostically characterized by having the combination of the following characters: presence of a comb of equal, flat spines surrounding the apex of the tibiae of all legs, a reduced genital segment, and an aedeagus lacking a basal lamina and having an elongate internal flagellum (*e.g.*, Jeannel, 1936; Gnaspini, 1993, 1996; Newton, 1998). Whereas the apical tibial comb is similar in all legs in Ptomaphagina, the protibiae in Ptomaphaginina and in the monotypic Baryodirina bear a row of spines extending along the outer edge. Ptomaphagini was traditionally considered to be close to the Neotropical tribe Eucatopini (*e.g.*, Jeannel, 1936, and see discus-

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Table 1. List of species of Leiodidae Cholevinae examined in this study [¹ specimens used in Antunes-Carvalho & Gnaspini (2016); ² specimens used in Gnaspini *et al.* (2017a); ³ specimens used in Antunes-Carvalho *et al.* (2019); ⁴ images from M. Perreau (either in Perreau, 2000 or unpublished)], with a summary of the main morphological characteristics observed on the mesotibial apex among the studied taxa. TC denotes if mesotibiae have ('Y' (yes) [or '~Y' if it bears small differences from that in Ptomaphagini]) or not ('N' (no), although a series of spines may be present) an apical crown of spines; IN denotes the number of 'interstitial spines'. See text for explanation of characters and character states and for discussion. '?' indicates that that feature was not examined in that species.

Systematic assignment		Species	Provenance	TC	IN	Fig.
Anemadini	Anemadina	<i>Anemadus italicus</i> Zoia, 1990	Italy: Calabria	~Y	4	4A-B
	Eocatopina	<i>Eocatops ophidianus</i> Szymczakowski, 1976 ³	Turkey: Akdamar Island, Van Gölü	~Y	3	5A, 9E
	Eunemadina	<i>Dissochaetus vanini</i> Gnaspini, 1993 ^{1,2,3}	Brazil: São Paulo: Iporanga	N	2	4C
Cholevini	Paracatopina	<i>Eunemadus chilensis</i> Portevin, 1914 ²	Chile: Osorno: near Puaucho	~Y	4	4D-E, 9F
		<i>Paracatops alacris</i> (Broun, 1880) ^{2,3}	New Zealand: AK: Duck Creek SR	~Y	3	5B-C, 9G-H
	Catopina	<i>Catops fuliginosus</i> Erichson, 1837 ^{1,2,3}	Italy: Friuli Venezia Giulia	N	4	6A-B
	Cholevina	<i>Catops nigricans</i> (Spence, 1815) ⁴	Italy: Liguria	N	?	—
Eucatopini	—	<i>Nargus</i> (s. str.) <i>badius</i> (Sturm, 1839) ^{1,3}	Italy: Liguria	~Y	4	6C
	—	<i>Eucatops</i> sp. ^{1,3}	Peru: Madre de Dios	Y	?	7D-E
Leptodirini	—	<i>Eucatops</i> (<i>Napocatops</i>) <i>giganteus</i> Salgado, 2005 ²	Ecuador: Napo: near Baeza	Y	4	2, 7F-H
	Bathysciina	<i>Oryotus ausmeieri</i> Perreau, 2003 ⁴	Slovenia: Slap ob Idrijci, Jama Krasnica	?	?	9C
	Bathysciotina	<i>Bathysciotes khevenhuelleri tergestinus</i> Müller, 1922 ^{1,3}	Italy: Friuli Venezia Giulia	~Y	1	3A, 9A-B
	Leptodirina	<i>Leptodirus hohenwarti</i> Schmidt, 1832 ^{1,3}	Italy: Friuli Venezia Giulia	~Y	1	3B
Pholeuina	—	<i>Diaprysius serullazi</i> Peyerimhoff, 1904 ²	France: Ardeche: grt. des Assiettes	~Y	2	3C
	—	<i>Quaestus noltei</i> (Coiffat, 1965) ⁴		?	?	—
Oritocatopini	—	<i>Afrocotops</i> sp. ²	South Africa: Natal; near Estcourt	N	2	6D
Ptomaphagini	Ptomaphagina	<i>Adelopsis leo</i> Gnaspini, 1993	Brazil: São Paulo: Guapiara	Y	0	1A-D, 8
		<i>Amplexella dimorpha</i> Gnaspini, 1996 ^{1,2,3}	Venezuela: Aragua	Y	0	1E-F
		<i>Parapaulipalpa</i> sp. ^{1,2,3}	Venezuela: Bolívar	Y	0	1G-H
	—	<i>Paulipalpa claudicans</i> (Szymczakowski, 1980) ^{1,2,3}	Brazil: Santa Catarina	Y	0	1I
	—	<i>Ptomaphagus</i> (<i>Appadelopsis</i>) <i>cumberlandus</i> (Peck, 1979)	U.S.A.: Alabama	Y	0	1J
	—	<i>Ptomaphagus</i> (s. str.) <i>sericatus</i> (Chaudoir, 1845) ³	Slovenia: Lower Carniola, Trebnje	Y	0	—
	Ptomaphagina	<i>Ptomaphagus palpalis</i> Szymczakowski, 1974 ³	Nepal: Kathmandu, Phulchoki	Y	0	—
		<i>Ptomaphaminus bengalominus</i> (Perreau, 1991) ⁴	India: Bengale occidental	Y	0	—
		<i>Ptomaphaminus chapmani</i> (Peck, 1981) ^{1,2}	Malaysia: Sarawak	Y	0	1K, 7A-B
		<i>Ptomaphaminus fagei</i> Perreau, 2009 ⁴	Indonesia: Marang Mts., Gua Sedepan	Y	0	—
—	<i>Proptomaphagus puertoricensis</i> Peck, 1971 ^{3,4}	Puerto Rico: Aguas Buenas, Aguas Buenas Cave	Y	0	7C	
Sciaphyini	—	<i>Sciaphyes sibiricus</i> (Reitter, 1887) ²	Russia: Siberia	~Y	2	3D, 9D

sion in Gnaspini, 1994), because of the presence of the tibial comb of spines, with a similar protibial pattern to that in Ptomaphagina and Baryodirina, and the lack of a tegmen on the aedeagus (although the general pattern of the male genitalia is very different between those tribes). But recent evidence places Ptomaphagini closer to Leptodirini and Sciaphyini, and places Eucatopini + Oritocatopini as an early branch in the evolution of Cholevinae (Antunes-Carvalho & Gnaspini, 2016; Antunes-Carvalho *et al.*, 2019). Actually, although Jeannel (1936) and followers used the comb of spines to unite Ptomaphagini and Eucatopini, they recognized combs and/or crowns of spines at the tibial apex in other groups (Anemadini Eocatopina and Paracatopina, Cholevini Cholevina, and some subtribes of Leptodirini).

Recent studies have shown that features of the legs such as the pretarsal structure and the shape and distribution of tenent setae are valuable character systems for the detection of phylogenetic signals to characterize supraspecific groups (*e.g.*, Antunes-Carvalho & Gnaspini, 2016; Gnaspini *et al.*, 2017a, b). We, therefore, study the fine morphology (as opposed to the more common approach of gross morphology) of the apex of the tibiae

to address these questions in a new comparative scenario. We dedicate this study to Dr. Cleide Costa, on her 80th birthday celebration, in recognition of her important contribution to knowledge of the morphology of Coleoptera.

METHODS AND MATERIALS

The methods used here follow the ones of previous studies on the group (*e.g.*, Antunes-Carvalho & Gnaspini, 2016; Gnaspini *et al.*, 2017a, b). We used part of the material studied in the cited references and additional specimens (Table 1). In addition to ptomaphagines and eucatopines, we analysed at least one representative of tribes/subtribes of Cholevinae where a tibial comb of spines was recorded in Jeannel (1936) and Newton (1998), namely (following the taxonomic structure used in Newton, 1998), Anemadini: Eocatopina and Paracatopina, Cholevini: Cholevina, Leptodirini: Leptodirina and Bathysciotina. For comparison, we examined additional taxa, namely, Anemadini: Anemadina and Eunemadina, Cholevini: Catopina, Leptodirini:

Pholeuina, and Oritocatopini. We studied the mesotibiae (as a representative of the patterns found on both midlegs and hindlegs) of the taxa listed above and we additionally studied the protibiae in Eucatopini and Ptomaphagini: Ptomaphaginina, where the comb of spines extends along the external margin. The dissected legs of additional specimens were mounted on metallic stubs using carbon adhesive pads, and sputter-coated with gold. The specimens were examined in a Zeiss DSM 940 scanning electron microscope in IBUSP [Instituto de Biociências, Universidade de São Paulo]. Except when noted, the left leg was illustrated.

Terminology

The apex of the tibiae is generally armed with spines and spurs. Both are elements of the integumental armature (*e.g.*, Jeannel, 1936). Spines are more robust than setae and frequently with similar length (*e.g.*, circle in Fig. 1A). Spurs, in turn, are much longer and stronger than spines, and frequently exhibit additional armature, for instance being serrate and/or bearing small spines, tubercles or microtrichia (*e.g.*, 'is' and 'os' [for inner spur and outer spur, respectively] in Figs. 1A, B). In some taxa, there is a row of short and equal (and frequently flat) spines placed at the apex of the tibiae. These spines may either appear (a) in the form of a 'periapical comb' (as in Newton, 1998, who also used 'apical basket', as in 'corbeille apicale' of Jeannel, 1936) (for instance on the meso- and metatibiae of Ptomaphagini and Eucatopini and the protibiae of Ptomaphagini: Ptomaphagina) – we here prefer to use 'apical crown'; or (b) as a 'comb along the external edge' (as in Newton, 1998, as in 'peigne apical externe' or 'peigne sur le bord externe' of Jeannel, 1936) (for instance on the protibiae of Ptomaphagini: Ptomaphaginina and Eucatopini) – for brevity, we here use 'external comb'.

Although Jeannel (1936) was precise in the use of 'corbeille apicale', he used 'peigne' to refer either to the 'external comb' (mostly) or to the 'apical crown' (frequently when he wanted to refer to both, for instance in the case of taxa which have both, as the Eucatopini), mainly in keys (where, for the opposite state, he was frequently precise in using 'sans peigne ni corbeille apicale' ['without either comb or basket']).

RESULTS AND DISCUSSION

Crown of spines on mesotibial apex

In the following descriptions, where we add some details about this feature, we will restrict our analysis of the structures of interest in this discussion, focusing on the mesotibiae (summarized in Table 1). It is important for our discussion to highlight the presence of a pair of spurs latero-ventrally on the apex of all tibiae of cholevines as a general rule. For a more complete description of the sets of spines and patterns of distribution of spines and spurs,

please refer, *e.g.*, to Jeannel (1936) or Newton (1998); spur patterns have also been discussed and illustrated in Fresneda *et al.* (2011) and Antunes-Carvalho *et al.* (2019).

In Ptomaphagini, the inner spur ['is'] is varyingly shorter than the outer spur ['os'] (Figs. 1E, K). The spines of the apical crown have a flat appearance [which is probably given by the parallel sides of the spines, except for the rounded or pointed tip] and of approximately the same height (Figs. 1B, F). The spines closer to the inner spur, however, are progressively reduced in size at both sides of the spur – for instance, 3-4 spines in *Adelopsis* Portevin, 1907 (Fig. 1A, circle), 6-7 spines in *Paulipalpina* Gnaspini & Peck, 1996 (Fig. 1I, circle) –, leaving a small gap ['gp'] for the insertion of the inner spur (Figs. 1G, J, K). On the other hand, the spines close to the outer spur do not change in size or position, and the outer spur is placed inside the apical crown of spines (Figs. 1A, B, E, H, J, K). The sockets of both spurs bear a (usually) long seta, here called the 'body guard' seta ['bg'] (Figs. 1A, K), placed more laterally towards the midventral axis of the mesotibiae in *Adelopsis* and *Ptomaphaminus* Perreau, 2000. In the latter, the sockets of the spurs seem to be fused together, probably because the spurs are placed closer to each other when compared to other species (Fig. 1K). The same pattern of the mesotibial apical crown appears on the metatibiae (Figs. 1C, D).

In the remaining groups analysed, the inner spur is longer than the outer spur (*e.g.*, Figs. 2A, 4C, D), except in Leptodirini (*e.g.*, Fig. 3C), and there is also a 'body guard' seta at the midline of the spurs (*e.g.*, Figs. 2B, 4C). Antunes-Carvalho *et al.* (2019) have a discussion about size, patterns and armature of spurs and their phylogenetic signals in Cholevinae.

The mesotibial apical crown of Eucatopini follows the same pattern as in Ptomaphagini, with some differences. Only the last spine closer to the spurs (in this case, of both spurs, and at both sides of the spurs) is shorter than the rest of the apical crown (Fig. 2B, circle). The apical crown is interrupted at both spurs, and there is a series of three spines placed between the spurs, and clearly inserted slightly away from the apical margin of the mesotibia – this series is here called the 'interstitial spines' ['in'] (Fig. 2C). Except in Ptomaphagini (as above), the 'interstitial spines' are present in all groups analysed (*e.g.*, Figs. 4D, 5A), in variable numbers (see Table 1), meaning that, when there is an apical row of spines, it is interrupted at both spurs.

In Leptodirini, the apical crown in Bathysciotina (Fig. 3A) follows a very similar pattern to that of Ptomaphagini, with the spines also having a flat appearance (with 1 dorsal spine longer than the neighbours in the species studied, Fig. 3A, ellipse). The apical crown in Leptodirina (Fig. 3B) and in the Pholeuina *Diaprysius* Abeille de Perrin, 1878 (Fig. 3C) is formed by slender, rounded and pointed spines, more spaced and of irregular size, and with 2-3 longer dorsal spines in the latter species (Fig. 3C, ellipse). The apical crown in Sciaphyini is like that of Bathysciotina, but the spines are somewhat conical and there are 2-3 longer dorsal spines (Fig. 3D, ellipse).

The apical crown in Anemadini: Anemadina and Eocatopina is also similar to that in Ptomaphagini, but the spines are conical (Figs. 4A-B, 5A; and they are more spaced on the dorsal margin in the former). In the Anemadini: Eunemadina: *Eunemadus* Portevin, 1914

(with the spines of the apical crown also having parallel sides; Figs. 4D, E) and in Paracatopina (Figs. 5B, C), the apical crown is similar to that in Ptomaphagini, but it is interrupted by (three or four, respectively) long spines, slightly displaced from the margin (Fig. 4E, ellipses).

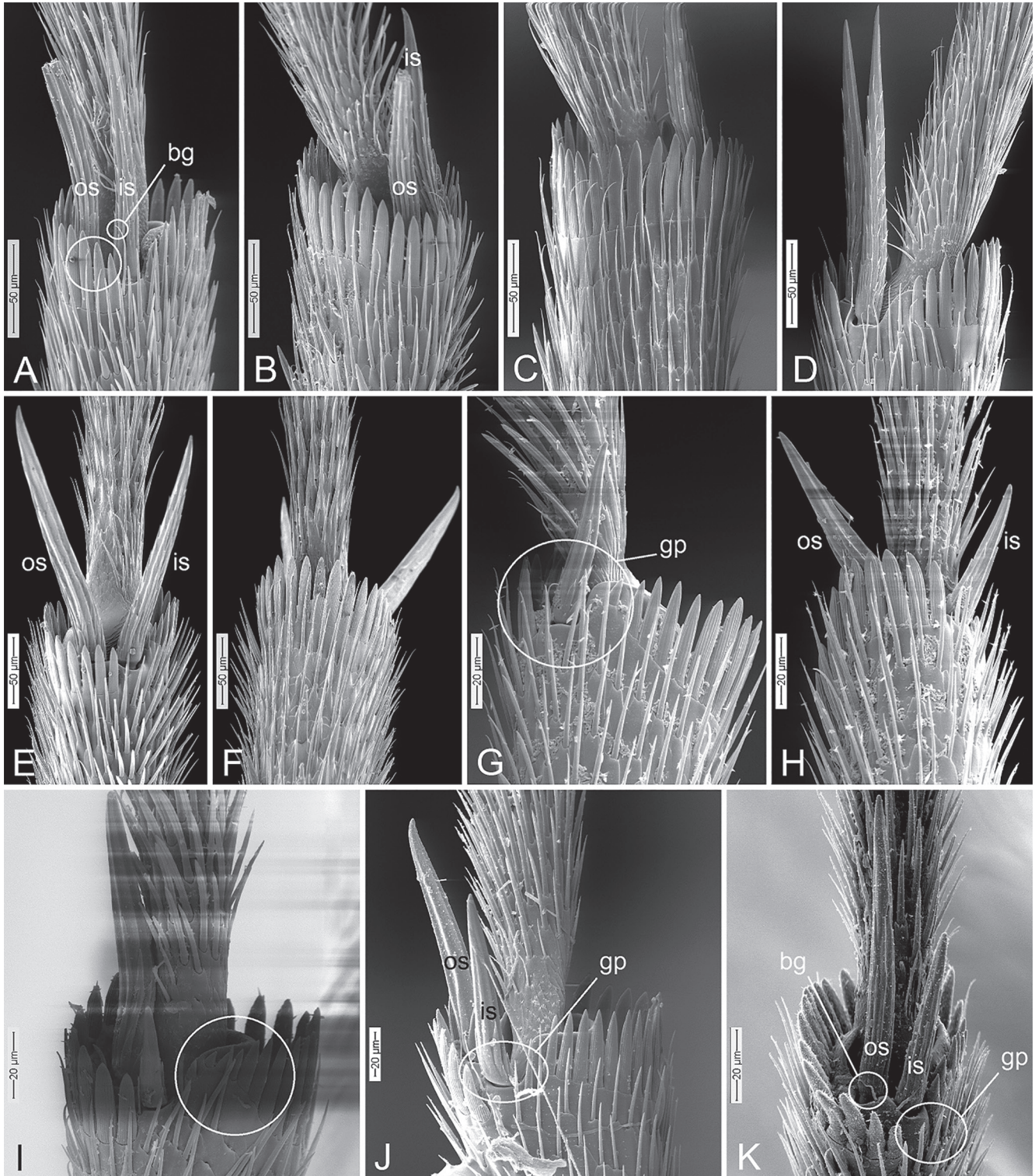


Figure 1. Apex of tibiae in Ptomaphagini. (A-D) *Adelopsis leo* Gnaspini, (A-B) Mesotibia, (A) ventro-lateral-internal and (B) lateral-external views; (C-D) Metatibia, (C) lateral-external and (D) lateral-internal views; (E-F) *Amplexella dimorpha* Gnaspini, mesotibia, (E) ventral and (F) dorso-lateral-external views; (G-H) *Parapaulipalpa* sp., right mesotibia (mirrored image), (G) lateral-internal and (H) ventral views; (I) *Paulipalpa claudicans* (Szymczakowski), right mesotibia (mirrored image), lateral-internal view; (J) *Ptomaphagus (Appadelopsis) cumberlandus* (Peck), mesotibia, ventro-lateral-internal view; (K) *Ptomaphaminus chapmani* (Peck), mesotibia, ventral view. bg = 'body guard' seta; gp = 'gap' on the apical crown of spines around base of spur; is = inner spur; os = outer spur; the circles highlight the progressively smaller spines near spur.

In the Eunemadina: *Dissochaetus* Reitter, 1884 (Fig. 4C), the apical row of spines also covers the dorsal and both lateral margins, but the spines do not have parallel sides and they do not form a proper apical crown,

with (four) long spines intercalated with a small series of short (rounded) spines (Fig. 4C, ellipse). A similar pattern was observed in Cholevini: *Catopina* (Figs. 6A, B) and *Oritocatopini* (Fig. 6D).

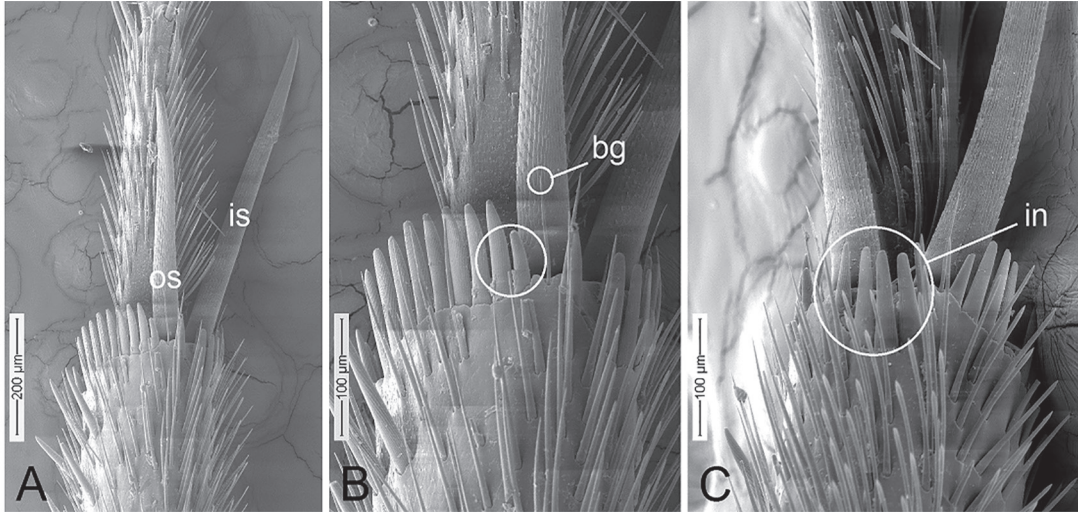


Figure 2. Apex of mesotibiae in Eucatopini, *Eucatops (Napocatops) giganteus* Salgado, (A-B) ventro-lateral-external (B = detail) and (C) ventral views. bg = ‘body guard’ seta; in = ‘interstitial spines’; is = inner spur; os = outer spur; circle = smaller spine near spur.

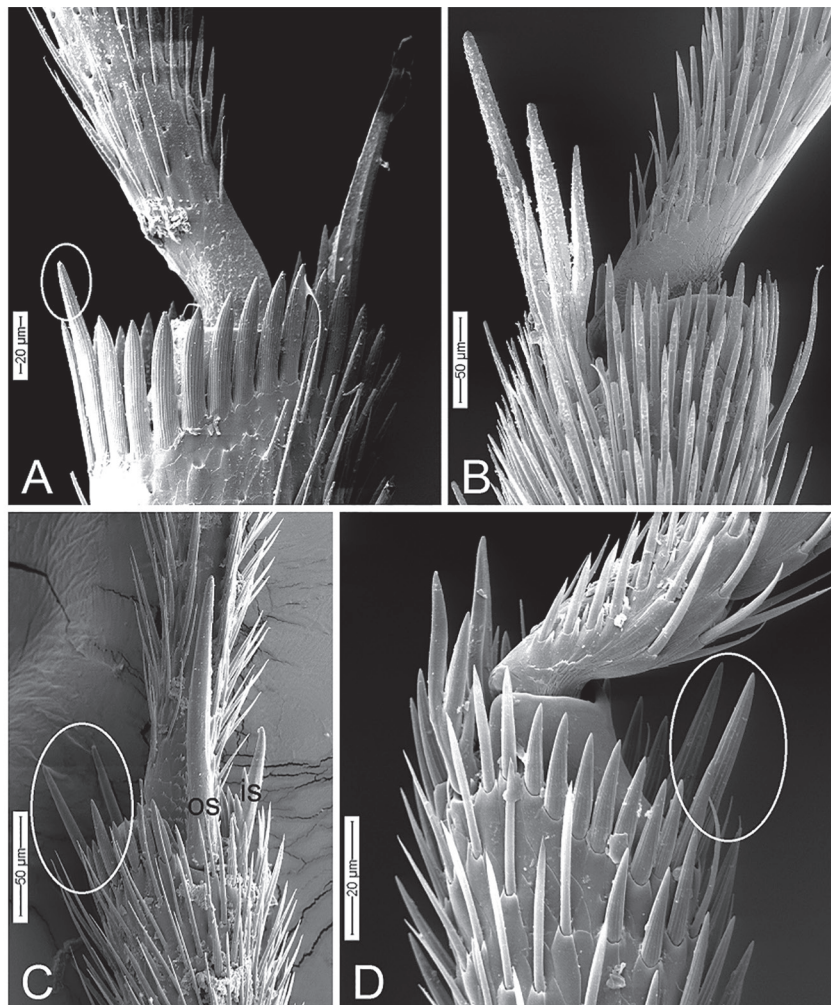


Figure 3. Apex of mesotibiae in Leptodirini (A-C) and Sciaphyini (D). (A) *Bathysciotes khevenhuelleri tergstinus* Müller, lateral-external view; (B) *Leptodirus hochenwarti* Schmidt, lateral-internal view; (C) *Diaprysius serullazi* Peyerimhoff, ventral view; (D) *Sciaphyes sibiricus* (Reitter), lateral-internal view. is = inner spur; os = outer spur; the ellipses highlight the longer spines on the dorsal portion of the apical crown of spines.

The apical crown in Cholevini: Cholevina (*Nargus* C.G. Thomson, 1867 – Fig. 6C) is formed by slender, rounded and pointed spines, with two dorsal spines (separated by three small spines) slightly longer than the neighbours in the species here studied (Fig. 6C, ellipse).

Row of spines on protibial apex in Ptomaphagini and Eucatopini

Jeannel (1936: 43) stated that the ‘apical comb’ of the protibiae occupies all the apical margin and the external lateral margin in Eucatopini and *Ptomaphagus* Portevin, 1914 (at that time the only genus of the later named subtribe Ptomaphaginina, of Ptomaphagini). Newton (1998: 132) offered a similar statement: ‘Protibia with comb of short spines along external edge as well as around apex’. We noticed that those statements were not precise, and we also noticed that the condition differs between those taxa.

In the Ptomaphagini: Ptomaphaginina: *Ptomaphaminus* (e.g., Figs. 7A, B; a species formerly placed in *Ptomaphagus*), starting from the inner spur, the protibial apical row of spines goes (following the

ventral margin) towards the outer spur, passes around it (as aforementioned for the meso- and metatibiae of Ptomaphagini) and continues until the dorsal region; at this point (Fig. 7A, arrow), instead of going back ventrad and forming a complete apical crown (as occurs on meso- and metatibiae [see above]), the spines follow the external margin of the protibiae. A similar pattern was observed in another species of *Ptomaphaminus* here studied, but we should note that the morphology is variable in species of this genus, which may be a non-monophyletic taxon (Schilthuizen et al., 2018). The same general pattern can be observed in the species of *Ptomaphagus*, but, in this genus, there is an additional row of strong setae following the external margin of the protibiae ventrally (Perreau, 2000: 30, fig. 18; Wang & Zhou, 2015, several figures). In other words, there is no complete periapical comb of spines, as described in Jeannel (1936) and Newton (1998). However, in *Proptomaphagus* Szymczakowski, 1969 (Fig. 7C), although the general pattern is similar to that described for *Ptomaphaminus*, it is possible to recognize a series of very small spines that extends from the internal portion of the apical crown of spines towards the external comb of spines (Fig. 7C, rectangle). These spines are not equally

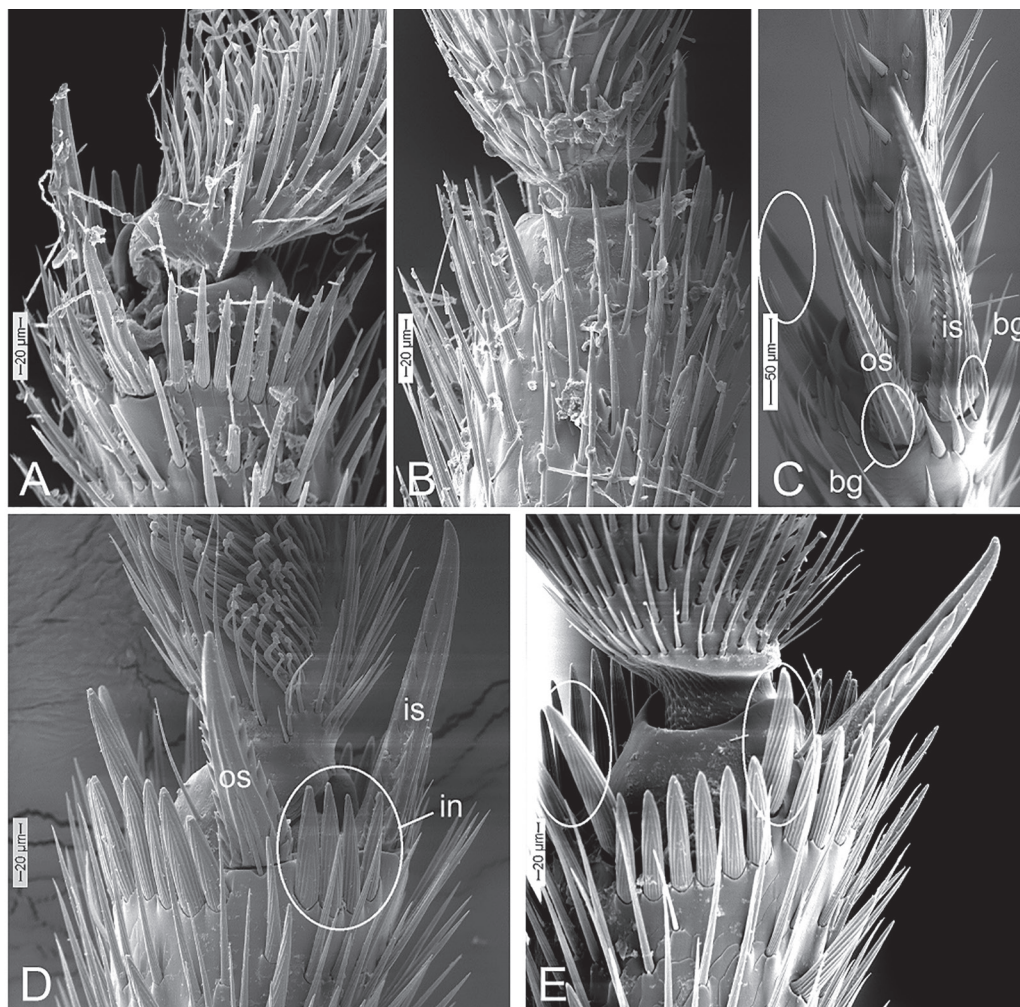


Figure 4. Apex of mesotibiae in Anemadini. (A-B) *Anemadus italicus* Zoia, (A) lateral-internal and (B) dorsal views; (C) *Dissochaetus vanini* Gnaspini, ventral view; (D-E) *Eunemadus chilensis* Portevin, (D) ventral and (E) lateral-external views. bg = ‘body guard’ seta; in = ‘interstitial spines’; is = inner spur; os = outer spur; the ellipses highlight the longer spines on the dorsal portion of the apical crown of spines.

sized and not specifically placed around the apex of the protibiae, but diagonally placed.

In *Eucatops* Portevin, 1903 (Figs. 7D-H), the protibial apical spines show the same distribution as above, with two differences. First, there is a gap in the spines close to

the outer spur (as described above for the mesotibiae of *Eucatopini*) (Fig. 7F). Second, there is indeed a series of spines at the inner face of the protibiae (Figs. 7E, G, H, circle), but it does not follow or even reach the apical margin, and therefore does not form an apical crown (or peri-

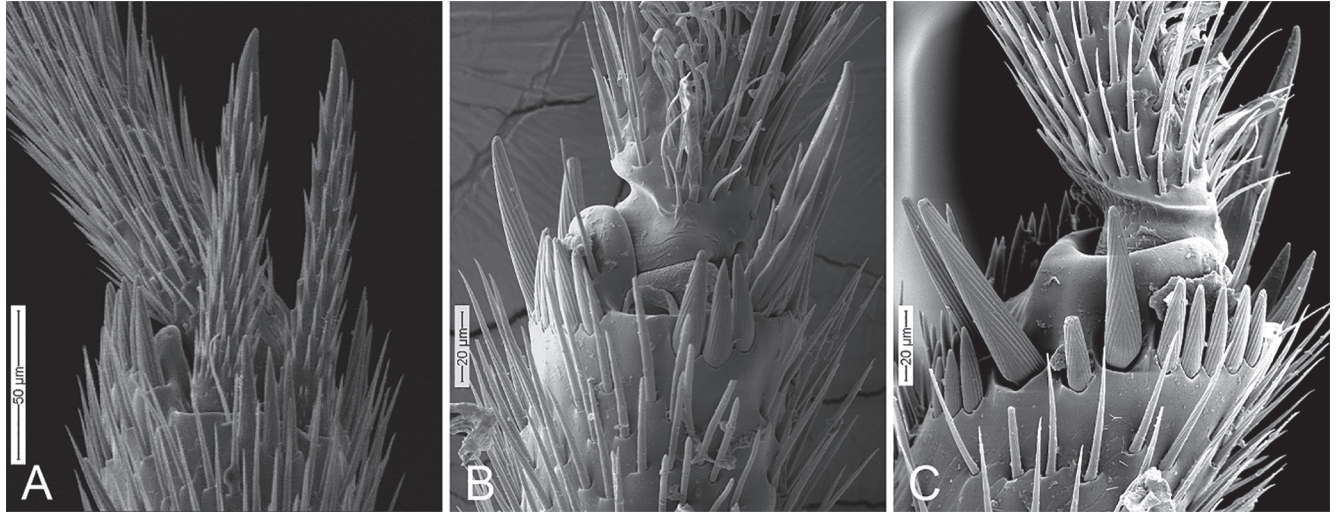


Figure 5. Apex of mesotibiae in Anemadini (continued). (A) *Eucatops ophidianus* Szymczakowski, ventral view; (B-C) *Paracatops alacris* (Broun), (B) ventral and (C) lateral-external views.

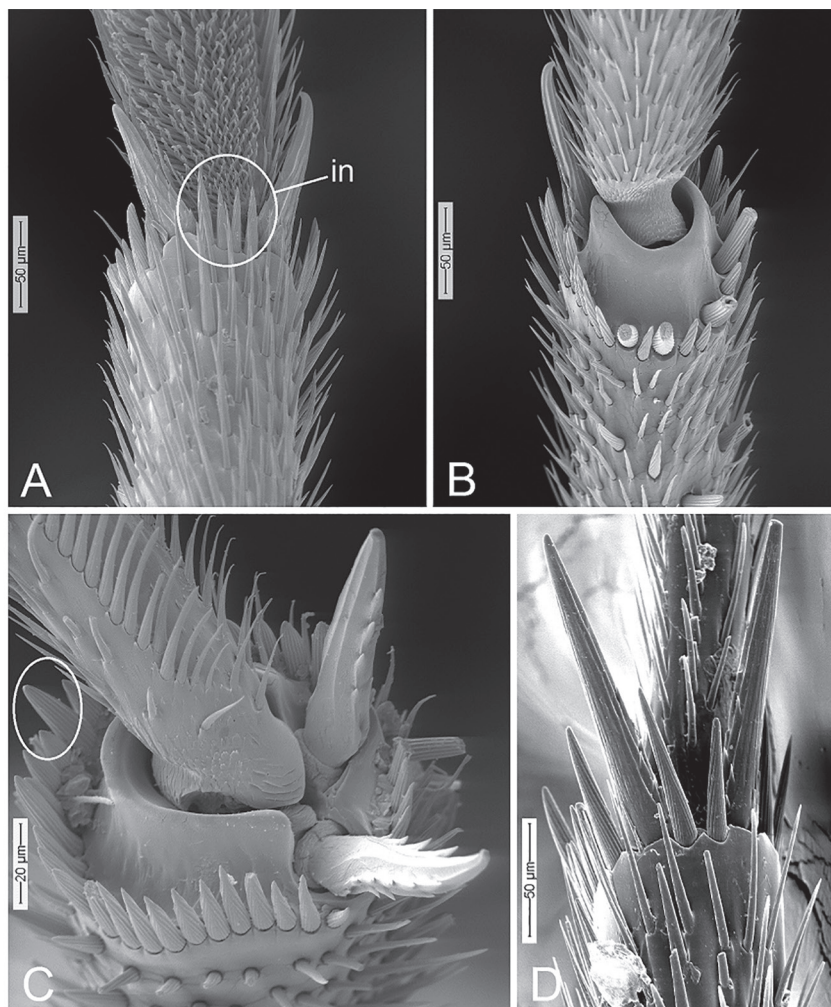


Figure 6. Apex of mesotibiae in Cholevini (A-C) and Oritocatopini (D). (A-B) *Catops fuliginosus* Erichson, (A) ventral and (B) dorsal views; (C) *Nargus* (s. str.) *badius* (Sturm), fronto-lateral-external view; (D) *Afrocatops* sp., ventral view. in = 'interstitial spines'; the ellipse highlights the longer spines on the dorsal portion of the apical crown of spines.

apical comb). Instead, originating close (but not next) to the inner spur, the series of spines continues away from the apex, makes a curved/parabolic trajectory, and ends

at a row of strong spines (Figs. 7E, H, ellipse) placed parallel to the comb of flat spines that comes from the dorsal portion of the protibial apex. The same pattern can be

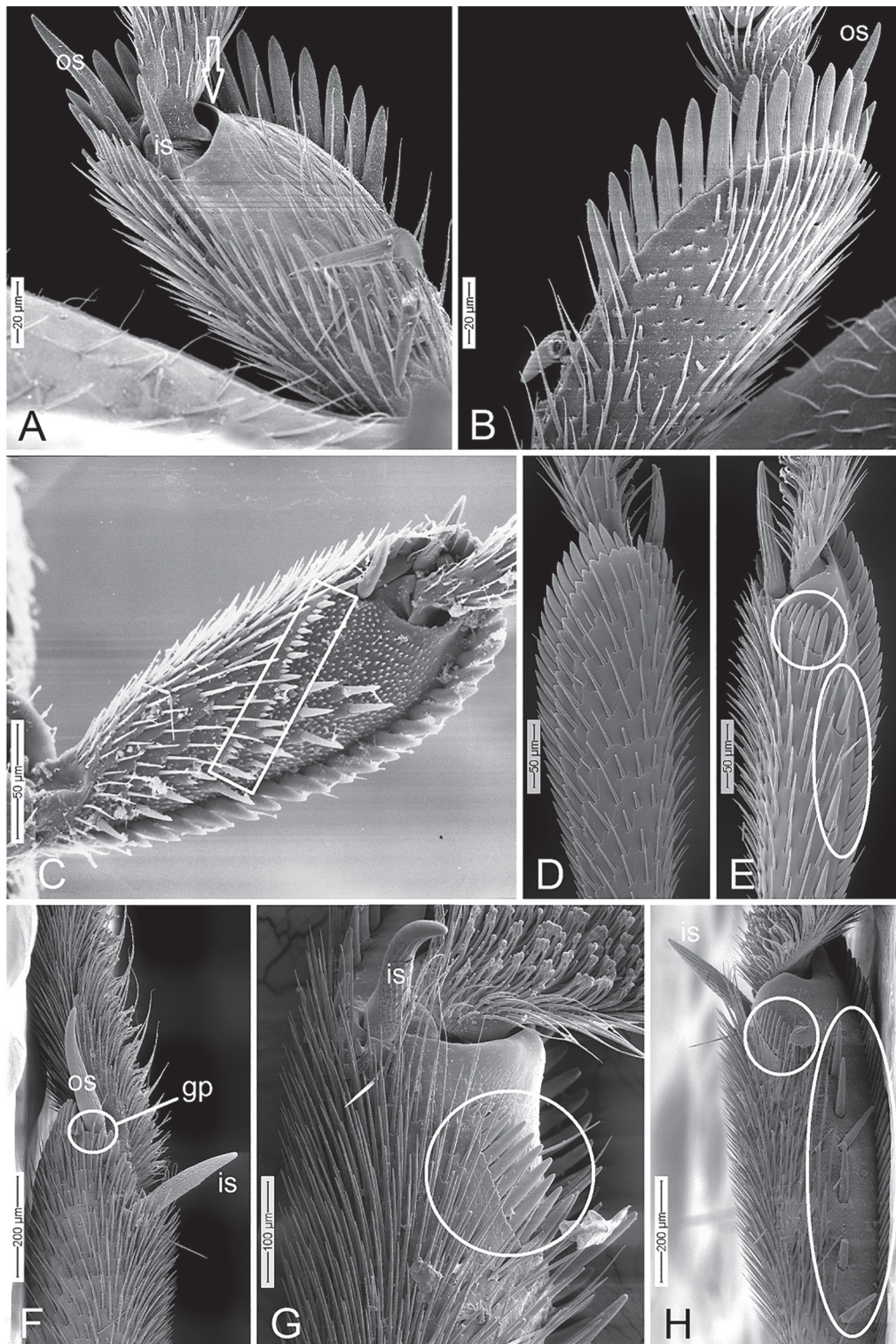


Figure 7. Apex of protibiae. (A-B) *Ptomaphaminus chapmani* (Peck), right leg (mirrored image), (A) ventral and (B) dorsal views; (C) *Proptomaphagus puertoriensis* Peck, ventral view (used with permission and modified from fig. 19 in Perreau, 2000); (D-E) *Eucatops* sp., (D) dorsal (note the serrate nature of the external margin of the protibia in this species) and (E) ventral views; (F-H) *Eucatops (Napotops) giganteus* Salgado, (F) lateral-external, (G) ventro-lateral-external, and (H) lateral-internal views. gp = 'gap' on the apical crown of spines around base of spur; is = inner spur; os = outer spur; the arrow shows the point where the spines stop circling the apical margin of the protibia and start to follow the external margin of the protibia, instead of making a complete apical crown; the rectangle highlights the presence of a series of small spines that extends from the internal portion of the apical crown of spines (to the left of the figure) towards the external comb of spines (to the right of the figure); the circle highlights the series of spines at the inner face of the protibia; the ellipse highlights the longitudinal row of strong spines along the outer margin of the protibia.

observed in the species of *Eucatops* studied in Perreau (2000: 30, fig. 17). Therefore, in this genus there is indeed a comb of spines that goes almost all around the protibiae, but not completely periapically.

In the Ptomaphagini: Ptomaphagina; these are known to have an apical crown not extending as an external comb also on the protibiae (e.g., Jeannel, 1936; Newton, 1998). Here we illustrate the subtribe based on *Adelopsis* (Figs. 8A-C; but a similar pattern was also observed, for instance, in *Paulipalpina* and *Ptomaphagus* Hellwig, 1795; and see also Perreau, 2000: 30, fig. 20, *Ptomaphagus subvillosus* Goeze). We observed that the protibial apical crown is complete – i.e., not interrupted at the inner spur, differently from the meso- and metatibiae of Ptomaphagini (see previous topic). In other words, both outer and inner spurs are placed inside the area delimited by the apical crown. The spines of the protibial apical crown are also progressively shorter in size closer to the inner spur, as observed for the mesotibiae (see previous topic). Also differently from the meso- and metatibiae, the apical crown of spines is not perpendicular to the axis of the protibiae, but is diagonal (as in Fig. 8A), in a similar manner to *Ptomaphaminus* (compare, e.g., to Fig. 7B), but it does not extend onto the lateral margin of the protibiae.

Concerning the other tribes, *Bathysciotes* Jeannel, 1910 (Leptodirini: Bathysciotina) clearly shows a protibial external comb of spines (Fig. 9A) and it also has a row of ventro-external spines (Fig. 9B, ellipse), which are not flat (as are those of the typical external comb of spines) but are clearly separated from the other setae and spines along the ventro-lateral face of the protibiae. The protibial external comb of spines appears in all genera of Bathysciotina (and in some Leptodirina) but with different degrees of development – very short combs, restricted to the apical portion of the protibiae have been recorded (as in Perreau, 2019). On the other hand, a similar ventral row of strong spines, although more diagonally placed, appears also in representatives of the leptodirine tribes Bathysciina and Pholeuina – e.g., *Oryotus* L. Miller, 1856, *Perriniella* Jeannel, 1910, *Quaestus* L.W. Schauffuss, 1861, and *Speonomus* Jeannel, 1908 (Fig. 9C; see also

Perreau, 2000: 30, fig. 21; Perreau & Tronquet, 2002: 483, fig. 10), but those tribes do not have a protibial external comb of spines. The leptodirines *Leptodirus* F.J. Schmidt, 1832 (Leptodirina) and *Diaprysius* (Pholeuina) and the sciaphyine *Sciaphyes* Jeannel, 1910 (Fig. 9D) show an apical row of spines (similar to the one on the mesotibiae) with a small gap close to the internal lateral margin.

In Anemadini, the Eocatopina *Eocatops* Peyerimhoff, 1924 (Fig. 9E) has a gap between the internal and the external portions of the apical crown, as in the Ptomaphaginina *Ptomaphaminus* (see Fig. 7A), and the spines of the external portion of the apical margin start to follow the external margin of the protibiae, but an external comb of spines is not formed (see also, e.g., Jeannel, 1936: 175, fig. 347 and Latella, 2010: 90, fig. 7). The Eunemadina *Eunemadus* (Fig. 9F) has a complete, diagonal apical crown of spines, similar to that of the Ptomaphagina *Adelopsis* (see Fig. 8). Finally, the Paracatopina *Paracatops* Portevin, 1907 (Figs. 9G-H) shares both (a) the series of spines (which does not follow or reach the apical margin or the external lateral margin of the protibiae) at the inner face of the protibiae (Figs. 9G-H, circle) observed in the Eucatopini *Eucatops* (see Figs. 7E, G, H, circle) and (b) the diagonal series of very small spines that connects the internal and the external portions of the apical crown of spines (Figs. 9G-H, rectangle), as the one observed in the Ptomaphaginina *Proptomaphagus* (Fig. 7C, rectangle).

General remarks

Considering the meso- and metatibiae, firstly, we here recognize apical crowns of spines similar to those of Ptomaphagini, in addition to Eucatopini, for instance also in some Anemadini Eunemadina (which were not previously cited as bearing apical crowns) and in Leptodirini: Bathysciotina and Leptodirina. On the other hand, other groups which were considered to bear apical crowns (for instance, Cholevini: Cholevina – e.g., in Jeannel, 1936) do not show the same pattern as the taxa cited above. Secondly, the apical crown of Ptomaphagini was found

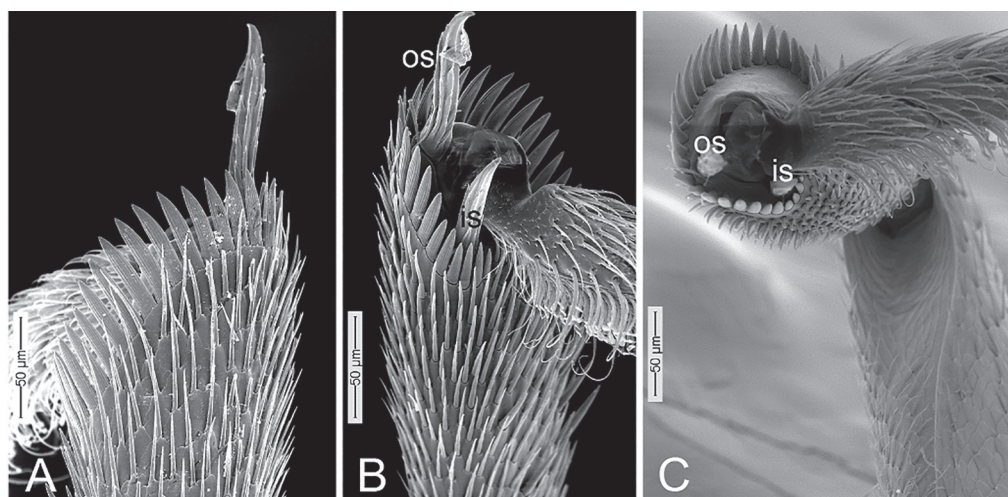


Figure 8. Apex of protibiae, *Adelopsis leo* Gnaspini, (A) dorsal, (B) ventral, and (C) frontal views. is = inner spur; os = outer spur.

here to differ from the others cited above (also from that of Eucatopini) by being uninterrupted at the outer spur. This seems to be an additional synapomorphy of Ptomaphagini. The general pattern discussed above is also valid for the protibiae, except in Eucatopini and Ptomaphagini: Ptomaphaginina and Baryodirina, when

the literature is considered, but see the previous topic and the next paragraphs.

In conclusion, we see no reason, as has historically been done, to identify the tibial apical crown of spines as evidence supporting Ptomaphagini and Eucatopini as closely related sister tribes. A second character that has



Figure 9. Apex of protibiae. (A-B) *Bathysciotes khevenhuelleri tergestinus* Müller, (A) dorsal and (B) ventral views; (C) *Oryotus ausmeieri* Perreau, right protibia (mirrored image), ventral view; (D) *Sciaphyes sibiricus* (Reitter), ventral view; (E) *Eocatops ophidianus* Szymczakowski, ventral view; (F) *Eunemadus chilensis* Portevin, ventral view; (G-H) *Paracatops alacris* (Broun), (G) ventro-lateral-external and (H) ventral views. The rectangle highlights the presence of small spines that connect the internal and external portions of the apical crown of spines; the circle highlights the series of spines at the inner face of the protibia; the ellipse highlights the longitudinal row of strong spines along the ventral outer margin of the protibia.

also been used as a character supporting the relationship between Ptomaphagini and Eucatopini is the lack of a tegmen on the aedeagus, which may be a convergence, considering the overall different pattern of the aedeagus in both tribes, as already commented in Newton (1998). Still concerning the aedeagus, Jeannel (1936) also cited an elongate flagellum in the endophallus as a typical character of both tribes, but this is very variable and many cases a short flagellum is recorded in both tribes, even in figures in Jeannel (1936). The only character that they consistently share is a protibial external comb of spines in Eucatopini and only part of Ptomaphagini.

However, differences were also observed in the structure of the series of spines forming the apical crown and the external comb of spines on the protibiae of Eucatopini and Ptomaphagini Ptomaphagina. This may be related to an independent acquisition of an external comb of spines, which would agree with a recent phylogenetic study placing those tribes far apart from each other (Antunes-Carvalho *et al.*, 2019). In that study, Eucatopini together with Oritocatopini appear as the sister group to the remaining Cholevinae. Moreover, an external comb of spines occurs also in Leptodirini: Bathysciotina. Thus, all these data suggest that the apical crown and the external combs in Ptomaphagini and Eucatopini may be convergent (homoplasies), deriving from separate ancestries, as similar adaptive responses to possibly similar selective pressures.

We also detected similarities between Ptomaphagini and other groups here studied as well as between Eucatopini and other groups. A good example is the Anemadini, whose subtribes were tentatively (and 'for convenience') grouped in a single tribe by Newton (1998), and which, in our study, show differences among themselves and similarities either to Ptomaphagini and/or to Eucatopini. A close relationship between Anemadini (or its subtribes, independently) and Ptomaphagini and/or Eucatopini has never been proposed in the literature, and is not assumed here (because we believe that a complete analysis should be conducted by adding the characters here studied to the ones already studied in the literature, and surely with a more robust sample of taxa).

Our goal here was not to exhaustively explore this system of characters, or to propose a new taxonomic scheme for Cholevinae, but to discuss the (in)convenience of or questionable grouping of Ptomaphagini and Eucatopini based mainly on the tibial comb of spines, as has been done in the literature. We suggest that we have shown the morphological complexity and potential for the character system to detect phylogenetic signals in Cholevinae (and also in Leiodidae and other coleopterans as a whole).

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