

## Identification of a new bacterial genus and multiple species from archived samples reveals early divergence in infectious agents

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### Abstract

Despite the many substantial advances and progress in understanding the internal evolutionary relations of several Lycidae groups, the divergence time estimation of the family within Elateroidea remains subject of debate, with estimates to as early as the early to mid-Jurassic. Herein we describe *Prototrichalus* gen. nov., and three new species: *Prototrichalus sepronai* sp. nov., *Prototrichalus meiyngae* sp. nov. and *Prototrichalus milleri* sp. nov., from Burmese amber of the Cenomanian Age (mid-Cretaceous), supporting an age for the tribe of at least 100 Ma. The new taxa belong to the tribe Metriorrhynchini and are the oldest recognizable beetles from this lineage, indeed, among the oldest known representatives of the Lycidae. The discovery and description of *Prototrichalus* and its three species provides direct evidence of the group's as a recognizable group in the mid-Cretaceous, much earlier than the recent predictions in the Late Cretaceous.

### 1. Introduction

With up to 4,600 extant species (Bocak and Bocakova 2008, 2010; Masek et al. 2018) and 12 fossil taxa described, including Dominican, Baltic and Burmese ambers (Ferreira and Ivie 2017; Bocak et al. 2019; Tihelka et al. 2019; Kazantsev 2019, and citations therein), the family Lycidae has representatives in both temperate and tropical regions with a nearly worldwide distribution (Bocak and Bocakova 2010; Ferreira and Ivie 2017; Masek et al. 2018.). In the species where males of both sexes attain a fully adult morphology, lycids can be recognized among other elateroids by the 7 (female) or 8 (male) ventrites and the conical, separated mesocoxae. In the vast majority of species, the pronotum is divided into 2 or more cells (areolae) by raised ridges, and the elytra with the presence of longitudinal costae on each, often reticulate elytron. Larvae and extreme pedomorphic females (lacking compound eyes, bifid tarsal claws, a multi-piece antennal flagellum and elytra) can be distinguished by the non-opposable, mandibles that are each divided longitudinally.

The family Lycidae is one of the better studied families of beetles in the superfamily Elateroidea. In the past decade several phylogenies (e.g. Kazantsev 2005; Bocak and Bocakova 2008; Bocak et al. 2008; Masek et al. 2018, Kusy et al. 2019), new descriptions of extinct and extant taxa and taxonomic revisions have appeared (e.g. Ferreira 2015; Kazantsev 2017; Ferreira and Ivie 2017; Ferreira and Ivie 2018a, 2018b; Li et al. 2018). However, despite the many advances and substantial progress in understanding the internal evolutionary relationships of several lycid groups (e.g. Masek et al. 2014; Sklenarova et al. 2014; Motyka et al. 2017), the divergence time estimation of Lycidae within Elateroidea (as well as many other beetle families) remains subject to debate.

Hunt et al. (2007) estimated the origin of Elateroidea, including Lycidae at over 140 Ma (early Cretaceous). McKenna et al. (2015) proposed a minimum estimation origin of Lycidae of about 50 Ma (early Cenozoic). Toussaint et al. (2016) and Zhang et al. (2018), added more

calibration points to their phylogenies and proposed minimum estimation dates of 95 Ma and 120 Ma (both mid-Cretaceous), respectively, for the origin of Lycidae as a whole. As predicted by Toussaint et al. (2016) and Zhang et al. (2018), two mid-Cretaceous lycid fossils from two different lineages were discovered and described by Bocak et al. (2019) and Tihelka et al. (2019) which provided evidence for an earlier diversification of Lycidae, pushing the origins of the family to a minimum of 100 Ma in the Cretaceous. In the most recent and exhaustive study to date, McKenna et al. (2019) push the origin of Lycidae into the Jurassic, with standard deviation going as early as the late Triassic.

Herein we report a new lycid genus with three new species from Cretaceous-age burmite belonging to the tribe Metriorrhynchini, the first fossil records for the tribe. The tribe is among the most diverse in number of genera and species in the family (Bocak 2002; Sklenarova et al. 2014). With an extraordinary diversity in Southeast Asia, Wallacea and New Guinea (Kazantsev 2012a; Sklenarova et al. 2014), it is also present in the Afrotropical and Australian regions (loc. cit.). Together with *Burmolycus compactus* Bocak, Li and Ellenberger, 2019 and *Cretolycus praecursor* Tihelka, Huang and Cai, 2019, our new genus and three species add to this the oldest assemblage of Lycidae.

## **2. Material and Methods**

The specimens (total number = 5) were obtained by FMO (2 specimens) and by Dr. Meiyang Lin (Institute of Zoology, Chinese Academy of Sciences, Beijing, P.R. China) (3 specimens), and Dr. Lin later donated the material to MAB. All specimens are amber inclusions. The specimens obtained by FMO and Dr. Lin are from Noije Bum Village, Hukawng Valley, Northern Myanmar (Kania et al., 2015: fig. 1A). Material from Hukawng Valley is comprised of folded sedimentary ( $\pm$ volcanic) rocks of Cretaceous and Cenozoic age, with a variety of clastic sedimentary rocks, with thin limestone beds and abundant carbonaceous material (Cruikshank and Ko 2003). Amber from this locality is dated to the earliest Cenomanian age in the mid-Cretaceous, with an estimated age of  $98.79 \pm 0.62$  Ma (Shi et al. 2012). Specimens are deposited in the National Museum of Natural History, Washington D.C., USA (USNM) and Montana Entomology Collection, Bozeman, MT, USA (MTEC).

Specimens were studied under a Leica® Wild M3C and a KYOWA SM stereoscopic microscope with magnification up to 40x. Specimens were photographed submerged in olive oil and taken with either an OPTIKAM PRO HDMI – 4083.13, 6 megapixels camera, using OptikaISview software, attached to a KYOWA SM microscope or a Canon T3i DSLR with an MP-E 65 mm lens and a Stackshot™ automated macro rail for focus stacking. Images were processed with Zerene Stacker® software version 1.04. Enhancements to digital images were made in Adobe Photoshop® CC 2019 and final plates prepared using Adobe Illustrator® CC 2019. The distribution map technique follows Ferreira (2016). Morphological terminology follows Bocak and Bocakova (1990), Kazantsev (2005) and Lawrence et al. (2011). The taxonomic acts proposed in this work have been registered in ZooBank (see below), together with the electronic publication LSIC: urn:lsid:zoobank.org:pub:0250D333-39FF-4D34-8DDD-CDA1D25B0661.

### 3. Systematic Paleontology

Order Coleoptera Linnaeus, 1758

Family Lycidae Laporte, 1836

Subfamily Metriorrhynchinae Kleine, 1926

Tribe Metriorrhynchini Kleine, 1926

Genus **Prototrichalus** Molino-Olmedo, Ferreira, Branham and Ivie gen. nov.

(urn:lsid:zoobank.org:act:278B85DB-F0C2-4C78-9F11-173815C1D956)

**Figs. 1–3.**

**Type species.** *Prototrichalus sepronai* Olmedo, Ferreira, Branham and Ivie gen. et sp. nov.

**Etymology.** The genus name is a combination of the name *Trichalus* Waterhouse, 1877, in reference to the new taxon's resemblance with trichaline beetles, and the Ancient Greek combining form *πρωτο-* (proto-) of the word *πρῶτος* (protos), meaning first, in reference to the possibility of this genus being the ancestor of some of the lineages of trichaline beetles. Gender masculine.

**Diagnosis.** *Prototrichalus*, have an unique combination of features not present in any other *Metriorrhynchini* group: pronotum imarginate, rectangular or subtrapezoidal, bearing five large cells (Figs. 1A–1D, 2A, 2B, 3A, 3B); the presence of a carina on the mesoventrite separating the mesocoxae; elongate maxillary palp, with terminal palpomere of males securiforme (Figs. 1A–1B, 1F) and of females elongate (Figs. 2D, 3C–3D)); and the absence of secondary elytral costae (Figs. 1A, 1C, 2A, 3A).

**Species Included.** *Prototrichalus sepronai* sp. nov., *Prototrichalus meiyingae* sp. nov. and *Prototrichalus milleri* sp. nov.

**Description.** Pronotum with a fine pubescence, elytra glabrous (Figs. 1A–1D, 2A, 2B, 3A, 3B), remainder of body with fine pubescence throughout (Figs. 2C, 3C). **Body. Head:** transverse, posteriorly partially covered by pronotum, apparently hypognathous (Figs. 1A–1C2, 2C, 3C–3D). Eyes hemispherical, slightly projecting anterolaterally when viewed dorsally (Figs. 3C–3D); coarsely granulate (Figs. 3C–3D). **Mouthparts:** Maxillary palp four-segmented, elongate, with last palpomere acuminate (Figs. 2D, 3C–3D) or securiforme (Figs. 1A–1B, 1F). Mandibles short, connate with labrum, hooked apically (Fig. 3D). Clypeus and labrum transverse (Fig. 3D), posterior margin of labrum emarginate (Fig. 3D). **Antennae:** 11-segmented, ranging from pectinate (Fig. 1E–1F) to subserrate (Figs. 2A, 2C) or filiform (Figs. 3C–3D) reaching middle of elytra (Fig. 1E, 3C) or apical third (Fig. 2A); scape subconic, pedicel short.

**Prothorax:** Pronotum transverse, rectangular (Figs. 2A, 2E) or trapezoidal (Figs. 1A–1B), emarginate (Figs. 1A–1B, 2A, 2E), narrower than humeral width, angles roundish (Figs. 1A–1B, 2A, 2E); disc region bearing 5 large cells (Figs. 1A–1B, 2E); median central areola reaching anterior and posterior margins of pronotum, enlarged in the center and slightly tapered towards margins (Figs. 1A–1B, 2E); posterior areolae one third shorter than anterior (Figs. 1A–1B, 2E). Hypomeron concave. **Mesothorax:** Mesoventrite bearing a carina (Fig. 2E). Scutellum

protruded, longer than wide, posteriorly bifurcated (Fig. 3A). **Metathorax:** Metaventricle convex, posterolateral angles pronounced, round; metadiscrimen complete (Figs. 2C, 3C). **Elytra:** irregularly punctate (Figs. 1A, 1C, 2A, 3A); subparallel, ranging from 3 to 4x longer than pronotum; with four primary elytral costae present; costa I short, only reaching basal third of elytra; costae II and III fused before apex (Fig. 3B) or free, not reaching apex (Figs. 1D, 2B); costa IV ranging from reaching third fourth of elytral length to first or second fourth of elytral length; secondary costae absent (Figs. 1D, 2B, 3B).

**Legs:** elongate; femora and tibiae ranging from clavate to subparallel (Fig. 1C, 2C, 3C); apex of tibiae bearing a pair of spines; mesocoxae separated; metacoxae transverse (Fig. 3C); tarsomeres 5-5-5, tarsomeres II-IV expanded, with pads strongly developed, the V bearing simple claws (Figs. 1C, 2B).

**Abdomen** of males presumably with eight visible ventrites (not visible), females with seven visible ventrites (Figs. 2C, 3C). Female genitalia, styli very slender, apically located; coxites broadening towards apex (Fig. 3E).

**Length (pronotum+elytra):** 7.5–9.0 mm. **Width (across humeri):** 2.0–2.8 mm.

**Distribution.** All known species are from Myanmar.

**Type horizon.** Cenomanian (ca. 98.79 ± 0.62 Ma), mid-Cretaceous (Shi et al. 2012).

#### Key to the species of *Prototrichalus*

1. Elytral costae II and III fused before reaching the apex (Fig. 3B); antennae filiform (Figs. 3C–3D).....**Prototrichalus meiyingae**
- 1'. Elytral costae II and III free, not fused and not reaching the apex of elytra (Figs. 1D, 2B); antennae pectinate or subserrate (Figs. 1C, 2A).....2
2. Pronotum bearing 5 areolae (Figs. 1A–1B); antennae pectinate (Fig. 1C); terminal maxillary palpomere securiforme (Figs. 1A–1B, 1F).....**Prototrichalus sepronai**
- 2'. Pronotum bearing 7 areolae, five larger ones and two small ones located adjacent to the posterior lateral margins of central areole (Fig. 2E); antennae subserrate (Fig. 2A); terminal maxillary palpomere elongate, longer than wide (Fig. 2D)..... **Prototrichalus milleri**

*Prototrichalus sepronai* Molino-Olmedo, Ferreira, Branham and Ivie sp. nov.

(urn:lsid:zoobank.org:act:8AB12E76-6FD7-473C-8936-6D49FC4F1B82)

**Figures.** 1 A, B, C, D, E, F.

**Material examined (2).** Holotype 1 ♂: MTEC 057001 (USNM). Paratype 1 ♂: MTEC 057002 (MTEC).

**Etymology.** The species is named in honor of the Servicio de Protección de la Naturaleza (SEPRONA) in Spain, the unit of the Spanish Guardia Civil in charge of the protection of nature and cultural assets.

**Diagnosis.** *Prototrichalus sepronai* can be separated from other *Prototrichalus* by the combination of pectinate antennae (Figs. 1A–1C, 1F), the subtrapezoidal pronotum (Figs. 1A–1B), the securiform terminal maxillary palpomere (Figs. 1A–1B, 1F) and by the elytral costa IV reaching the posterior half of the elytra (Fig. 1C).

**Description.** Head: transverse, posteriorly partially covered by pronotum, apparently hypognathous (Figs. 1A–1C, 1F). Maxillary palp four-segmented, elongate, with last palpomere securiform (Figs. 1A–1C, 1F). Antennae: reaching middle of elytra; antennomere III 3x longer than pedicel; IV–IX pectinate (Figs. 1C, 1E–1F), antennomere XI elongate. Pronotum transverse, trapezoidal (Figs. 1A–1B), imarginate, narrower than humeral width, angles roundish (Figs. 1A–1B); disc bearing 5 large areole (Figs. 1A–1B); median central areola reaching anterior and posterior margins of pronotum, enlarged in the center and slightly tapered towards margins (Figs. 1A–1B); posterior areolae one third shorter than anterior (Figs. 1A–1B). Elytra: subparallel, ranging from 3x longer than pronotum; costa I short, only reaching basal third of elytra; costae II and III free, not reaching apex (Fig. 1D); costa IV ranging from reaching third fourth of elytral length (Fig. 1C) to half of elytral length. Legs: somewhat slender, elongate; femora and tibiae subparallel (Figs. 1C–1E).

**Length (pronotum+elytra):** 7.5–8.5 mm. **Width (across humeri):** 2.8 mm.

*Prototrichalus milleri* Molino-Olmedo, Ferreira, Branham and Ivie sp. nov.

(urn:lsid:zoobank.org:act:602EFFB2-B9C1-48A9-AD9A-54A655DD0433)

**Figures.** 2 A, B, C, D and E.

**Material examined (1).** Holotype 1 ♀: MTEC 057003 (USNM).

**Etymology.** This species is named in honor of the North American Lycidae systematist, Richard Stuart Miller.

**Diagnosis.** *Prototrichalus milleri* is unique among *Prototrichalus* by the combination of subserrate antennae (Fig. 2A), the elongate maxillary palp that is as long as half the length of antenna, the last palpomere acuminate (Fig. 2D); the elytral costae II and III free not reaching the elytral apex (Fig. 2B), and by the rectangularly emarginate transverse pronotum bearing 7 areolae (Fig. 2E, note the two small areolae laterally adjacent to the central areole).

**Description.** Head: transverse, posteriorly partially covered by pronotum, anteriorly with a small explained projection, apparently hypognathous (Figs. 2A, 2C–2D). Maxillary palp elongate, as long as half the length of antennae, with last palpomere acuminate, subequal in length with III; the sum of the length of the last two palpomeres subequal in length with palpomere II (Fig. 2D). Antennae: reaching 3/4 of elytral length, subserrate. Pronotum transverse, rectangular, imarginate (Fig. 2A), narrower than humeral width, angles roundish (Fig. 2A, 2E); disc region bearing 5 areole (Fig. 2E) and one extra small areole in each posterolateral margin of central areole (Fig. 2E); Elytra: subparallel, 4x longer than pronotum; costa I short, only reaching basal third of elytra; costae II and III or free, not reaching apex (Figs. 2A–2B); costa IV reaching third fourth of elytral length. Female genitalia damaged (Fig. 2B).

**Length (pronotum+elytra):** 9.06 mm. **Width (across humeri):** 2 mm.

*Prototrichalus meiyngae* Molino-Olmedo, Ferreira, Branham and Ivie sp. nov.

(urn:lsid:zoobank.org:act:29F5C27D-EE42-4F91-A98B-2DBD4C673C6E)

**Figures.** 3 A, B, C, D, E.

**Material examined (1).** Holotype 1 ♀: MTEC 057004 (USNM).

**Etymology.** This species is named for Dr. Mei-Ying Lin (Institute of Zoology, Chinese Academy of Sciences) who donated three of the five known specimens of *Prototrichalus* that were used in this study.

**Diagnosis.** *Prototrichalus meiyingae* can be separated from other *Prototrichalus* by the combination of filiform antennae (Figs. 3C–3D), the subtrapezoidal pronotum, elongate maxillary palps that is half as long as the antenna, with last palpomere acuminate (Fig. 3D) and by the elytral costae II and III fused before reaching apex (Figs. 3A–3B).

**Description.** Head: transverse, posteriorly partially covered by pronotum, apparently hypognathous (Figs. 3C–3D). Maxillary palp four-segmented, elongate, as long as half the length of antennae, with last palpomere acuminate (Figs. 3C–3D). Antennae: reaching middle of elytra, filiform; antennomere III 4x longer than pedicel. Pronotum transverse, trapezoidal, imarginate, narrower than humeral width, angles roundish; disc region bearing 5 large areole; median central areola reaching anterior and posterior margins of pronotum, enlarged in the center and slightly tapered towards margins; Elytra: subparallel, 3x longer than pronotum; costa I short, only reaching basal third of elytra; costae II and III fused before reaching apex (Figs. 3A–3B); costa IV reaching second half elytral length. Legs: somewhat slender, elongate; femora and tibiae subparallel (Fig. 3C). Female genitalia, styli very slender, apically located, coxites broadening towards apex (Fig. 3E).

**Length (pronotum+elytra):** 8 mm. **Width (across humeri):** 2.2 mm.

## Discussion

### Comparison with extant taxa and tribal assignment

*Prototrichalus* possesses all the characteristics that define a member of the family Lycidae (see introduction). The new genus has unique characters present only in the *Metriorrhynchini* and *Erotinae*, most specifically the pronotum with multiple carinae forming a pattern of multiple areolae (Kazantsev 2012a, 2012b; Sklenarova et al. 2014). The pectinate male antenna of *Prototrichalus*, present in some *Metriorrhynchini*, excludes it from being placed in the *Erotinae*, which have filiform or subserrate antennae (Kazantsev 2012a, 2012b; Sklenarova et al. 2014; Bocek and Bocak 2017; Bocek and Adamkova 2019).

Additionally, with the *Metriorrhynchini*, *Prototrichalus* shares the presence of four primary distinct elytral costae, with costa I short, specifically present in members of the trichaline group (Bocek and Bocak 2017). However, *Prototrichalus* is very different from any extant genus of *Metriorrhynchini*, lacking the secondary elytral costae and reticulation present in members of this tribe. The morphologically most similar genus is the trichaline genus *Schizotrichalus* Kleine, 1926, but despite the superficial similarity between these genera, *Prototrichalus* has a very different habitus and only convergently resembles *Schizotrichalus*.

*Prototrichalus* exhibits a unique mixture of lycid characters states, not currently known by us to co-exist in modern taxa. Because of this, we choose to place *Prototrichalus* as incertae

sedis in the Metriorrhynchini, as it cannot be placed in any of the current subtribes, in full understanding of the weakness of our conclusion. An alternative choice would be to erect a new Lycidae subfamily, proposing that Prototrichalus is a basal lineage, however, at only 100 Ma old, it is not of an age to be ancestral, as these fossils are at least 1/3 of the way from the most recently proposed origin of the lycids to today (McKenna et al., 2019).

### **Metriorrhynchini as a recognizable group in the mid-Cretaceous**

The Metriorrhynchini are a clade with a Palaeotropical distribution, that hypothetically began its diversification in Eastern Gondwana (Sklenarova et al. 2013). Sklenarova et al. (2013) supported an origin of the group in the late Cretaceous using as their starting point the only calibrated publication available at the time by Bocak et al. (2008). Bocak et al. (2008) proposed the first dated phylogeny of Lycidae based on a penalized likelihood approach, a relaxed-clock method (Ho et al., 2005), and set the in-group age arbitrarily to 100 Ma (Bocak et al. 2008, Supplementary Figure S3) estimating Metriorrhynchini to be as old as  $60.8 \pm 2.36$  Ma.

Sklenarova et al. (2013, 2014) and Bocek and Bocak (2019), using Bocak et al. (2018) as a starting point for their own studies, added further DNA and morphology data in their analyses and were able to hypothesize a more in-depth evolutionary relationship of Metriorrhynchini and date subsequent clades within the in-group. Some of the trichaline lineages estimated to be as old as  $31.95 \pm 3.02$  Ma for Trichalus+Microtrichalus Pic, 1921;  $26.75 \pm 3.02$  Ma for Leptotrichalus Kleine, 1925, and as old as  $51.78 \pm 3.02$  Ma for the clade containing Trichalus sp., Microtrichalus spp., Leptotrichalus spp., Synchronnus sp. and Wakarumbia spp. (Sklenarova et al. 2013). Bocek and Bocak (2019), in a similar but slightly different group delimitation, retrieved very similar age estimates for the trichaline lineage, with an estimation of the origin of the group of at least 50 Ma.

The discovery and description of Prototrichalus and its three species provides direct evidence of Metriorrhynchini as a recognizable group in the mid-Cretaceous, earlier than the original predictions in the Late Cretaceous by Bocak et al. (2008) and the studies that followed. Future phylogenetic analysis of Metriorrhynchini using Prototrichalus as a calibration point should dramatically change the current estimation dates for the tribe and how we understand the origin, dispersal and evolutionary history of the group.

### **Concluding Remarks**

Prototrichalus exhibits a unique mixture of lycid characters states, not known to us to co-exist in any modern Lycidae, and we choose to place it as incertae sedis in the Metriorrhynchini based on similarities on the pronotum and antennae. The discovery of Prototrichalus provides direct evidence of Metriorrhynchini as a recognizable group in the mid-Cretaceous, pushing the origins of the group to be at least 40 Ma beyond what has been previously discussed.

### **Acknowledgments**

The authors wish to express their gratitude to the editor, E. Koutsoukos and to the two anonymous reviewers who greatly contributed for the improvement of this paper. FMO is grateful to Bárbara and Mauricio Odifredi for sending to him some of the specimens used in this study and to Dr. Reyes Peña Santiago (University of Jaén, Spain) for his help with the photographs of his specimens. VSF is grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of Brazil for a PhD scholarship (process 202559/2015-7) and

to the Idea Wild Project Award, that allowed him to acquire part of the photographic equipment utilized in this study. MAB gratefully acknowledges Dr. Mei-Ying Lin for her donation of multiple amber fossils to this study. MAI thanks Ladislav Bocak (Palacký University Olomouc, Czech Republic) for useful discussions about the manuscript in an earlier form. This is a contribution of the Montana Agricultural Experiment Station.

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### **Figure Captions**

**Figure 1.** *Prototrichalus sepronai* sp. nov. Holotype MTEC 057001. **A.** Dorsal habitus. **B.** Pronotum and terminal maxillary palpomere details. Paratype MTEC 057002. **C.** Lateral view and antennae. **D.** Elytral costae III and IV detail; elytral punctuation detail. **E.** Lateral view and antennae. **F.** Antennae and terminal maxillary palpomere details. Scale bars: 2 mm.

**Figure 2.** *Prototrichalus milleri* sp. nov. Holotype MTEC 057003. **A.** Dorsolateral habitus. **B.** Elytral costae III and IV detail; elytral punctuation detail; tarsus detail. **C.** Ventrolateral view. **D.** Maxillary palp detail. **E.** Pronotum and maxillary palpomere details. Scale bars: 2 mm.

**Figure 3.** *Prototrichalus meiyingae* sp. nov. Holotype MTEC 057004. **A.** Dorsolateral habitus. **B.** Elytral costae III and IV details. **C.** Ventral view. **D.** Ventral view of the head and antennae. **E.** Female genitalia and abdomen detail. Scale bars: 2 mm.