

## ***Malalcahuello ocaresi* gen. & sp. n. (Elateridae, Campyloxeninae)**

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### **Abstract**

*Malalcahuello ocaresi* **gen. n. & sp. n.**, from Chile, is described and compared with *Campyloxenus pyrothorax* Fairmaire & Germain, 1860.

### **Keywords**

Coleoptera, Elateridae, Campyloxeninae, *Malalcahuello*, *Campyloxenus*, Chile

### **Introduction**

Fairmaire and Germain (1860) described *Campyloxenus pyrothorax*. Costa (1975) transferred the species to his newly erected monotypic subfamily Campyloxeninae based on the following character states: claws lacking setae near base, hind wings with a wedge cell, female genitalia with a stylus and a very elongate baculum. Stibick (1979) placed it within the Agrypninae based on the presence of prothoracic luminous organs. All authors (Golbach 1994; Lawrence et al. 2010a; Bouchard et al. 2011; and Arias-Bohart and Elgueta 2012) have followed Costa (1975) in retaining *Campyloxenus pyrothorax* within its monotypic subfamily Campyloxeninae. During an ongoing canopy forest fogging surveys over the last decade in Chile (Arias et al. 2008; Richardson and Arias-Bohart 2011) we collected an unknown click beetle which I describe here and place within the Campyloxeninae.

## Materials and methods

Specimens and primary types repositories are from institutional and private collections. Acronyms follow those provided by the institution or Arnett et al. (1993).

<b>ANIC</b>	Australian Insect Collection, Canberra, Australia;
<b>BMNH</b>	British Museum of Natural History, London, England;
<b>EMEC</b>	Essig Museum of Entomology, University of California, Berkeley, USA;
<b>ETA</b>	Elizabeth Arias-Bohart (private collection) Sacramento, USA;
<b>FMNH</b>	The Field Museum of Natural History, Chicago, Illinois USA;
<b>MNHN</b>	Muséum national d'Histoire naturelle, Paris, France;
<b>MNNC</b>	Museo Nacional de Historia Natural, Santiago, Chile;
<b>JEB</b>	Juan Enrique Barriga Tuñón, (private collection) Curicó, Chile;
<b>RBINS</b>	Collections Nationales Belges d'Insectes et d'Arachnides, Institut royal des Sciences Naturelles de Belgique, Brussels, Belgium;
<b>SRC</b>	Sergio Riese (private collection) Genova, Italy.

The following procedure as detailed by Becker (1958) was used for examining male and female genitalia: The last few abdominal segments were removed and placed overnight in a Petri dish with soapy in order to soften the tissues. Male genitalia were extracted, examined and stored in small genitalia vials with 90% alcohol, or glued to a card pinned under the specimen. Measurements using a calibrated ocular micrometer are as follows: total body length from the frontal margin to elytral apex; pronotal length and maximum width of the pronotum and elytral length and maximum width of elytra. Adult morphology follows Gur'yeva (1974), Platia (1994), Calder (1996), Arias (2008), Lawrence and Arias (2009), Lawrence et al. (2010b), Arias-Bohart (2013, 2014). Wing vein nomenclature follows that of Dolin (1975), Kukalova-Peck and Lawrence (1993, 2004). Locality data were taken directly from labels where / = line separation and // = new label. Approximate GPS, when not available, its provided underlined. Locality data for JEB material can be accessed at <http://www.coleoptera-neotropical.org>. Drawings were made using a camera lucida on a Leica MZ7 dissecting scope. Drawings were made using a camera lucida on a Leica MZ7 dissecting scope. Type material has been databased with a unique number indicated on the label information consisting of the acronym EMEC and the identification number. For example, the holotype of *Malalcahuello ocaresi* sp. n. has the unique number EMEC117539 that can be accessed at <http://essigdb.berkeley.edu>.

## Taxonomy

### *Malalcahuello* gen. n.

<http://zoobank.org/E87CF381-6C94-49F2-AFB4-832AF2EF264B>

**Type species.** *Malalcahuello ocaresi* sp. n., here designated.



**Etymology.** The generic name Malalcahuello (gender masculine) is derived from the type locality of origin of the genus, Malalcahuello, in southern Chile. The word Malalcahuello derives from Mapudungun language *malal* = barnyard and *kahuellu* horse (Musigraf 2003).

**Diagnosis.** This genus differs from all other elaterid genera by the following combination of characters: strongly serrate antennae from antennomere 3 onwards, antennomere 2 very small, length about 0.4 times as long as antennomere 3; pronotum 0.76–0.99 as long as wide, convex, without deep impressions basally, lacking bioluminescent organs; stout, and protruding posterior angles with apex truncate; mesocoxal distance about 0.16 times mesocoxal cavity; wing venation with R cell elongate 4.2 times its width and wedge cell length 4 times its maximum width.

**Description.** Body about 3.27–3.87 times as long as wide; pronotal sides slightly sinuated, narrower than elytral sides. Elytral maximum width at posterior third; elytral apices softly rounded, not meeting at mid-line. Dorsal vestiture short, spare, fine, with some erect and decumbent short, well distributed hairs (Fig. 1).

Head slightly declined at base, transverse, ratio of median length to greatest postocular width 0.30–0.42. Eyes medium size, protuberant in both sexes, faceted, without interfacetal hairs. Supra-antennal ridges raised above, each antennal fossa with deep curved invagination between antennal insertion and eye; short (Fig. 5). Fronto-clypeal region completely carinate, produced forward, not concealing clypeus; frontoclypeal carina rugulose; clypeus length about 4.8 times its width. Labrum elongate, sclerotized, sinuate basally.

Antennae in male surpassing posterior angles, antennomeres 3–10 strongly serrate, antennomere 11 elongate, longer than preceding ones; all antennomeres clothed with short, semi-decumbent goldish hairs and few erect, goldish long hairs. Female antennae shorter than male antennae (Fig. 2).

Prothorax subquadrate, sides slightly sinuated, carinate and emarginate, visible for their entire lengths from above; posterior angles stout, protruding, with apex truncate and produced posterolaterally embracing humeral area; posterior edge without scutellar notch; disc finely punctate, clothed with gold sinuated vestiture; prosternum strongly combed, with deep punctures; notosternal suture complete, strongly sinuated, open at anterior end, curved at posterior end; prosternum puncticulate, with semi-erect, sparse hairs; prosternal process slightly narrower near base, then gradually expanded posteriorly, following procoxae in lateral view, extending well behind procoxae. Hypomeron simple, depressed medially, with deep punctures. Procoxae subglobular (Fig. 17).

Scutellum not elevated, flat, anteriorly simple, posteriorly rounded, notched on the sides, all borders well defined, tongue-shaped. Elytra about 2.81–2.54 times as long at midline as greatest width and 4.43–5.02 times as long as pronotum; anterior edge carinate; humeri well developed; parallel-sided at anterior third, gradually enlarging towards posterior third, converging posteriorly, apices rounded, not meeting and central midline. Disc with 10 defined puncture rows.

Mesoventrite on same plane as metaventrite; mesocoxae projecting, mesocoxal cavities narrowly separated, open laterally to mesepisternum; mesocoxal distance 0.25

times mesocoxal diameter; mesosternal posterior region excavated and 0.23 times mesocoxal diameter length (Fig. 7); metacoxae obliquely oriented, with plates extending narrowing towards body side; posterior region of mesosternite length 0.35 times as mesocoxal diameter length.

Hind wing about 2.63–2.66 times as long as wide; apical field about 0.6 times as long as total wing length, with 2 pigmented oblique linear sclerites; radial cell well developed, elongate, length 4.1 times as long as wide, with inner posterobasal angle acute; cross-vein r3 long, length about 2.2 times length of radial cell, horizontal and arising away from r4, which is slightly straight and complete; base of RP very long, extending to wing base; R-M loop forming narrowly acute angle; medial spur arise and then straight; medial field with five free veins; MP3+4 branching in 2 long veins; wedge cell length about 2.8 times its width (Fig. 9).

Tarsomeres 1–3 elongate, tarsomere 4 smaller than precedents; pretarsal claws simple; empodium short, not extending between claws; tarsomeres 2, 3 and 4 lobate (Fig. 18).

Female genitalia: bursa copulatrix globular, space shuttle shape from above, one spherical thicker gold gland medially, sides of bursa with 2–3 spinules semi-curved (Figs 11, 12, 13).

Male genitalia: aedeagus symmetrical, phallobase broadly rounded; each paramere with a lateral hook at apex; median lobe attached to parameres both dorsally and ventrally (Fig. 22).

**Distribution.** Southern Chile: provinces Ñuble and Malleco.

***Malalcahuello ocaresi* sp. n.**

<http://zoobank.org/B58F21AD-7022-415E-AA42-521FF0F97973>

Figs 1, 2, 5, 7, 9, 11–13, 17, 18, 20, 22

**Etymology.** This species is named in honor of Sergio Ocares Figueroa, long time friend, and excellent insect collector from Los Lleuques, Region VIII, Chile.

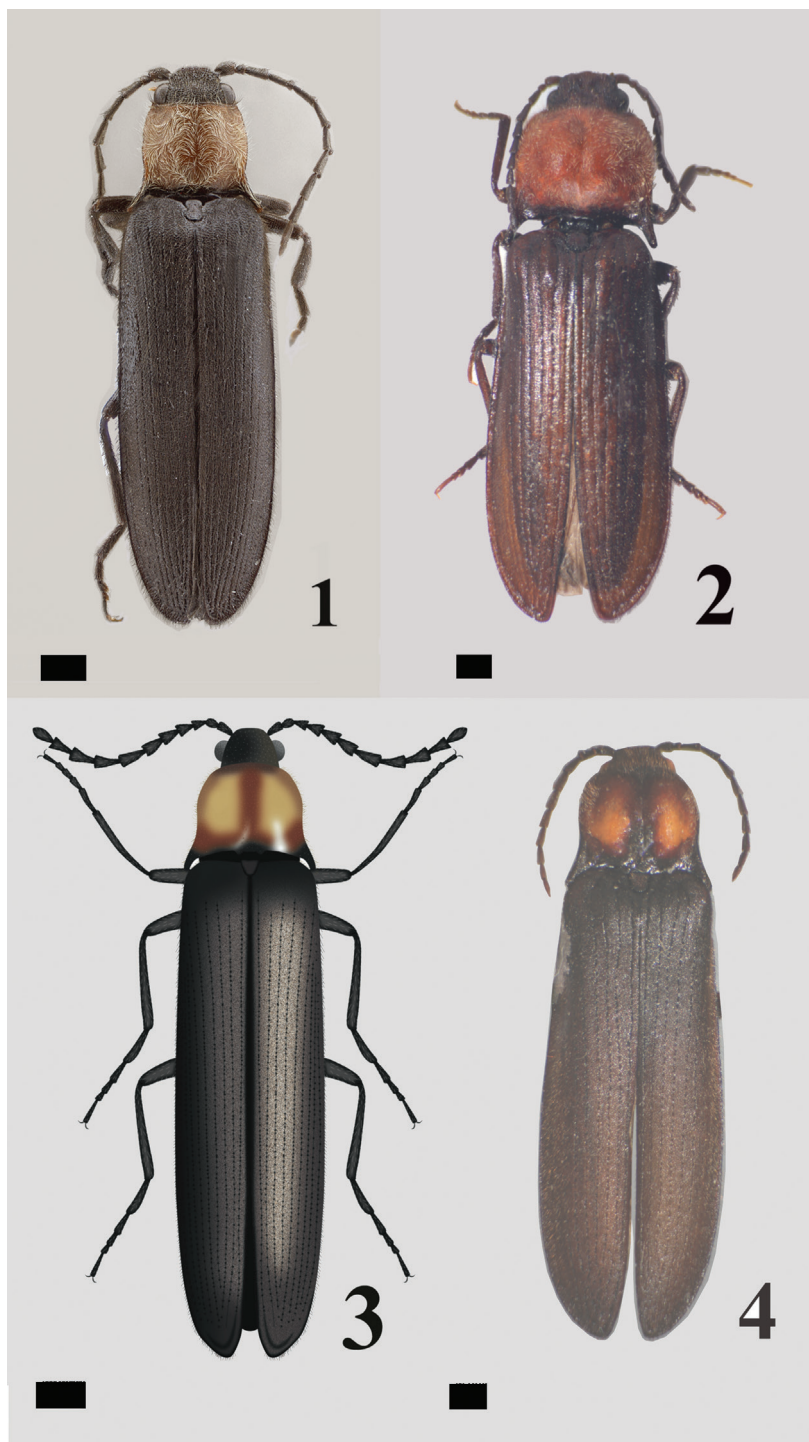
**Description.** Holotype: male, total body length 11.63 mm, width 3.00 mm. (Fig. 1).

Head and mandibles dark brown; antenna same color as head.

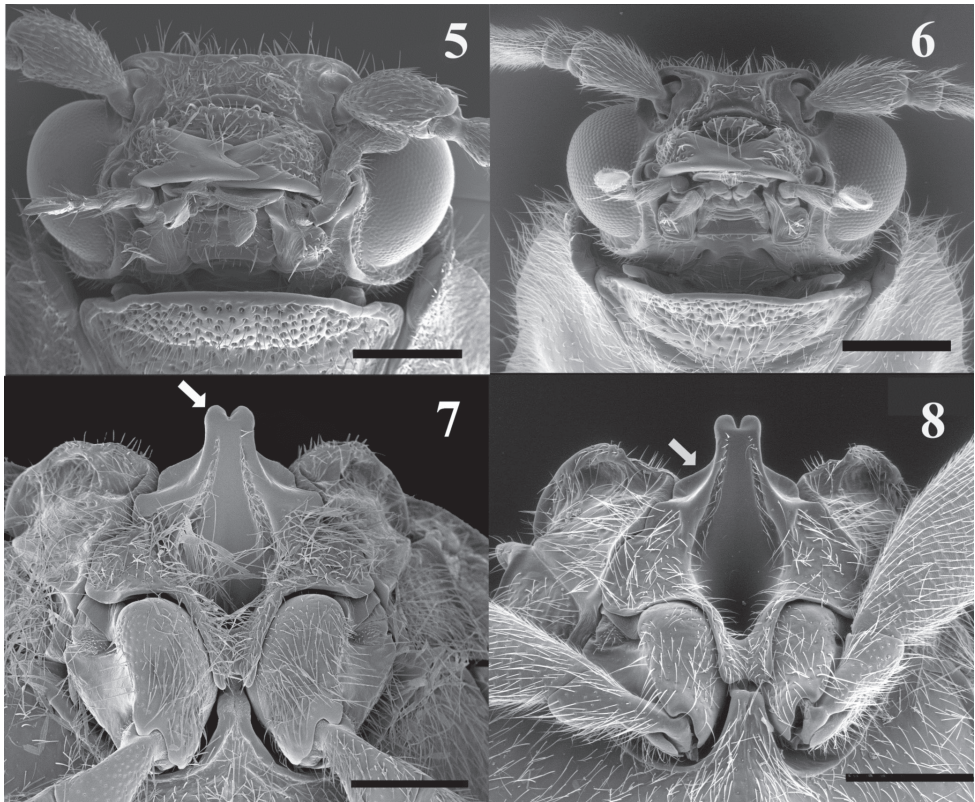
Pronotum about 0.76–0.99 times as long at midline as its greatest width; reddish, with long, gold semi-decumbent curved hairs; punctate, punctures separated for more than one own diameter; prosternal spine about 0.63 times as long as diameter of procoxal cavity.

Scutellum black or same color than elytra, anterior border sometimes darker; with long, thin, gold semi-decumbent hairs; Elytra about 2.54–2.81 times as long at midline as its greatest width; black or dark brown; elytral punctures closely aligned in rows forming a defined striae; posterior edge of mesosternal cavity excavate. Legs brown, vestiture black; tarsomeres 2, 3 and 4 with lobes, lobes of tarsomeres 3 and 4 spongiose and extending well beyond tarsomere length apically (Fig. 18).

Aedeagus. Length 1.89 mm, and 0.35 mm wide; parameres apex globose with a hook, with at least 3 strong setae (Fig. 22).



**Figures 1–4.** 1–2 Adult *Malalcahuello ocaresi* sp. n.: male (1), female (2) 3–4 Adult of *Campyloxenus pyrothorax*: male illustration by Nancy Arias Tobar (3), female (4).



**Figures 5–8.** 5–6 SEM illustration of frontal head of: *Malalcahuello ocaresi* (5), *Campyloxenus pyrothorax* (6) 7–8 SEM illustration of mesoventral cavity of, *Malalcahuello ocaresi* (7), *Campyloxenus pyrothorax* (8). Scale bar = 0.5 mm.

**Distribution.** Southern Chile. Provinces: Ñuble and Malleco.

**Remarks.** Variation within species ranges in males 11.06–13.09 mm in length, and females 15.25–15.75 mm in length; elytra color black to dark brown.

**Type material.** HOLOTYPE: ♂ Chile VIII Region/ Las Trancas 18/24/xii/2005 Malaise Trap/ Arias & Ocares UC Berkeley 36°54'26"S, 71°29'36"W // EMEC117539// [MNNC]

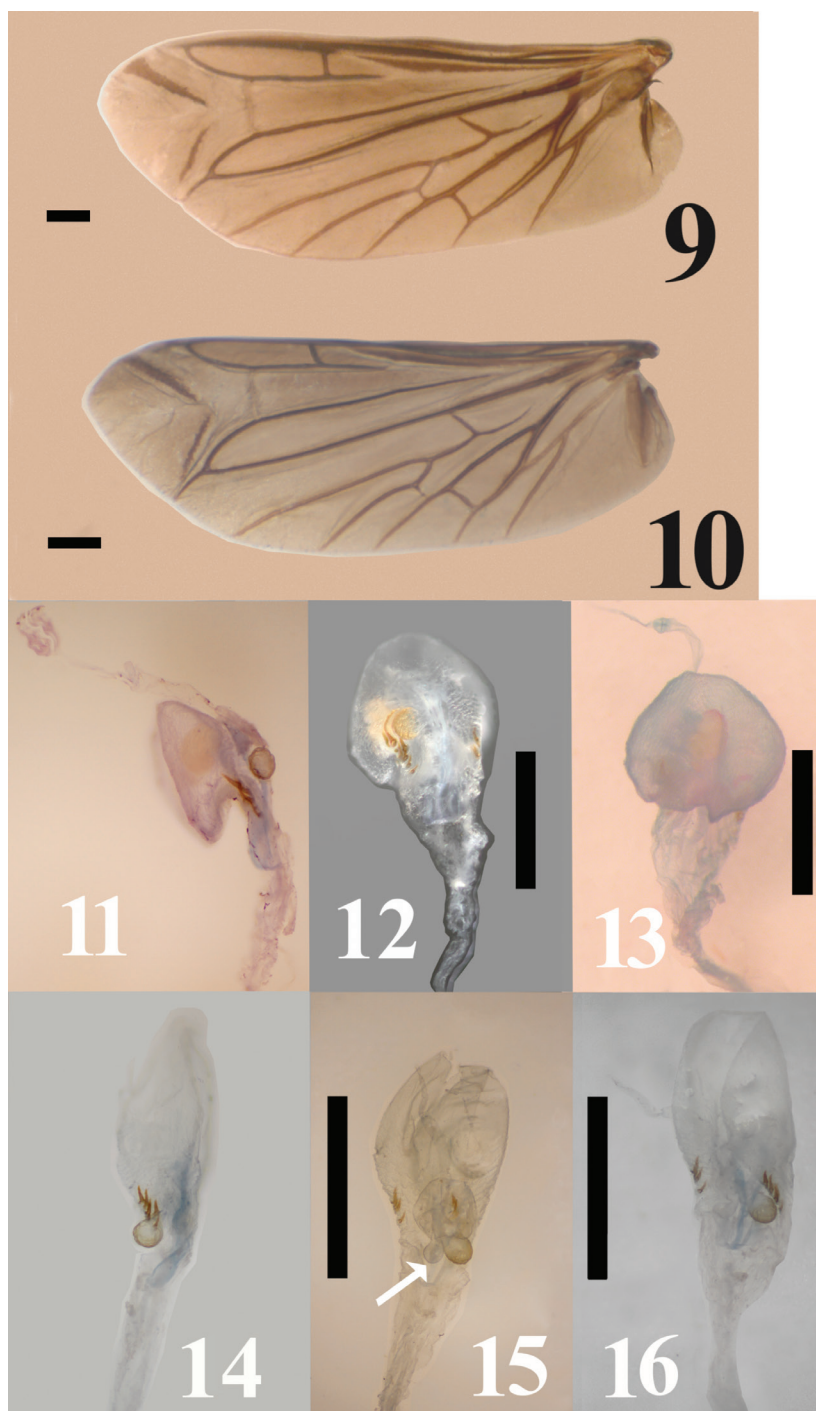
PARATYPES: ♀ Chile Malleco/ Tolhuaca Curac./ 15.i.1950 L. Peña// Alotipo (red) 38°18'36"S, 71°38'42"W // EMEC113596// [SRC]

♀ Chile Malleco/ Río Blanco Cur. / 15.ii.1954 L. Peña E. // 38°13'00"S, 72°20'00"W // EMEC10006010// [JEB]

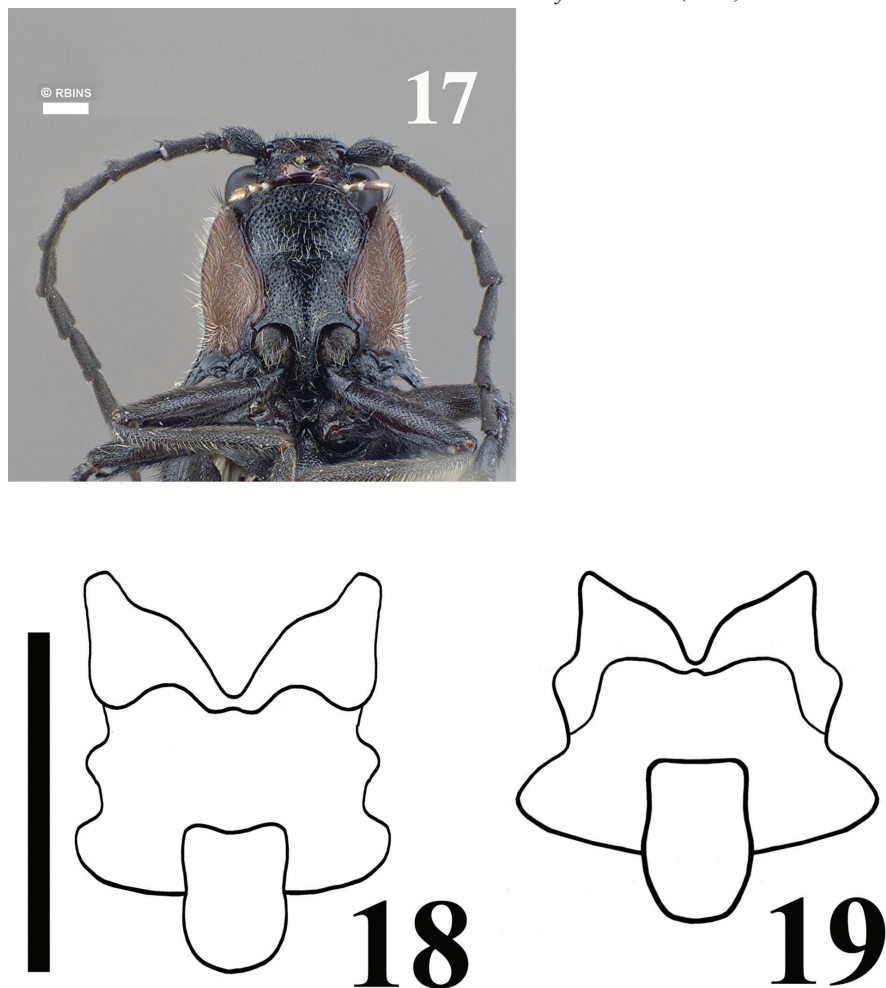
♀ Chile Malleco/ Río Blanco Termas / 15.i.1974 L. Peña // 38°13'00"S, 72°20'00"W // Paratipo// (wings on a card) // Ex-COLECCION / Jorge Valencia / JVCC / Chile 003256 // EMEC10006013// [JEB]

♀ Chile Malleco / I 1996/ A. / Ugarte // 38°13'00"S, 72°20'00"W // EMEC10006017// [MNHN]





**Figures 9–16.** 9–10 Wing venation illustration of *Malalcahuello ocaresi* (9), *Campyloxenus pyrothorax* (10) 11–13 Female genitalia *Malalcahuello ocaresi* 14–16 Female genitalia *Campyloxenus pyrothorax*. Scale bar = 0.5 mm. (11–16); 1 mm (9–10).



**Figure 17–19.** 17 Ventral view of *Malalcahuello ocaresi*. 18–19 SEM illustration of tarsomeres of: *Malalcahuello ocaresi* (18), *Campyloxenus pyrothorax* (19). Scale bar = 0.5 mm.

♂ CHILE Malleco Pr.:/ Malalcahuello, 13.7 km E/ of on road to Lonquimay./ 1565m 38°26.15'S/ 71°29.26'W// 24.xii.1996–6.ii.1997. /Nothofagus pumilio/Araucaria araucana/ forests w/Chusquea // FMHD #96-234. Flight intercept trap/ A. Newton/ & M. Thayer 1978// EMEC117552 // [MNNC]

♂ Chile VIII Region/ Las Trancas 18/24/xii/2005 Malaise Trap/ Arias & Ocares UC Berkeley 36°54'26"S, 71°29'36"W //EMEC10006015// [RBINS]

♂ Chile VIII Region/ Las Trancas 18/24/xii/2005 Malaise Trap/ Arias & Ocares UC Berkeley 36°54'26"S, 71°29'36"W //EMEC113597// [ETA]

♂ Chile Ñuble Shangrila/ 6-11/XII.1998. / J Mondaca / 36°54'26"S, 71°29'36"W //EMEC109681// [EMEC]

♂ CHILE Malleco Pr.:/ Malalcahuello, 13.7 km E/ of on road to Lonquimay./ 1565m 38°26.15'S/ 71°29.26'W// 24.xii.1996–6.ii.1997. /Nothofagus pumilio/Araucaria



**Figures 20–23.** 20–21 Tarsomeres of *Malalcahuello ocaresi* (20), *Campyloxenus pyrothorax* (21) 22–23 Male genitalia of *Malalcahuello ocaresi* (22), *Campyloxenus pyrothorax* (23). Scale bar = 0.5 mm.

araucana/ forests w/Chusquea // FMHD #96-234. Flight intercept trap/ A. Newton/ & M. Thayer 1978// EMEC110090 // [FMNH]

♂ Las Trancas / VIII Region Chile. Enero 1982/ Coll. T. Curkovic. // 36°54'26"S, 71°29'36"W // EMEC10006011// [BMNH]

♂ Chile Ñuble Prov. / Las Trancas 19.5 km/ E Recinto. 1250, / trap site 647/ 10.xii.82–3.i.1983/ Nothofagus Forests/ A. Newton & M. Thayer // Window/trap 647 36°54'26"S, 71°29'36" // EMEC10006012 // [FMNH]

♂ Chile Malleco/ Rio Blanco Cur./ 15.ii.1954 L. Peña E. [JEBT] Ex-COLECCION / Jorge Valencia / JVCC / Chile 001619 // COLECCION JEBC / Juan Enrique / Barriga-Tuñon / Chile 0203579 // 38°13'00"S, 72°20'00" // EMEC10006013// [JEB]

♂ Chile 1400 mts/ prov. Malleco/ vn. Lonquimay/ 22-Dic-1994/ Leg. J. E. Barriga// Coleccion JE Barriga/ // 38°22'36"S, 71°35'00"W// 46023//EMEC10006014// [ANIC]

♂ CHILE REGION IX (LA ARAUCANIA)/ P.N. Villarrica. Paso Mamuil Malal/ Araucaria Picnic area/ S39°34'283, W71°29'908, 1100 m/ 19.January.2006 sweeping & beating/ A.B.T. Smith, M. J.Paulsen // in a vial// EMEC10006016// [ETA].

Other Material studied: *Campyloxenus pyrothorax* Fairmaire et Germain, 1860.

♂ Chile Bío Bío / Los Angeles/ 26-12-1940 /37°28'S 72°21'W; CHILE Region IX/ Parque Huerquehue, 2825'39°92'S/71°43.323'W, xii-11-2001. Malaise trap / Arias et al Berkeley; Chile Bío Bío/ Los Angeles/ 26-12-1940/ B Orellana Colector 37°28'S 72°21'W; CHILE Region IX/ Parque Huerquehue, 2825'39°92'S/71°43.323'W xii-11-2001. Malaise trap / Arias et al., Berkeley; Chile VIII Region/ Las Trancas 18/24/ xii/2005 Arias & Ocares UC Berkeley 36°54'26"S, 71°29'36"W; 52.- Chile X Region/ Oncol Park/ Calfuco Way/ Fogging 14:13PM. 150cc / I/ 07 / I / 2007. 515m 14°C / 39°42.114/ 73°19.244/ *Saxegothaea conspicua* 35m/ Arias et al., UCB; 8-CHILE VIII Region/ PN Nahuelbuta. Pichinahuel Exit/ 37°48.341'S/ 73°02.112'W /1215m/ 05.XII.2001. Canopy Fogging GT/ *Araucaria araucana* F/ Arias & Andrews et al., UCB; ♀ (2): CHILE REGION IX (LA ARAUCANIA)/ P.N. Villarrica. Paso Mamuil Malal/ Araucaria Picnic area/ S39°34'283"W 71°29'908", 1100 m/ 19 January 2006 sweeping & beating/ A.B.T. Smith, M.J. Paulsen.

### Key to separate Chilean genera of Campyloxeninae

- 1 Clypeus more than 4 times as long as its width (Fig. 7); pronotum with long, decumbent vestiture, lacking luminous spots and a deep circular impression basally (Figs 1, 2); lobe of tarsomere 4 extending apically about 0.7 times length of tarsomere 4 (Fig. 20).....***Malalcahuello* gen. n.**
- Clypeus less than 4 times as long as its width (Fig. 8); pronotum with short, erect vestiture, with luminous spots and with a deep circular impression basally (Figs 3, 4); lobe of tarsomere 4 extending apically less than 0.5 times length of tarsomere 4 (Fig. 21) .....***Campyloxenus* Fairmaire & Germain, 1860**

### Discussion

*Campyloxenus* and *Malalcahuello* belong to the subfamily Campyloxeninae since they share the following characters (additionally to those of Costa 1975): body somewhat soft; long serrate antennae from antennomere 3; stout and protruding posterior angles; anterior region of mesosternum anteriorly produced and bilobate (arrow in Fig. 7), anterior articulating surfaces of mesosternum well-developed (arrow in Fig. 8); mesosternal cavity oval, not deep, open to mesepimerum and to mesepisternum; pre-scutum v shape, scutellum not notched and somewhat subrectangular (Figs 18, 19); tarsomeres 1–4 with lobes, tarsomeres 1–2 with very small lobes (Figs 20, 21); sexual dimorphisms,



females are larger than males and present shorter antennae. Both *Campyloxenus* and *Malalcahuello* are monotypic genera.

*Malalcahuello* differs from *Campyloxenus* by the following (contrasting characters for *Campyloxenus* in parentheses): frontoclypeal carina frontally rugulose (frontoclypeal carina frontally not rugulose); lacking bioluminescent organs (exhibits bioluminescent organs); clypeus about 4.8 times as long as wide (clypeus about 3.6 times as long as wide); clypeus and labrum with thin hairs (clypeus and labrum with thick hairs); pronotal sides slightly sinuated (Figs 1, 2) (pronotal sides strongly sinuated (Figs 3, 4)); lobe of tarsomere 4 extending apically about 0.7 times length of tarsomere 4 (lobe of tarsomere 4 extending apically less than 0.4 times length of tarsomere 4); bursa copulatrix shuttle space-shape (Figs 11–13) (bursa copulatrix elongate (Figs 14–16)).

Members of the Elateridae generally exhibit a hard body, but members of the subfamily Campyloxeninae exhibit a soft-body trait that is also found within the Elateriformia, in Dascillidae, Elmidae, Ptilodactylidae and Psephenidae (Bocakova et al. 2007). Within the subfamily Campyloxeninae, only *Campyloxenus* exhibits bioluminescent organs. These are lacking in *Malalcahuello*. Bioluminescence is limited to the tribes Pyrophorinae and Hapsodrilini within the Elateridae (Colepiccolo-Neto et al. 1986), and the genus *Balgus* (Costa, 1984) that has been placed in Thylacosterninae (Vahtera et al. 2009). Most species of Coleoptera possessing bioluminescent organs exhibit soft bodies are members of the cantharoid section of Elateriformia. Kundera et al. (2014) indicated multiple origins for the soft-bodied trait and bioluminescent organs. *Malalcahuello* lacks bioluminescent organs and its body is harder than *Campyloxenus*. Future molecular studies of endemic Campyloxeninae may elucidate their systematic position within the Elateriformia.

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# The *Mycetophila ruficollis* Meigen (Diptera, Mycetophilidae) group in Europe: elucidating species delimitation with COI and ITS2 sequence data

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## Abstract

European species of the *Mycetophila ruficollis* group are compared on the basis of morphology and sequences of mitochondrial cytochrome oxidase subunit one (COI) and the ITS2 region of nuclear ribosomal DNA. The study represents the first evaluation of morphology-based species delimitation of closely related fungus gnat species by applying molecular information. Detailed descriptions and illustrations of the male terminalia are presented along with a key for the identification of all nine European species of the group. Phylogenetic analyses of molecular data generally supported the morphological species discrimination. The barcoding region of COI superseded ITS2 rDNA in resolving species. In the COI barcoding region interspecific differences ranged from 2.9 to 10.6% and the intraspecific distance from 0.08 to 0.8%. Only COI data distinguished between the similar and closely related *M. ichneumonea* and *M. uninotata* of which the latter was observed to include cryptic species. The host range of some species is suggested to be narrower than previously considered and to depend on the forest type. Presented evidence indicates the importance of analysing sequence data of morphologically very similar mycetophages reared from identified host fungi for elucidating species delimitation as well as their geographic and host ranges. New country records, viz. Estonia for *M. evanida*, Georgia for *M. ichneumonea*, *M. idonea* and *M. ruficollis*, and Norway for *M. strobli*, widen the known distribution ranges of these species.

## Keywords

Fungus gnats, *Mycetophilini*, mycetophages, morphology, phylogenetic analysis, taxonomy, DNA barcoding

## Introduction

*Mycetophila* Meigen, 1803 is one of the largest and earlier described genera among fungus gnats (Diptera: Mycetophilidae). The first fungus gnat ever described is today known as *Mycetophila fungorum* (De Geer, 1776), a widespread and common species in the Palaearctic region. Since then more than 650 species from all biogeographical realms have been described in the genus (Bechev 2000, Zaitzev 2003, Oliveira and Amorim 2014). Based on morphological characters, the genus has been divided into several subgenera (particularly in the Neotropical region: cf. Lane 1955) and species-groups (particularly in the Holarctic region: cf. Laffoon 1957, Laštovka 1963, 1972, Zaitzev 1999). However, neither analyses addressing the intrageneric phylogeny nor keys to all species have yet been provided, with the most exhaustive presented by Laffoon (1957) for the Nearctic species and Zaitzev (2003) for the Palaearctic species.

One of the most clearly delimited and supposedly monophyletic intrageneric subdivisions is the *M. ruficollis* Meigen species-group, introduced by Laštovka (1972). Members of the group (see Figs 1–3) are morphologically characterised by 1) mid tibiae without ventral bristles, 2) bM-Cu setose, with 8 or more setae and 3) wings with central spot only (except one Nearctic species; Laštovka and Kidd 1975). The general outline of male terminalia and absence of ventral setae on mid tibia are shared with *M. fungorum* and allied species, which are otherwise devoid of setae on bM-Cu and form another intrageneric group of species. Within the limits of the *ruficollis*-group, there are 19 currently recognised species: 17 of them are from the Holarctic (Laštovka 1972, Laštovka and Kidd 1975, Chandler and Ribeiro 1995, Wu 1997) and two from the Oriental region (Wu 1997). The records of *M. ruficollis* Meigen, 1818 in the Afrotropical region are based probably on misidentifications (cf. Matile 1980) and represent obviously undescribed species. Laštovka (1972) discussed about 30 closely related species in the group which also include supposedly undescribed species, especially those from the Oriental region, known to him at that time. Eleven Holarctic species were covered by a detailed study by Laštovka (1972) including a key to species, while seven species from the Palaearctic region were described subsequently by Laštovka and Kidd (1975), Chandler and Ribeiro (1995) and Wu (1997). All species with known biology are mycetophagous in their larval stage, colonising a variety of Agaricales, Russulales and to lesser extent Boletales, with one species reared from Polyporales (e.g. Yakovlev 1994, Ševčík 2010). However, little is known about the host range of species in this group. On the basis of published data, their larvae seem to be most frequent in fruit bodies of *Russula*, *Lactarius*, *Cortinarius* and *Pholiota*, tending to avoid species of Boletales (e.g. Hackman and Meinander 1979, Krivosheina et al. 1986, Kurina 1994, Ševčík 2010, Pöldmaa et al. 2015).

The *M. ruficollis* species-group includes morphologically similar species which are reliably identifiable only by comparing details of male terminalia. While discussing intraspecific variability, Laštovka (1972) noted that in spite of variable coloration of the body, the characters in male terminalia are generally constant but vary in some details. Moreover, Laštovka and Kidd (1975) described *M. britannica* in two morphological





**Figures 1–3.** *Mycetophila ichneumonea* Say, 1823, a typical member of the *M. ruficollis* group. **1** male habitus **2** head with maxillary palpi, closer view **3** male terminalia, closer view. Scale bar = 1 mm (**1**), 0.5 mm (**2**) and 0.2 mm (**3**). Abbreviations: plp = segments of maxillary palpus; gc = gonocoxite; gst d = dorsal branch of gonostylus; gst v = ventral branch of gonostylus.

forms and noted that *M. ichneumonea* may be polytypic consisting of 2–3 subspecies. This led us to suggest that the species-group may hide some undiscovered diversity, i.e. cryptic species with possibly different larval diet.

One of the most important and frequently used set of characters for delimiting cryptic species is that obtained from DNA sequence analyses. In studies of fungus gnats, DNA sequence data are so far mostly used to clarify phylogenetic relationships of subfamilies and/or genera (e.g. Rindal et al. 2009, Martinsson et al. 2011, Ševčík et al. 2014) but also to associate sexes of one species (Kurina et al. 2011) and compensate for the deficiencies of the morphological component in species identification of otherwise obscure material (Pöldmaa et al. 2015). For delimiting species, molecular characters have thus far been incorporated only in the genus *Neuratelia* (Kurina et al. 2015). In addition to the widely used 'DNA barcode' fragment (COI) (e.g. Hebert et al. 2003, Hajibabaei et al. 2006), ITS2 sequences have successfully been applied for species delimitation. The aim of this study was to 1) test morphological species delimitation by the application of molecular methods, 2) present a key to European species of the *M. ruficollis* group supplemented by modern illustrations of male terminalia, and 3) search for possible cryptic species. Because this group includes morphologically extremely similar species, they are frequently being identified only to species-group level even by specialists. Our intention was to provide also a reliable DNA reference dataset that could be used in further DNA based identification of fungus gnats.

## Material and methods

### Morphological analyses

The study is based on material collected throughout the Europe during 1984 to 2014 mostly by Malaise traps, light traps and sweepnetting. In addition, several specimens from Lebanon and Georgia are also included. A part of the Estonian material was reared from macrofungi. For that, fruit bodies were isolated into plastic containers and covered with nylon gauze, while peat was used as a pupation substrate. Containers were incubated in a lab facility and checked every other day while emerged adults were collected by an aspirator (see also Pöldmaa et al. 2015). Altogether 116 male specimens of the *M. ruficollis* group were morphologically studied.

The majority of the included material was initially collected into 70 % ethyl alcohol and studied under stereomicroscopes Olympus SZ61 or Leica S8APO. For detailed study of male terminalia they were detached and macerated in a solution of KOH followed by neutralization in acetic acid and washing in distilled water (for details see Kurina 2003). The remaining chitinous structure was thereafter separated to several anatomical units which were: 1) inserted into glycerine for study and preserved as glycerine preparations or 2) slide-mounted individually in Euparal between two pieces of coverslip allowing them to be studied from both sides under a compound microscope (for details see Hippa and Kurina 2012). The preservation method of each specimen is indicated in the material sections. The habitus photo was taken from a specimen in alcohol. All photos of male terminalia were taken from preparations in Euparal and combined by software LAS V.4.1.0. from multiple gradually focused images taken by



a camera Leica DFC 450 attached to the compound microscope Leica DM 6000 B (see also Kurina and Oliveira 2013). Morphological terminology follows generally that of Sølvi et al. (2000) while several specific terms of male terminalia (see Table 1) are used according to Laštovka (1972). The term “bristle” is used for a seta that is significantly larger in length and diameter than surrounding setae (see also Merz and Haenni 2000). Detailed revised descriptions of male terminalia figured by Laštovka (1972) and Laštovka and Kidd (1975), supplemented by illustrations, are provided herein.

**Table 1.** Terminology used for describing male terminalia with synonyms from earlier studies and references to corresponding figures.

Present study	Laštovka 1972, Laštovka and Kidd 1975	Corresponding figures and used abbreviations
gonocoxite	gonocoxopodite	Figs 3, 33, 34 – gc
posterior margin of gonocoxite	posterior margin of gonocoxopodite	Fig. 34 – gc pm
posterior impression of gonocoxite	posterior impression of gonocoxopodite	Fig. 34 – gc pi
anterior impression of gonocoxite	anterior impression of gonocoxopodite	Fig. 34 – gc ai
dorsal branch of gonostylus	dististyle	Fig. 3 – gst d; Figs 6–14
ventral branch of gonostylus	basistyle	Fig. 3 – gst v; Figs 15–32
posterior margin, lateral margin, basal margin and basal angle of dorsal branch of gonostylus	posterior margin, lateral margin, basal margin and basal angle of dististyle	Fig. 6 – pm, lm, bm, ba
distal posterior process and proximal posterior process of dorsal branch of gonostylus	distal posterior process and proximal posterior process of dististyle	Fig. 6 – dpp, ppp
medial bristle of dorsal branch of gonostylus		Fig. 6 – mb
posterior process of ventral branch of gonostylus	posterior process of basistyle	Figs 15, 16 – pp
spines 1–4 on the ventral branch of gonostylus	spines 1–4 on the basistyle	Figs 15, 16 – sp 1, sp 2, sp 3, sp 4
aedeagal complex	intromittent organ	Figs 33–44
aedeagus	aedeagus	Figs 33, 35 – aed
ejaculatory apodeme	penis tube	Fig. 35 – ej ap, ej ap b
rim of ejaculatory apodeme	rim of penis tube	Fig. 35 – ej ap r
aedeagal guide	penis sheath	Fig. 33 – aed gd
lateral impression on aedeagal guide	lateral impression on penis sheath	Fig. 33 – aed gd li
aedeagal apodeme	thecal apodeme	Figs 33, 35 – aed ap

The following acronyms are used for depositories:

- IZBE** Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences [former Institute of Zoology and Botany], Tartu, Estonia;  
**JSPC** Jukka Salmela private collection (Rovaniemi, Finland);  
**PCPC** Peter Chandler private collection (Melksham, United Kingdom).

## Molecular techniques

Based on the preliminary morphological determination, at least one male specimen from every species from each locality was allocated for DNA sequencing. For that, after detaching terminalia, the rest of the abdomen or a leg was placed in the lysis buffer, preserving the rest of the specimen. DNA was extracted by incubating the material overnight at 56 °C in 10X Reaction Buffer B (Solis Biodyne, Tartu, Estonia) with the addition of 2.5 µl (20 mg/ml) proteinase K (Fermentas, Lithuania). After 15 min at 98 °C the material was centrifuged and DNA solution pipetted into a new tube.

In 66 specimens, the 658 bp barcode region at the 5' end of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified and sequenced with primers Lep-F1 and Lep-R1 (Hebert et al. 2004) or LCO1490 and HCO2198 (Folmer et al. 1994). In most of the specimens an additional 790 bp, following the barcoding region, were amplified and sequenced with primers C1-J-2195 and TL2-N-3014 (Simon et al. 1994). In 33 specimens, the second fragment of the internal transcribed spacer region (ITS2) was amplified and sequenced using primers ITS2A and ITS2B (Beebe and Saul 1995). PCR was performed in a total volume of 25 µl, with the reaction mixture containing 1X HOT FIREPol® Blend Master Mix Ready to Load (Solis BioDyne, Tartu, Estonia), 10 pmol of primers and 20–80 ng of DNA. PCR was carried out in an Eppendorf Mastercycler epigradient thermocycler (Eppendorf AG, Hamburg, Germany). The initial denaturation at 95 °C for 15 min was followed by 35 cycles of 30 s at 95 °C, 30 s at 45–60 °C (depending on primers) and 1 min at 72 °C, followed by a final extension at 72 °C for 10 min. PCR products were visualised on a 1.2% agarose gel, and the remaining PCR product was purified with fast alkaline phosphatase and exonuclease I (Thermo Scientific, Pittsburgh, USA). DNA sequencing was performed at MacroGen Europe (Amsterdam, Netherlands) or at the Estonian Biocentre (Tartu, Estonia). All sequences obtained in this study were deposited in GenBank under accession numbers KR997602–KR997703.

## Phylogenetic analyses

The sequences were edited and assembled with Sequencher 5.1 (Gene Codes, Ann Arbor, MI, USA), aligned with Mafft 6 online version (Kato and Toh 2008) and edited manually using GeneDoc 2.6.0.3. Phylogenetic analyses were performed on the separate and combined COI and ITS2 datasets. Four sequences of *Mycetophila fungorum* were used as an outgroup in all analyses. Bayesian analyses were performed on all three datasets using MrBayes at the Cipres website (<http://www.phylo.org>) with default settings of the online version and invgamma model. Each analysis was run for 10 mln generations, of which every 1000th was sampled. The first 25% of sampled trees were discarded as burn-in. Posterior probabilities were calculated from remaining 7500 trees. Parsimony analyses were performed in PAUP (Swofford 2003). During the 1000 random searches 50 best trees were kept in each search that were all used for

additional swapping. This was done until the limits of available computer memory was reached. The most parsimonious trees obtained were used to calculate the strict consensus. 1000 bootstrap replications were performed to assess branch support. Intra- and interspecific distance variance was calculated in MEGA6 (Tamura et al. 2013) using Kimura 2-parameter model (see e.g. Waugh 2007, Öunap and Viidalepp 2009).

## Results

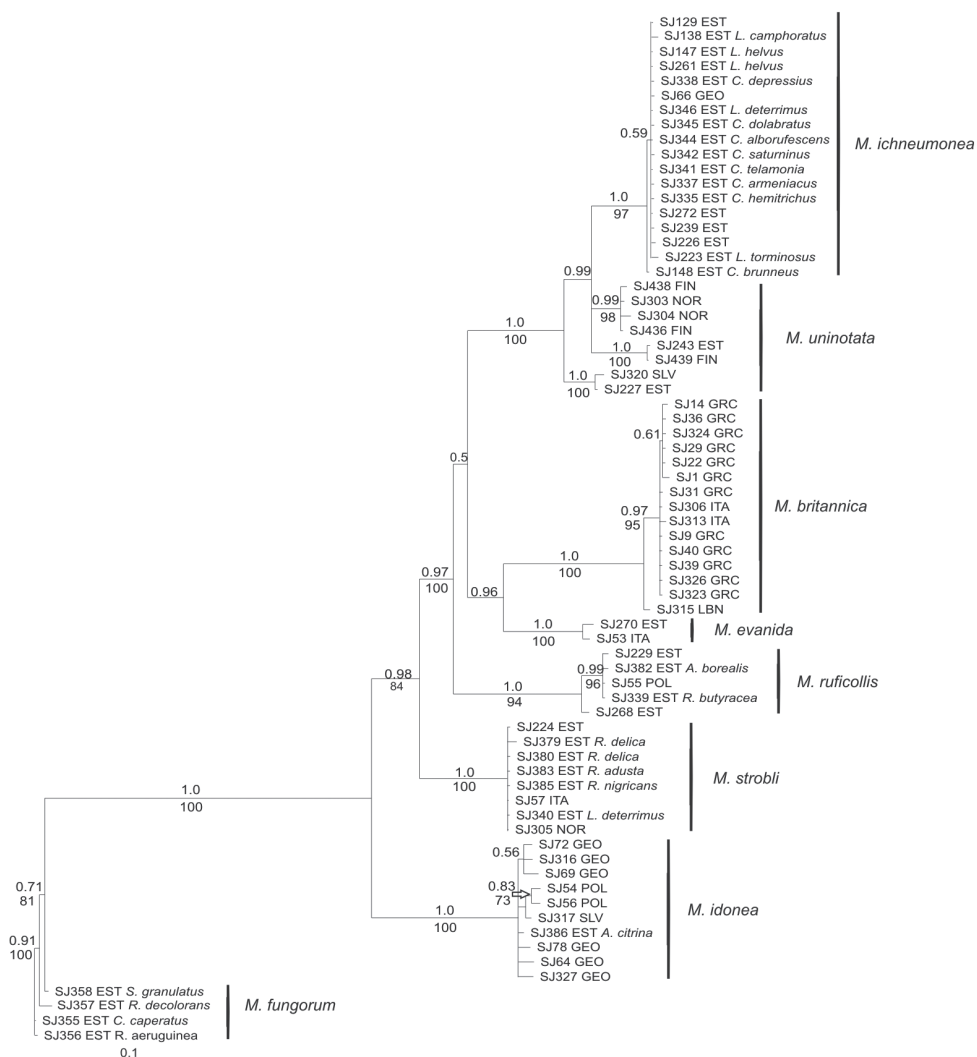
Based on the morphology, mainly that of male terminalia, the studied material was identified to belong to all nine species of the *M. ruficollis* group known from Europe. Deviation in morphological characters of some specimens suggested that these might represent additional undescribed species. Phylogenetic analyses, based on molecular data, recognised seven well supported clades, corresponding to the morphologically distinguished species. Fresh material, suitable for molecular analyses, was not available for two European species: *M. sepulta* and *M. suffusala*. The phylogenies led to reconsideration of morphology-based identification in several specimens. Consequently, the colouration and dimensions of the gnats' body were realised to be variable but the characters of male terminalia mostly constant within a species.

The interspecific genetic distance among species of the *M. ruficollis* group was calculated for the COI barcoding region, including 647 bp (Table 2). Interspecific differences ranged from 2.9% (between *M. ichneumonea* and *M. uninotata*) to 10.6% (between *M. evanida* and *M. idonea*), with the mean interspecific distance of 8.1%. The intraspecific distance ranged from 0.08 to 0.8% except for 2.3% in *M. uninotata*. The second region of COI, following the barcoding region, distinguished the seven analysed species based on 4.0–9.3% of interspecific variation (data not shown).

The COI datamatrix comprised 66 sequences and 1432 characters (1108 constant, 287 parsimony informative). The ITS2 datamatrix comprised 37 sequences and 584 characters of which 535 were constant and 47 parsimony informative. The combined COI and ITS2 datamatrix comprised 37 sequences and 2016 characters (1662 constant, 331 parsimony informative). When comparing the ITS2 and COI regions in

**Table 2.** Genetic distances between species of the *M. ruficollis* group and *M. fungorum*, used as an out-group, quantified according to the Kimura 2-parameter model from the COI barcoding region.

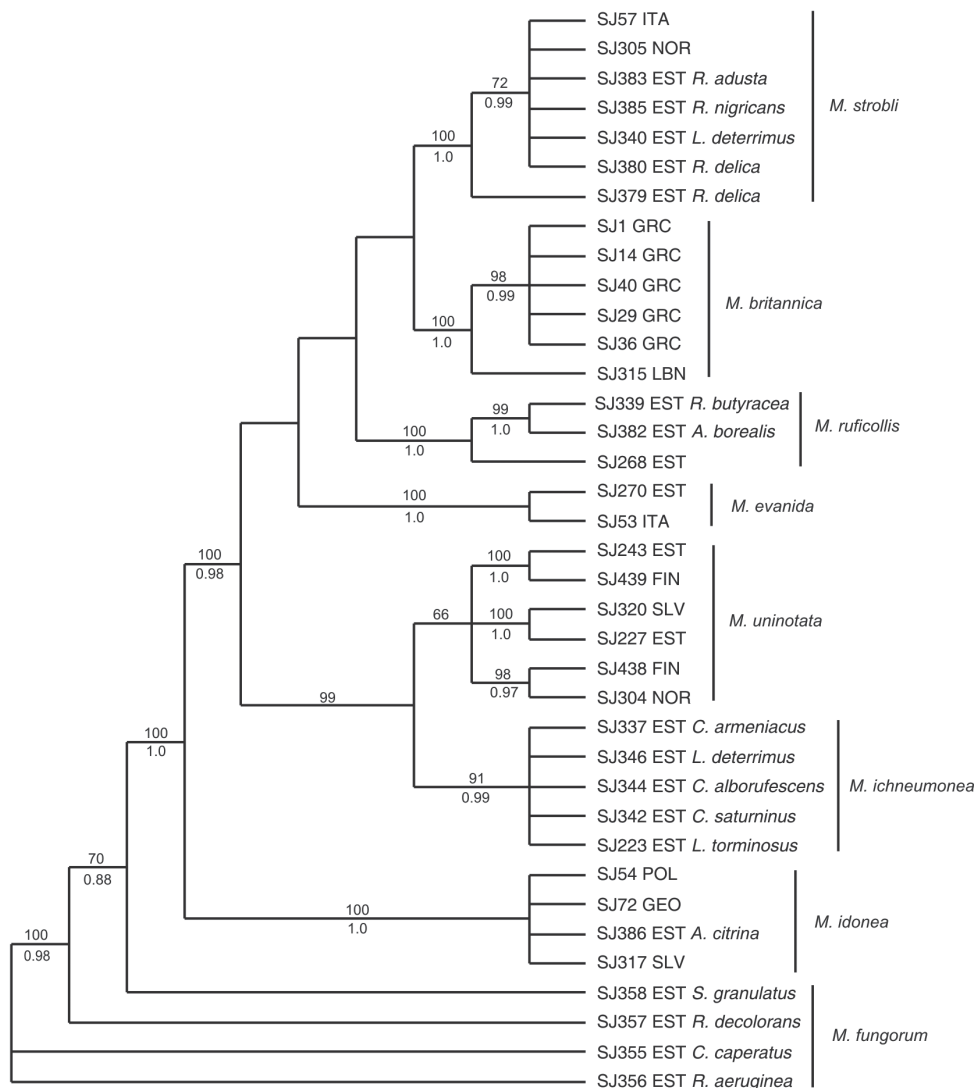
		1	2	3	4	5	6	7	8
1	<i>M. ichneumonea</i>	<b>X</b>							
2	<i>M. uninotata</i>	2.9%	<b>X</b>						
3	<i>M. strobli</i>	8.2%	8.1%	<b>X</b>					
4	<i>M. ruficollis</i>	8.8%	9.3%	7.2%	<b>X</b>				
5	<i>M. evanida</i>	7.3%	7.2%	6.7%	7.4%	<b>X</b>			
6	<i>M. britannica</i>	8%	7.8%	6.9%	8.1%	6.6%	<b>X</b>		
7	<i>M. idonea</i>	9.9%	9.5%	9.8%	9.7%	10.6%	10.1%	<b>X</b>	
8	<i>M. fungorum</i>	11.1%	11.7%	11.8%	12.3%	11.9%	12.5%	12.4%	<b>X</b>



**Figure 4.** Bayesian consensus tree of the COI regions of *Mycetophila ruficollis* species group. Posterior probability values are presented above the branches and bootstrap support values below the branches. Scale bar indicates substitutions per site. For gnats reared from fungal fruitbodies, the host is indicated.

the 37 sequences for which both data were available, the proportion of variable sites in COI (21.3%) exceeded that in ITS2 (8.4%) more than twice. In the COI dataset of 66 sequences variable sites represented 22.6% of the total amount, with the first (barcoding) part of 647 basepairs (available for 61 sequences) including 29.9% and the following 785 basepairs (47 sequences) 22.3% of variable sites.

Phylogenetic reconstructions of COI data (Fig. 4) distinguished six strongly supported species (bootstrap support 94% to 100%, posterior probabilities 0.94–1.0) in the ingroup. However, *M. uninotata* appeared paraphyletic with three strongly sup-



**Figure 5.** Consensus of most parsimonious trees calculated from combined COI and ITS2 rDNA sequence data of the *Mycetophila ruficollis* species group. Bootstrap support values are presented above the branches and posterior probability values below the branches. For gnats reared from fungal fruitbodies, the host is indicated.

ported lineages. The analyses of COI data also support close relationship between *M. ichneumonea* and *M. uninotata* as well as *M. evanida* and *M. britannica*. By contrast, the ITS2 trees (not shown) were much less resolved, with four well-supported groups recognised. The ITS2 phylogenies did not distinguish *M. ichneumonea* from *M. uninotata* with one *M. strobli* specimen from Italy also placed in this clade. The consensus of most parsimonious (Fig. 5) as well as the Bayesian trees calculated from 37 combined

COI and ITS2 sequences were well resolved and with higher support values for most of the clades than obtained in analyses of individual gene regions. The main difference was observed in the three lineages of *M. uninotata* forming a monophyletic group, yet receiving only low support.

Members of the *M. ruficollis* species group were selected from the material of adults reared from mushrooms collected from Estonia during 1988–1990 (Kurina 1991) and extensive sampling in five pine dominated boreal forests in 2011 (Põldmaa et al. 2015) as well as scattered localities in the following years. Among the >11 000 adults reared from the pine forest material most gnats from the *M. ruficollis* species group were sequenced and identified as belonging to *M. ichneumonea*. Among the 100 fungal species, this gnat emerged only from 12 fruit bodies of *Lactarius* and 4 fruit bodies of *Cortinarius*. Only one specimen, reared from *Rhodocollybia butyracea*, represented *M. ruficollis*. The study presents new species records for Estonia (*M. evanida*), Georgia (*M. ichneumonea*, *M. idonea*, *M. ruficollis*) and Norway (*M. strobli*).

### Key to the species of the *M. ruficollis* group in Europe based on characters of male terminalia

The key is compiled on the basis of original data, Laštovka (1972), and Laštovka and Kidd (1975).

- 1      4<sup>th</sup> palpal segment wider than the 3<sup>rd</sup> and about twice as wide as 5<sup>th</sup>, and about as long as the 5<sup>th</sup> (Laštovka and Kidd 1975: Fig.1). Posterior margin of gonocoxite ventromedially undulating or with diminutive central prominence... **2**
- 4<sup>th</sup> palpal segment about as wide as the 3<sup>rd</sup> and only slightly wider than the 5<sup>th</sup>, and distinctly shorter than the 5<sup>th</sup> (Laštovka and Kidd 1975: Figs 7–15). Posterior margin of gonocoxite ventromedially convex or with a clear prominence..... **3**
- 2      Wing with central spot only. Posterior margin of gonocoxite ventromedially undulating (Fig. 42). Lateral margin of dorsal branch of gonostylus almost straight (Fig. 10)..... ***Mycetophila ruficollis* Meigen**
- Wing with central spot and apical dark shade. Posterior margin of gonocoxite ventromedially with diminutive central prominence which is somewhat sunken into the posterior impression (Figs 47, 48). Lateral margin of dorsal branch of gonostylus with clear concavity (Fig. 13)..... ***Mycetophila suffusala* Chandler & Ribeiro**
- 3      Central spot of wing narrow and indistinct (sometimes almost absent). Dorsal branch of gonostylus with: lateral margin almost stright and distal posterior process subequal to proximal posterior process, both separated by wide and deep notch (Fig. 9)..... ***Mycetophila sepulta* (Laffoon)**
- Central spot of wing distinct. Dorsal branch of gonostylus with different combination of characters: with clear concavity at lateral margin and/or with distal and proximal posterior processes in different height..... **4**

- 4 Posterior impression of gonocoxite wide and compressed with oblique lateral projections (Figs 49, 50); posterior process of ventral branch of gonostylus with well distinguished warts (Fig. 31); posterior processes of dorsal branch of gonostylus about the same height (Fig. 14)..... ***Mycetophila uninotata* Zetterstedt**
- Posterior impression of gonocoxite cup-shaped, with vertical lateral projections; posterior process of ventral branch of gonostylus without or with only small warts; posterior processes of dorsal branch of gonostylus unequal in height ..... **5**
- 5 Lateral margin of dorsal branch of gonostylus with distinct and deep concavity (Fig. 9). Aedeagal guides distally divided, lateral impressions small or absent (Fig. 37)..... ***Mycetophila ichneumonea* Say**
- Lateral margin of dorsal branch of gonostylus without or with a shallow concavity. Aedeagal guides distally only shallowly bifurcated, lateral impressions wide..... **6**
- 6 Posterior margin of dorsal branch of gonostylus sinuate (Fig. 6). Posterior margin of gonocoxite ventromedially concave or with indistinct convexity (Fig. 34)..... ***Mycetophila britannica* Laštovka & Kidd**
- Posterior margin of dorsal branch of gonostylus straight. Convexity on posterior margin of gonocoxite ventromedially well outlined ..... **7**
- 7 Distance between spines 2, 3 and 4 on ventral branch of gonostylus nearly equal (Figs 27, 18). Posterior process of ventral branch of gonostylus with very small warts. Lateral margin of dorsal branch of gonostylus straight or slightly convex (Fig. 7)..... ***Mycetophila idonea* Laštovka**
- Distance between spines 3 and 4 on ventral branch of gonostylus much shorter than distance between spines 3 and 2. Posterior process of ventral branch of gonostylus with warts and setulae. Lateral margin of dorsal branch of gonostylus concave ..... **8**
- 8 Posterior margin of gonocoxite laterally from ventromedial convexity slanting (Fig. 46). Posterior margin of dorsal branch of gonostylus proximally from medial bristle with 4 gradually diminishing rather weak bristles (Fig. 12)..... ***Mycetophila strobli* Laštovka**
- Posterior margin of gonocoxite laterally from ventromedial convexity straight (Fig. 40). Posterior margin of dorsal branch of gonostylus proximally from medial bristle with 5–6 gradually diminishing strong bristles (Fig. 8) ..... ***Mycetophila evanida* Laštovka**

## The species

### *Mycetophila britannica* Laštovka & Kidd, 1975

Figs 6, 15, 16, 33, 34

**Material.** ITALY. 4♂♂, Sardinia, Alghero, near Nuraghe Palmavera, 40°35'N, 08°14'E, 63m, 21.xi.2005, sweeping, O. Kurina leg. (IZBE0200050, IZBE0200131,



in alcohol with terminalia in glycerine; IZBE0200129, IZBE0200130, in alcohol, abdomen used for DNA sequence: SJ306, SJ313). **GREECE.** 1♂, Village Kerkini, Krousia Mts., 41°11'32,4"N, 23°03'59,5"E, 190m, 5.ix–11.ix.2007, Malaise trap, G. Ramel leg. (IZBE0200132, in alcohol, abdomen used for DNA sequence: SJ326); 1♂, Elodia, Cafe site, 41°12'46,8"N, 23°05'42,9"E, 10.iii–16.iii.2008, Malaise trap, G. Ramel leg. (IZBE0200134, in alcohol, abdomen used for DNA sequence: SJ17); 1♂, Village Kerkini, Cafe Elodia, 41°12'46,8"N, 23°05'42,9"E, 40m, 25.ii–2.iii.2008, Malaise trap, G. Ramel leg. (IZBE0200135, in alcohol, abdomen used for DNA sequence: SJ323); 1♂, Village Vironia, Beabies site, 41°19'15,4"N, 23°13'39,6"E, 1150m, 9.vi–15.vi.2008, Malaise trap, G. Ramel leg. (IZBE0200146, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ39); 1♂, Village Neo Petritsi, Sultanitsa site, 41°19'02,1"N, 23°12'05,0"E, 1485m, 30.vi–6.vii.2008, Malaise trap, G. Ramel leg. (IZBE0200136, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ9); 1♂, Village Neo Petritsi, Sultanitsa site, 41°19'02,1"N, 23°12'05,0"E, 1485m, 8.ix–14.ix.2008, Malaise trap, G. Ramel leg. (IZBE0200137, in alcohol, abdomen used for DNA sequence: SJ31); 1♂, Village Promohonas, Procom, 41°22'38,1"N, 23°21'58,8"E, 60m, 25.ii–2.iii.2008, malaise trap, G. Ramel leg. (IZBE0200138, in alcohol, abdomen used for DNA sequence: SJ324); 1♂, Village Neo Petritsi, Sultanitsa site, 41°19'02,1"N, 23°12'05,0"E, 1485m, 15.ix–21.ix.2008, Malaise trap, G. Ramel leg. (IZBE0200139, in alcohol, abdomen used for DNA sequence: SJ22); 1♂, Village Vironia, Ramna site, 41°17'42,5"N, 23°11'33,1"E, 750m, 17.xi–23.xi.2008, Malaise trap, G. Ramel leg. (IZBE0200140, in alcohol, abdomen used for DNA sequence: SJ29); 1♂, same as earlier, (IZBE0200141, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ40); 1♂, Village Vironia, Beabies site, 41°19'15,4"N, 23°13'39,6"E, 1150m, 15.ix–21.ix.2008, Malaise trap, G. Ramel leg. (IZBE0200142, in alcohol, abdomen used for DNA sequence: SJ14); 1♂, Village Vironia, Ramna site, 41°17'42,5"N, 23°11'33,1"E, 750m, 10.xi–16.xi.2008, Malaise trap, G. Ramel leg. (IZBE0200143, in alcohol, abdomen used for DNA sequence: SJ1); 1♂, Village Vironia, Ramna site, 41°17'42,5"N, 23°11'33,1"E, 750m, 8.xii–14.xii.2008, Malaise trap, G. Ramel leg. (IZBE0200144, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ36). **LEBANON.** 1♂, Kesrouane Mar Elias, 33°54'N, 35°32'E, 27.v–4.vi.2012, light trap, J. Kullberg leg. (IZBE0200145, in alcohol, abdomen used for DNA sequence: SJ315); 1♂, Kesrouane Mar Elias, 33°54'N, 35°32'E, 30.v.2012, light trap, J. Kullberg leg. (IZBE0200153, in alcohol, abdomen used for DNA sequence: SJ322).

**Male terminalia.** Posterior margin of gonocoxite slightly concave ventromedially, and with abrupt and blunt projections laterally. Posterior impression wide. Anterior impression with anteriorly evenly divergent wide arms. Ventral branch of gonostylus with narrow, short and asymmetrical posterior process which bears minute warts; ventral surface with 6–9 long bristles deviating from other setosity; spine 1 and spine 2 of almost equal height and width; spine 1 sharply pointed; spine 2 blunt; spines 3 and 4 smaller, pointed and close to each other. Dorsal branch of gonostylus abruptly narrowed beyond the medial bristle; lateral margin with shallow concavity or almost



straight; distal posterior process very small, separated from proximal posterior process by a narrow but distinct notch, both processes apically rounded. Posterior margin proximally from medial bristle with 5 gradually diminishing bristles followed by 2 small setae. Basal angle slightly rounded, basal margin with few setae. Distal posterior process apically with small seta, proximal posterior process apically bare. Ejaculatory apodeme with semi-rounded or proximally truncated base and without rim. Aedeagal guides wide, apically widened, extending beyond aedeagus distally, lateral impressions wide. Aedeagal apodemes laterally angular.

**Intraspecific variation.** Laštovka and Kidd (1975) figured the ventromedial margin of the gonocoxite with a small convexity medially that was not observed in the studied material. They also described two different forms of the dorsal branch of the gonostylus: 1) slender and slightly narrowing beyond the medial bristle, and 2) shorter and abruptly narrowing beyond the medial bristle. The last character resembles that of *M. ruficollis*, *M. laffooni* Laštovka, 1972 and somewhat also *M. suffusala*. All studied specimens had the dorsal branch of the gonostylus slender, corresponding to the first form. Laštovka and Kidd (1975) found *M. britannica* to be most similar to *M. evanida*. In some studied specimens from Greece, the spine 2 on the ventral branch of the gonostylus is more massive, being longer than the spine 1. In Greek specimens the spine 2 in the ventral branch of the gonostylus is blunt while it is sharply pointed in Italian specimens.

**Hosts and distribution.** *Mycetophila britannica* has been earlier reared from *Polyporus squamosus*, *Armillaria mellea*, *Hebeloma crustuliniforme*, *Russula nigricans*, *Hypholoma* sp. and *Lactarius resimus* (Laštovka and Kidd 1975, Yakovlev 1994, Chandler 2010), while we have studied sweepnetted and trapped material only. Having been described from the British Isles, the species is widely distributed in Western Europe extending also to Norway and the Middle East (Kjørandsen 2012, Chandler 2013). Except for a finding in Russian Karelia (Kjørandsen 2012) it is not found in Eastern Europe. According to Chandler and Ribeiro (1995) and Chandler et al. (2005), *M. britannica* is common in the Mediterranean region including Morocco.

### *Mycetophila evanida* Laštovka, 1972

Figs 8, 21, 22, 39, 40

**Material. ESTONIA.** 1♂, Jõgeva county, Pataste, 58°34'52,2"N, 26°46'42,3"E, 5.x–19.x.2009, Malaise trap, J. Elts leg. (IZBE0200069, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ270); 1♂, Tartu county, Meliste, 58°19'43,8"N, 26°56'25,1"E, 4.x–18.x.2008, Malaise trap, O. Kurina leg. (IZBE0200070, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ230). **ITALY.** 1♂, Südtirol, N. Park Stilfser Joch, Unt. Tartscher Tal (S von Trafoi), 46°32'33,9"N, 10°30'17,2"E, 1630m, 27.vi–4.vii. 2005, Malaise trap, C. Lange and J. Ziegler leg. (IZBE0200071, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ53).

**Male terminalia.** Posterior margin of gonocoxite ventromedially with clear convexity, and with abrupt and blunt projections laterally. Posterior impression rather wide. Anterior impression with anteriorly evenly divergent narrow arms. Ventral branch of gonostylus with semicircular posterior process which bears minute warts and few setae; spine 1 slender, evenly tapering and sharply pointed; spine 2 shorter, thicker and rather blunt; spines 3 and 4 much smaller than spine 1, sharply pointed and close to each other. Dorsal branch of gonostylus steeply tapering; lateral margin with shallow concavity; distal posterior process about half as high as proximal posterior process, both separated by shallow concavity. Distal posterior process with few setae; proximal posterior process bare with 2–3 setae deviating from other setosity on its base. Posterior margin proximally from medial bristle with 6 gradually diminishing bristles; internal surface with one somewhat stronger seta next to the medial bristle. Basal angle right-angled, basal margin with setae. Ejaculatory apodeme with subquadrate base and very wide rim. Aedeagus widened apically, apical margin slightly convex. Aedeagal guides with wide lateral impressions; apically narrow and rounded, not extending upper margin of aedeagus. Aedeagal apodemes laterally angular.

**Intraspecific variation.** In some cases the base of the ejaculatory apodeme resembles *M. idonea*, because of having its basal margin somewhat concave. The holotype has an additional small spine on the ventral branch of the gonostylus close to spines 3 and 4 (cf. Laštovka (1972: fig. 12). In studied material this spine was not observed.

**Hosts and distribution.** *Mycetophila evanida* has been reared from species of *Russula*, *Lactarius*, and *Tubaria* (Yakovlev 1994). The species is widely distributed in Europe extending also to the Eastern Palearctic (Laštovka 1972, Chandler 2013, Jakovlev 2014). The material from Estonia represents a new country record.

### *Mycetophila ichneumonea* Say, 1823

Figs 1, 2, 3, 9, 19, 20, 37, 38

**Material. FINLAND.** 1♂, Sodankylä, Syväkuru, 67°25'N, 026°35'E, 21.viii.2013, sweeping, J. Salmela leg. (IZBE0200150, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ434). **ESTONIA.** 1♂, Tartu county, Melliste, 58°20'N, 26°59'E, 20.viii–4.ix.2008, Malaise trap, O. Kurina leg. (IZBE0200073, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ272); 1♂, same as earlier except 24.x–16.xi.2008 (IZBE0200074, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ226); 1♂, Saare county, Orissaare, 58°33'19"N, 23°05'12"E, 18.x–5.xi.2008, Malaise trap, H. Jäe leg. (IZBE0200075, in alcohol, abdomen used for DNA sequence: SJ239); 1♂, Tartu county, Maiorg near Annikoru, 58°16'41,6"N, 26°20'03,6"E, 1.v–16.v.2009, Malaise trap, O. Kurina leg. (IZBE0200076, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ129); 1♂, Jõgeva county, Kaiu, 58°39'20,4"N, 26°52'43,2"E, reared from *Cortinarius traganus*, coll. 21.ix.2011, emerg. 24.x.2011, S. Jürgestein leg. (IZBE0200077, in alcohol); 1♂, Tartu county, Järvelja, 58°17'45"N, 27°15'41,7"E,

reared from *Lactarius helvus*, coll. 23.ix.2011, emerg. 12.x.2011, S. Jürgenstein leg. (IZBE0200078, in alcohol, abdomen used for DNA sequence: SJ261); 1♂, Tartu county, Järvselja, 58°17'45"N, 27°15'41,7"E, reared from *Lactarius torminosus*, coll. 23.ix.2011, emerg. 10.x.2011, S. Jürgenstein leg. (IZBE0200079, in alcohol, abdomen used for DNA sequence: SJ223); 1♂, Põlva county, Viira, 58°0'N, 27°11'E, reared from *Cortinarius hemitrichus*, coll. 10.x.2012, emerg. 31.x.2012, S. Jürgenstein leg. (IZBE0200081, in alcohol, abdomen used for DNA sequence: SJ335); 1♂, Jõgeva county, Kaiu, 58°39'20,4"N, 26°52'43,2"E, reared from *Cortinarius armeniacus*, coll. 1.x.2012, emerg. 29.x.2012, S. Jürgenstein leg. (IZBE0200082, in alcohol, abdomen used for DNA sequence: SJ337); 1♂, Jõgeva county, Kaiu, 58°39'20,4"N, 26°52'43,2"E, reared from *Cortinarius depressus*, coll. 1.x.2012, emerg. 31.x.2012, S. Jürgenstein leg. (IZBE0200083, in alcohol, abdomen used for DNA sequence: SJ338); 1♂, Lääne county, Haapsalu, 58°57'N, 23°32'E, reared from *Cortinarius saturninus*, coll. 25.ix.2012, emerg. 22.x.2012, S. Jürgenstein leg. (IZBE0200085, in alcohol, abdomen used for DNA sequence: SJ342); 1♂, Võru county, Rõuge, 57°44'N, 26°55'E, reared from *Cortinarius* subgenus *Telamonia*, coll. 3.ix.2012, emerg. 21.ix.2012, S. Jürgenstein leg. (IZBE0200086, in alcohol, abdomen used for DNA sequence: SJ341); 1♂, Tartu county, Uniküla, 58°16'N, 26°55'E, reared from *Cortinarius dolabratus*, coll. 2.x.2012, emerg. 2.xi.2012, S. Jürgenstein leg. (IZBE0200087, in alcohol, abdomen used for DNA sequence: SJ345); 1♂, Jõgeva county, Kõduküla, 58°34'N, 26°31'E, reared from *Lactarius deterrimus*, coll. 15.ix.2012, emerg. 2.x.2012, S. Jürgenstein leg. (IZBE0200088, in alcohol, abdomen used for DNA sequence: SJ346); 1♂, Valga county, Miti, 58°06'16,7"N, 26°22'28,9"E, reared from *Lactarius rufus*, coll. 14.ix.2011, emerg. 3.x.2011, S. Jürgenstein leg. (IZBE0200089, in alcohol with terminalia in glycerine); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius rufus*, coll. 16.ix.2011, emerg. 12.x.2011, S. Jürgenstein leg. (IZBE0200090, in alcohol); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius rufus*, coll. 16.ix.2011, emerg. 3.x.2011, S. Jürgenstein leg. (IZBE0200091, in alcohol); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius rufus*, coll. 16.ix.2011, emerg. 6.x.2011, S. Jürgenstein leg. (IZBE0200152, in alcohol); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius camphoratus*, coll. 16.ix.2011, emerg. 5.x.2011, S. Jürgenstein leg. (IZBE0200092, in alcohol); 1♂, Põlva county, Ihamaru, 58°06'00,40"N, 26°55'55,45"E, reared from *Lactarius rufus*, coll. 18.ix.2011, emerg. 21.x.2011, S. Jürgenstein leg. (IZBE0200093, in alcohol); 1♂, Põlva county, Ihamaru, 58°06'00,40"N, 26°55'55,45"E, reared from *Rhodocollybia butyracea*, coll. 18.ix.2011, emerg. 5.x.2011, S. Jürgenstein leg. (IZBE0200094, in alcohol); 1♂, Jõgeva county, Kaiu, 58°39'20,4"N, 26°52'43,2"E, reared from *Cortinarius* sp., coll. 21.ix.2011, emerg. 17.x.2011, S. Jürgenstein leg. (IZBE0200095, in alcohol); 1♂, Jõgeva county, Kaiu, 58°39'20,4"N, 26°52'43,2"E, reared from *Cortinarius alborufescens*, coll. 21.ix.2011, emerg. 5.x.2011, S. Jürgenstein leg. (IZBE0200096, in alcohol); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius helvus*, coll. 16.ix.2011, emerg. 6.x.2011, S. Jürgenstein leg. (IZBE0200097, in alcohol); 1♂, Jõgeva county, Kaiu, 58°39'20,4"N,

26°52'43,2"E, reared from *Cortinarius* sp., coll. 21.ix.2011, emerg. 12.x.2011, S. Jürgenstein leg. (IZBE0200098, in alcohol); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius helvus*, coll. 16.ix.2011, emerg. 12.x.2011, S. Jürgenstein leg. (IZBE0200099, in alcohol); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius rufus*, coll. 16.ix.2011, emerg. 10.x.2011, S. Jürgenstein leg. (IZBE0200101, in alcohol with terminalia in glycerine); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius rufus*, coll. 16.ix.2011, emerg. 6.x.2011, S. Jürgenstein leg. (IZBE0200102, in alcohol); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius rufus*, coll. 16.ix.2011, emerg. 12.x.2011, S. Jürgenstein leg. (IZBE0200103, in alcohol); 1♂, Võru county, Rõuge, 57°44'N, 26°55'E, reared from *Cortinarius alborufesens*, coll. 3.ix.2012, emerg. 24.ix.2012, S. Jürgenstein leg. (IZBE0200104, in alcohol, abdomen used for DNA sequence: SJ344); 1♂, Lääne county, Haapsalu, 58°57'N, 23°32'E, reared from *Cortinarius cotoneus*, coll. 25.ix.2012, emerg. 17.x.2012, S. Jürgenstein leg. (IZBE0200105, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ343); 1♂, Lääne county, Vormsi 59°0'N, 23°15'E, reared from *Russula vinosa*, coll. 25.viii.1991, emerg. 7. ix.1991, O. Kurina leg. (IZBE0200106, pinned); 1♂, Pärnu county, Nigula NR, 58°0'41"N, 24°40'60"E, reared from *Megacollybia platyphylla*, coll. 5.viii.1990, emerg. 17.viii.1990, O. Kurina leg. (IZBE0200107, pinned); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius helvus*, coll. 16.ix.2011, emerg. 12.x.2011, S. Jürgenstein leg. (IZBE0200108, in alcohol); 1♂, Põlva county, Ihamaru, 58°06'00,40"N, 26°55'55,45"E, reared from *Lactarius rufus*, coll. 18.ix.2011, emerg. 17.x.2011, S. Jürgenstein leg. (IZBE0200109, in alcohol); 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Lactarius helvus*, coll. 16.ix.2011, emerg. 5.x.2011, S. Jürgenstein leg. (IZBE0200033, in alcohol, abdomen used for DNA sequence: SJ147; published earlier by Põldmaa et al. 2015); 1♂, Põlva county, Ihamaru, 58°06'00,40"N, 26°55'55, 45"E, reared from *Lactarius camphoratus*, coll. 18.ix.2011, emerg. 19.x.2011, S. Jürgenstein leg. (IZBE0200156, in alcohol, abdomen used for DNA sequence: SJ138); 1♂, Tartu county, Järvselja, 58°17'45"N, 27°15'41,7"E, reared from *Cortinarius brunneus*, coll. 23.ix.2011, emerg. 10.x.2011, S. Jürgenstein leg. (IZBE0200157, in alcohol, abdomen used for DNA sequence: SJ148). **SLOVAKIA.** 1♂, NP Slovensky kras, Silická Ladnica, 48°33'00,0"N, 020°30'14,4"E, 505m, 3.vi.2009, sweeping, O. Kurina leg. (IZBE0200111, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ318). **GEORGIA.** 1♂, Surami, 42°01'34,2"N, 43°29'52,5"E, 941m, 18.v.2012, sweeping, O. Kurina leg. (IZBE0200112, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ66); 1♂, Bakuriani, 41°45'46,2"N, 43°30'16,7"E, 1626m, 31.viii.2014, sweeping, O. Kurina leg. (IZBE0200242, slide mounted in Euparal); 1♂, same as earlier except 1.ix.2014 (IZBE0200243, in alcohol); 3♂♂, Bakuriani 1.2 km-W, 41°44'13,0"N, 43°30'45,1"E, 1741m, 1.ix.2014, sweeping, O. Kurina leg. (IZBE0200244–IZBE0200246, in alcohol).

**Male terminalia.** Posterior margin of gonocoxite ventromedially with shallow convexity, and with abrupt and blunt projections laterally. Posterior impression with

narrow base and well widened posterior part. Anterior impression with anteriorly evenly divergent narrow arms. Ventral branch of gonostylus with symmetrical semi-oval posterior process, which bears minute warts and few setae on ventral surface; spine 1 wide, sharply pointed; spine 2 similar or somewhat longer but more slender; spines 3 and 4 about twice as small, evenly tapering and with about equal distance between each other and spine 2. Ventral branch of gonostylus with 4–5 strong bristles deviating from other setosity laterally on ventral surface. Dorsal branch of gonostylus steeply tapering; lateral margin with deep concavity; proximal posterior process about twice as high as distal posterior process, both separated by deep concavity. Distal posterior process with apical small setula and with a basal strong seta deviating from other setosity; proximal posterior process apically rounded with 1–2 strong basal setae deviating from other setosity. Posterior margin proximally from medial bristle with 4–5 gradually diminishing bristles followed by 3–4 smaller setae; internal surface with a stronger seta next to the medial bristle. Basal angle clearly outlined, angular or somewhat rounded; basal margin with few setae. Ejaculatory apodeme with campanulate base and without rim. Aedeagus oval or cross shaped. Aedeagal guides extending over apical part of aedeagus; apically rounded and divided into two lamellae; with lateral impressions very small. Aedeagal apodemes laterally slightly angular or arched and pointed apically.

**Intraspecific variation.** In some cases the lateral margin of the dorsal branch of the gonostylus is shallower, and the distal posterior process and the posterior margin of the dorsal branch of the gonostylus resemble those of *M. uninotata*. The spine 2 on the ventral branch of the gonostylus compared to the spine 1 can be more prominent than described by Laštovka (1972).

**Hosts and distribution.** Known from many species of Agaricales and Russulales (Kurina 1991, Yakovlev 1994, Chandler 2010, Ševčík 2010). Our material from pine dominated boreal forests in Estonia indicates specialisation to *Lactarius* (Russulales) and *Cortinarius* (Agaricales). Widely distributed in Europe extending also to the Eastern Palaearctic, the Middle East and the Nearctic region (Laštovka 1972, Chandler 2013). The material from Georgia represents a new country record.

### *Mycetophila idonea* Laštovka, 1972

Figs 7, 17, 18, 35, 36

**Material. ESTONIA.** 1♂, Valga county, Soontaga, 58°01'42,6"N, 26°04'29,3"E, reared from *Amanita citrina*, coll. 17.ix.2013, emerg. 2.x.2013, S. Jürgenstein leg. (IZBE0200158, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ386). **POLAND.** 2♂♂, Białowieża 2 km SW, village Gródek, 52°41'02,8"N, 23°49'33,1"E, 17.viii.2007, sweeping, O. Kurina leg. (IZBE0200027, IZBE0200035, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ54, SJ56). **SLOVAKIA.** 1♂, Muranska planina, near Klak, 48°46'53"N, 019°59'21,3"E, 1211 m, 28.v.2009, sweeping, O. Kurina leg. (IZBE0200047, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ317). **GEORGIA.** 1♂, Dgnali NE



of Zhinvali, 42°13'25,9"N, 44°40'12,1"E, 914m, 15.v.2012, sweeping, O. Kurina leg. (IZBE0200055, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ69); 2♂♂, Marelisi SW of Surami, 41°57'56,0"N, 43°17'20,7"E, 412m, 19.v.2012, sweeping, O. Kurina leg. (IZBE0200056, IZBE0200057, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ72, SJ78); 1♂, Kintrishi NP, 41°45'11,7"N, 041°58'38,4"E, 453m, 21.v.2013, sweeping, O. Kurina leg. (IZBE0200058, in alcohol, abdomen used for DNA sequence: SJ327); 1♂, Kintrishi NP, 41°45'11,7"N, 041°58'38,4"E, 453m, 22.v.2013, sweeping, O. Kurina leg. (IZBE0200149, in alcohol, abdomen used for DNA sequence: SJ316); 1♂, Saguramo N of Tbilisi, 41°53'04,3"N, 44°46'46,5"E, 915m, 15.v.2012, sweeping, O. Kurina leg. (IZBE0200059, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ64); 1♂, same as earlier, (IZBE0200060, in alcohol with terminalia in glycerine); 1♂, Saguramo N of Tbilisi, 41°53'08,0"N, 44°46'44,2"E, 889m, 4.ix.2014, sweeping, O. Kurina leg. (IZBE0200237, slide mounted in Euparal); 1♂, Lagotekhi, 41°49'N, 46°17'E, 15.vi–25.vi.2014, Malaise trap, G. Japoshvili leg. (IZBE0200238, in alcohol with terminalia slide mounted in Euparal).

**Male terminalia.** Posterior margin of gonocoxite with slight convexity ventromedially, and with abrupt and blunt projections laterally. Posterior impression with considerably narrow base and widened posterior part. Anterior impression with narrow, anteriorly evenly divergent arms. Ventral branch of gonostylus with posterior process wide and shallow (asymmetrical), with minute warts; spine 1 and spine 2 almost the same high; spine 1 slender, evenly tapering and sharply pointed; spine 2 thicker and blunt; spines 3 and 4 smaller, blunt, with about equal distance between each other and spine 2. Dorsal branch of gonostylus evenly tapering, lateral margin without concavity; distal posterior process about half as high as proximal posterior process, both separated by deep concavity. Distal posterior process bears apical and subapical setae, proximal posterior process bare and apically rounded. Posterior margin proximally from medial bristle with 3–4 gradually diminishing bristles; internal surface with one somewhat stronger seta next to the medial bristle; otherwise the setosity has no special arrangement. Basal angle slightly rounded, basal margin with few setae. Ejaculatory apodeme with concave base and narrow rim. Aedeagus widened apically and truncated. Aedeagal guides with well outlined, wide lateral impressions; apically widened and rounded covering edges of aedeagus. Aedeagal apodemes laterally angular.

**Intraspecific variation.** In some specimens from Georgia and Poland the base of the ejaculatory apodeme is blunt, resembling that of *M. strobli*. In a few cases the spine 2 on the ventral branch of the gonostylus is slender.

**Hosts and distribution.** *Mycetophila idonea* has been reared from about 65 species of Agaricales and Russulales and also from *Boletus impolitus* (Yakovlev, 1994). The species is widely recorded from Europe extending to the Middle East and Eastern Palearctic (Chandler 2013). The species was erroneously reported as overwintering in Estonian caves by Kurina (1996); after critical study, the material was found to belong to *M. uninotata*. However, the occurrence in Estonia is confirmed by a new rearing record presented herein. The material from Georgia represents a new country record.

***Mycetophila ruficollis* Meigen, 1818**

Figs 10, 23, 24, 41, 42

**Material. POLAND.** 1♂, Białowieża 2 km SW, Gródek, 52°41'02,8"N, 23°49'33,1"E, 17.viii.2007, sweeping, O. Kurina leg. (IZBE0200119, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ55). **ESTONIA.** 1♂, Tartu county, Maiorg near Annikoru, 58°16'41,6"N, 26°20'03,6"E, 17.ix.–2.x.2008, Malaise trap, O. Kurina leg. (IZBE0200120, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ229); 1♂, Jõgeva county, Pataste, 58°34'52,2"N, 26°46'42,3"E, 18.x–30.x.2008, Malaise trap, J. Elts leg. (IZBE0200121, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ268); 1♂, Lääne county, Oonga, 58°0'41"N, 24°40'60"E, reared from *Armillaria mellea*, coll. 8.ix.1994, emerg. 21.ix.1994, O. Kurina leg. (IZBE0200123, pinned); 1♂, Saare county, Abruka, 58°9'50"N, 22°30'14"E, reared from *Megacollybia platyphylla*, coll. 11.ix.1991, emerg. 27.ix.1991, O. Kurina leg. (IZBE0200124, pinned); 1♂, Tartu county, Järvelja, 58°17'45"N, 27°15'41,7"E, reared from *Pholiota aurivella*, coll. 4.ix.1989, emerg. 25.ix.1989, O. Kurina leg. (IZBE0200125, pinned); 1♂, Tartu county, Järvelja, 58°17'45"N, 27°15'41,7"E, reared from *Entoloma* sp., coll. 27.viii.1989, emerg. 11.ix.1989, O. Kurina leg. (IZBE0200126, pinned); 1♂, Jõgeva county, Kõduküla, 58°34'N, 26°31'E, reared from *Rhodocollybia butyracea*, coll. 15.ix.2012, emerg. 2.x.2012, S. Jürgenstein leg. (IZBE0200127, in alcohol, abdomen used for DNA sequence: SJ339); 1♂, Saare county, Abruka, 58°9'50"N, 22°30'14"E, 21.ix.2013 reared from *Armillaria borealis*, coll. 21.ix.2013, emerg. 4.x.2013, O. Kurina leg. (IZBE0200128, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ382). **GEORGIA.** 1♂, Lagotekhi, 41°49'N, 46°17'E, 15.vi–25.vi.2014, Malaise trap, G. Japoshvili leg. (IZBE0200247, in alcohol).

**Male terminalia.** Posterior margin of gonocoxite undulate or slightly concave ventromedially, and with abrupt and blunt projections laterally. Posterior impression wide and uncompressed. Anterior impression with wide arms which are abruptly divergent anteriorly. Ventral branch of gonostylus with short and semicircular posterior process, with minute warts; ventral surface with long and slender bristles deviating from other setosity; spine 1 slender, sharply pointed, about half as wide as spine 2; spine 2 blunt, about half as high as spine 1; spines 3 and 4 smaller, sharply pointed, close to each other. Dorsal branch of gonostylus abruptly narrowed beyond the medial bristle. Posterior margin proximally from medial bristle with 7–9 gradually diminishing bristles; internal surface with a stronger seta next to the medial bristle. Lateral margin without concavity, almost straight. Basal angle slightly rounded, basal margin with few setae. Distal posterior process very shallow, almost unnoticeable; proximal posterior process high and massive, apically rounded. Distal posterior process with setae, proximal posterior process apically bare. Dorsal surface with a distinct band of setae from base of posterior processes to basal angle. Ejaculatory apodeme proximally narrows, with campanulate base and narrow rim. Aedeagal guides wide, apically widened and rounded, not extending beyond aedeagus distally; lateral impressions wide. Aedeagal apodemes laterally angular.

**Intraspecific variation.** The combination of wide 4<sup>th</sup> and considerably short 5<sup>th</sup> palpal segments, the ventroapical margin of the gonocoxite without any medial projections and the apically abruptly narrowed dorsal branch of the gonostylus are unique among European species of the group.

**Hosts and distribution.** *Mycetophila ruficollis* is reared from 35 species of macrofungi (Yakovlev 1994). However, some of the rearing records in the literature may possibly refer to entire group: e.g. records from *Lactarius* and *Russula* by Ribeiro (1990) and some records in Yakovlev (1994). Our records reveal saprotrophic members of the Agaricales as the host of this species. The species is widely distributed in Europe (Chandler 2013, Jakovlev 2014, Kjærandsen 2012) extending also to the Middle East and Eastern Palearctic (Chandler 2013). The material from Georgia represents a new country record.

### *Mycetophila sepulta* Laffoon, 1957

Figs 11, 25, 26, 43, 44

**Material. UNITED KINGDOM.** 1♂, Berks, California, Country Park, 1.xi.2001, sweeping, P. J. Chandler leg. (PCPC, pinned, terminalia slide mounted in Euparal); 1♂, Oxon, Spartum Fen, 15.x.1999, sweeping, P. J. Chandler leg. (PCPC, pinned, terminalia slide mounted in Euparal).

**Male terminalia.** Posterior margin of gonocoxite with clear convexity ventromedially, and with abrupt and blunt projections laterally. Posterior impression considerably narrow at base but well widening posteriorly. Anterior impression with anteriorly evenly divergent narrow arms. Ventral branch of gonostylus with posterior process narrow, asymmetrical and high, with minute warts; spine 1 very slender and sharply pointed; spine 2 somewhat wider than spine 1, but also pointed and of same length; spines 3 and 4 smaller, pointed and rather close to each other. Dorsal branch of gonostylus slightly tapering, somewhat constricted at the medial bristle; lateral margin without or with very shallow concavity; distal posterior process and proximal posterior process almost of same height, apically rounded, separated by a rather wide notch. Distal posterior process with apical seta, proximal posterior process subapically with 3 setae. Posterior margin proximally from medial bristle with 4–5 gradually diminishing bristles followed by 2–3 setae; internal surface with a stronger seta next to the medial bristle. Basal angle slightly rounded, basal margin with few setae. Ejaculatory apodeme with heart-shaped base and with very narrow or barely visible rim. Aedeagus apically concave. Aedeagal guides rather wide, apically widened, not extending beyond aedeagus distally, lateral impressions wide. Aedeagal apodemes laterally angular.

**Intraspecific variation.** In comparison with figures by Laštovka and Kidd (1975: fig. 30), the studied specimens have the distal posterior process of the dorsal branch of the gonostylus higher and the notch between the processes more clearly outlined. Laffoon (1957) mentioned *M. sepulta* to be closely allied to *M. ichneumonea*, *M. ruficollis* and *M. parvimaculata* Van Duzee, 1928.



**Hosts and distribution.** The species, described from North America, has rather scattered distribution in Western Europe, extending to Sweden (Chandler 2013, Kjærandsen 2012). In North America, it has a wide distribution from Alaska to California and Texas (Laffoon 1957). The only rearing record is that by Laštovka and Kidd (1975) from *Hypholoma elongatum*.

***Mycetophila strobli* Laštovka, 1972**

Figs 12, 27, 28, 45, 46

**Material.** **NORWAY.** 1♂, Troms, Svensby, 69°40'01,2"N, 019°49'58,8"E, 18.vii.2008, sweeping, O. Kurina leg. (IZBE0200061, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ305). **ESTONIA.** 1♂, Tartu county, Vapramäe, 58°15'9,28"N, 26°27'46,1"E, reared from *Russula delica* (No 1057), coll. 8.ix.1995, emerg. 21.ix.1995, O. Kurina leg. (IZBE0200239, pinned with terminalia in Euparal); 1♂, Pärnu county, Nigula NR, 58°9'N, 24°58'E, reared from *Lactarius torminosus*, coll. 22.viii.1993, emerg. 2.ix.1993, O. Kurina leg. (IZBE0200240, pinned with terminalia in glycerine); 2♂♂, Tartu county, Mustametsa, Välg, 58°36'53,09"N, 26°53'56,1"E, reared from *Russula delica*, coll. 1.x.2013, emerg. 14.x.2013 and 16.x.2014, S. Jürgenstein leg. (IZBE0200062, IZBE0200063, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ379, SJ380); 1♂, Saare county, Muhu, Igaküla, 58°36'3,5"N, 23°07'42"E, 4.x–18.x.2008, Malaise trap, H. Jäe leg. (IZBE0200064, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ224); 1♂, Jõgeva county, Kaiu, 58°39'20,4"N, 26°52'43,2"E, reared from *Russula nigricans*, coll. 25.ix.2013, emerg. 7.x.2013, S. Jürgenstein leg. (IZBE0200065, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ385); 1♂, Põlva county, Palojärvi, 58°2'57,3"N, 27°7'35,73"E, reared from *Russula adusta*, coll. 01.x.2013, emerg. 7.x.2013, S. Jürgenstein leg. (IZBE0200066, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ383); 1♂, Rapla county, Vardi, 59°1'27,07"N, 24°26'28,9"E, reared from *Lactarius deterrimus*, coll. 1.xi.2012, emerg. 5.xi.2012, S. Jürgenstein leg. (IZBE0200067, in alcohol, abdomen used for DNA sequence: SJ340). **ITALY.** 1♂, Südtirol, N. Park Stilfser Joch, Schmelz (SW von Prad), 46°36'42,1"N, 10°34'35,6"E, 940m, 15.viii–24.viii.2005, Malaise trap, C. Lange and J. Ziegler leg. (IZBE0200068, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ57).

**Male terminalia.** Posterior margin of gonocoxite ventromedially with clear angular convexity, and with abrupt and blunt projections laterally. Posterior impression with narrow base and well widened posterior part. Anterior impression with divergent arms which are sinuate at anterior fourth. Ventral branch of gonostylus with asymmetrical posterior process which bears minute warts and few setae; spine 1 short and sharply pointed; spine 2 blunt, almost twice as high and thick as spine 1; spines 3 and 4 sharply pointed, close to each other and similar in size to spine 1. Ventral branch of gonostylus with a few strong bristles deviating from other setosity laterally on ventral

surface. Dorsal branch of gonostylus slightly tapering, lateral margin with shallow concavity; proximal posterior process about three times as high as distal posterior process, both separated with a shallow concavity. Distal posterior process apically and subapically with few setae; proximal posterior process bare and apically rounded. Posterior margin proximally from medial bristle with 4–6 gradually diminishing bristles; internal surface with one somewhat stronger seta next to the medial bristle. Basal angle slightly rounded, basal margin bare. Ejaculatory apodeme with semi-oval base and wide rim. Aedeagus slightly widened apically and with apical margin convex. Aedeagal guides with wide lateral impressions; subapically constricted and apically rounded, not extending upper margin of aedeagus. Aedeagal apodemes laterally angular.

**Intraspecific variation.** Occasionally the spine 2 on the ventral branch of the gonostylus is somewhat slender and pointed.

**Hosts and distribution.** *Mycetophila strobli* has been reared from species of *Russula*, *Lactarius*, *Suillus*, *Collybia*, *Armillaria*, *Kuehneromyces* and *Cortinarius* (Yakovlev 1994, original data). The species is widely distributed in Europe extending to the Middle East (Chandler 2013). The material from Norway represents a new country record.

### *Mycetophila suffusala* Chandler & Ribeiro, 1995

Figs 13, 29, 30, 47, 48

**Material. PORTUGAL.** 2♂♂, **Madeira**, Queimadas, 10.ix–11.ix.1986, P. Ohm leg. (PCPC, pinned with terminalia in Euparal). **SPAIN.** 2♂♂, **Tenerife**, near top of west ridge at Izaña, 2350m a.s.l., 29.iii.1984, N.P. Ashmole leg. (PCPC, pinned with terminalia in Euparal).

**Male terminalia.** Posterior margin of gonocoxite ventrally straight except for diminutive central prominence, which is somewhat sunken into the posterior impression, and with abrupt and blunt projections laterally. Posterior impression wide and uncompressed. Anterior impression with evenly divergent arms anteriorly. Ventral branch of gonostylus with posterior process wide and angular, with minute warts; ventral surface with long and slender bristles deviating from other setosity; spine 1 sharply pointed, about as wide as spine 2; spine 2 geniculate, blunt, about as high as spine 1; spines 3 and 4 smaller, sharply pointed, close to each other. Dorsal branch of gonostylus abruptly narrowed beyond the medial bristle; lateral margin with concavity; distal posterior process very shallow, almost unnoticeable; proximal posterior process high and massive, apically rounded. Distal posterior process with setae, proximal posterior process apically bare. Dorsal surface with an indistinct band of setae from base of posterior processes to basal angle; the setae near basal angle are deviating from other setosity. Posterior margin proximally from medial bristle with 10–13 gradually diminishing bristles; internal surface with a stronger seta next to the medial bristle. Basal angle slightly rounded, basal margin with few setae. Ejaculatory apodeme with rectangular base and without rim. Aedeagus obovoid, with apical concavity. Aedeagal guides wide, apically hooked, extending beyond aedeagus distally, lateral impressions wide. Aedeagal apodemes laterally angular.

**Intraspecific variation.** Because of its larger size and details of the maxillary palpus, the species resembles *M. ruficollis* and by general structure of the male terminalia also *M. britannica*. However, the dark apical shade on the wing and details of the terminalia allow it to be safely distinguished.

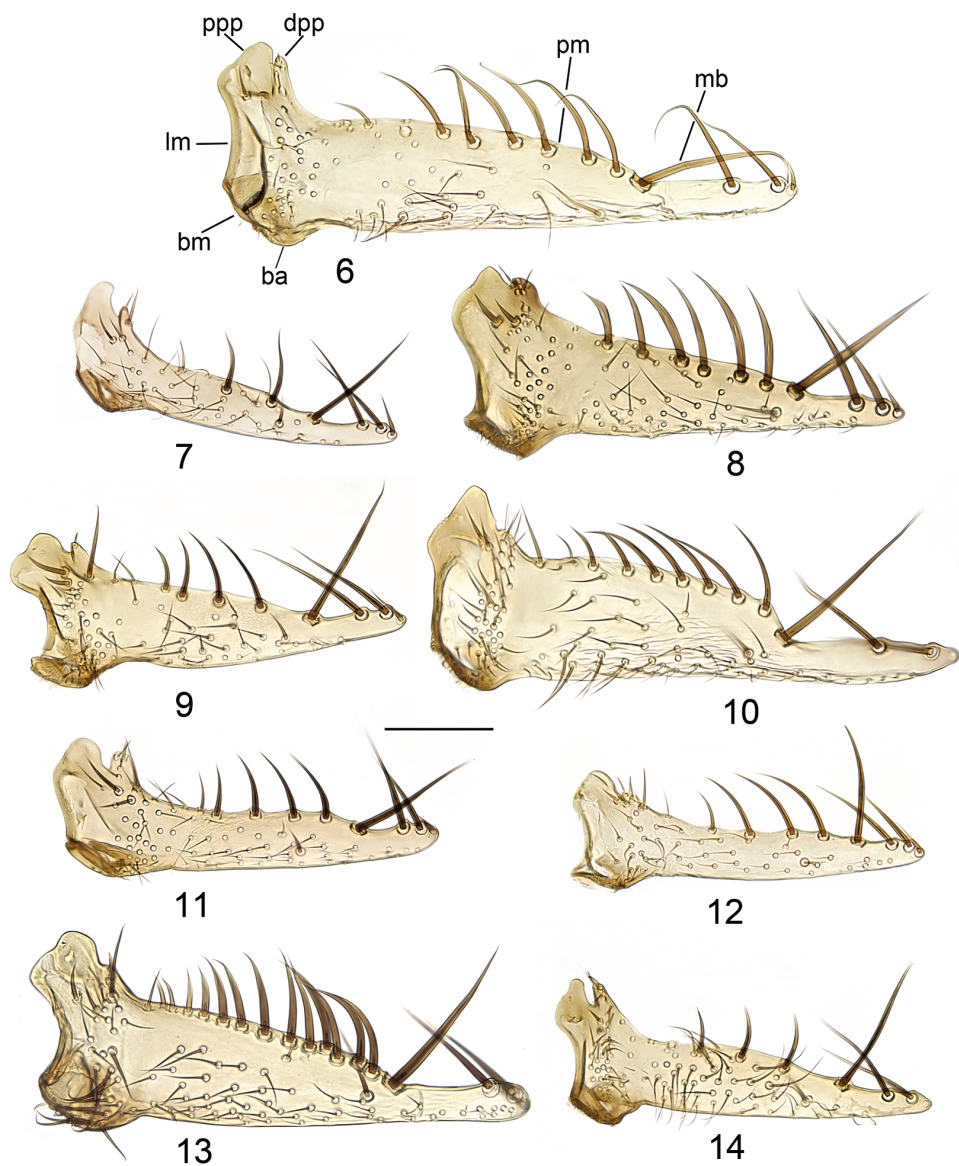
**Hosts and distribution.** So far recorded only from Madeira and the Canary Islands (Chandler and Ribeiro 1995). Hosts unknown.

### *Mycetophila uninotata* Zetterstedt, 1852

Figs 14, 31, 32, 49, 50

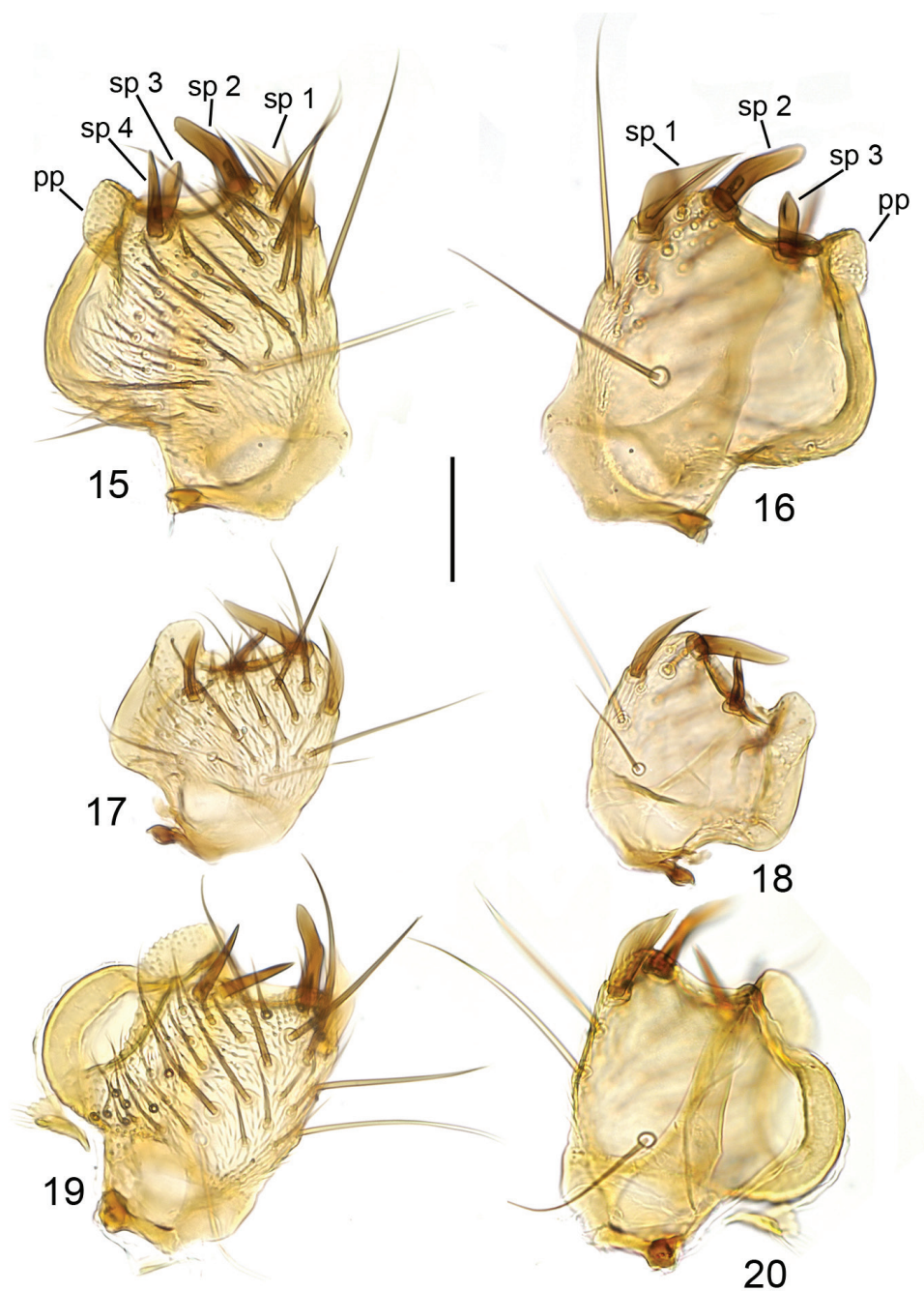
**Material.** **NORWAY.** 2♂♂, Troms, Svensby, 69°40'01,2"N, 019°49'58,8"E, 18.vii.2008, sweeping, O. Kurina leg. (IZBE0200113, IZBE0200072, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ303, SJ304). **FINLAND.** 3♂♂, Sodankylä, Paistipuolet, 75°319'15"N, 34°66'98,8"E, 1.vi–29.vi.2009, sweeping, J. Salmela leg. (IZBE0200114, IZBE0200115, IZBE0200116, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ436, SJ438, SJ439). **ESTONIA.** 1♂, Põlva county, Piusa cave, 57°54'N, 27°28'E, 1.ii.1996, sweeping, O. Kurina leg. (IZBE0200241, pinned with terminalia in glycerine); 1♂, Jõgeva county, Pataste, 58°34'52,2"N, 26°46'42,3"E, 10.ix–20.ix.2008, Malaise trap, J. Elts leg. (IZBE0200151, in alcohol with terminalia in glycerine, abdomen used for DNA sequence: SJ243); 1♂, Harju county, Üksnurme, 59°17'42,5"N, 24°37'41,1"E, 22.ix–12.x.2008, Malaise trap, E. Ilumäe leg. (IZBE0200117, in alcohol with terminalia in glycerine; abdomen used for DNA sequence: SJ227). **SLOVAKIA.** 1♂, NP Slovenský kras, Silická Ladnica, 48°33'00,0"N, 020°30'14,4"E, 505m, 4.vi.2009, sweeping, O. Kurina leg. (IZBE0200118, in alcohol, abdomen used for DNA sequence: SJ320).

**Male terminalia.** Posterior margin of gonocoxite with shallow convexity ventro-medially, and with blunt and oblique projections laterally. Posterior impression very wide and compressed, with emarginated anterior margin. Anterior impression with anteriorly evenly divergent narrow arms. Ventral branch of gonostylus with asymmetrical, narrow and elongated posterior process with dense and long warts; spine 1 sharply pointed; spine 2 about the same size, pointed; spines 3 and 4 smaller, pointed, closer to each other than to spine 2. Dorsal branch of gonostylus steeply tapering; lateral margin with shallow concavity; distal posterior process and proximal posterior process about the same height, both separated by a deep notch. Distal posterior process with 1–2 apical small setae and one bigger subapical seta; proximal posterior process bare and angular. Posterior margin proximally from medial bristle with 3–4 bigger gradually diminishing bristles followed by 2–3 smaller setae; internal surface with a stronger seta next to the medial bristle. Basal angle almost right-angled; basal margin with few setae. Ejaculatory apodeme with campanulate base and without rim. Aedeagus mostly triangular-shaped, apically widened. Aedeagal guides: 1) with two lamellae, 2) with wide and shallow lateral impressions, and 3) apically rounded, not extending beyond aedeagus distally. Aedeagal apodemes laterally angular.

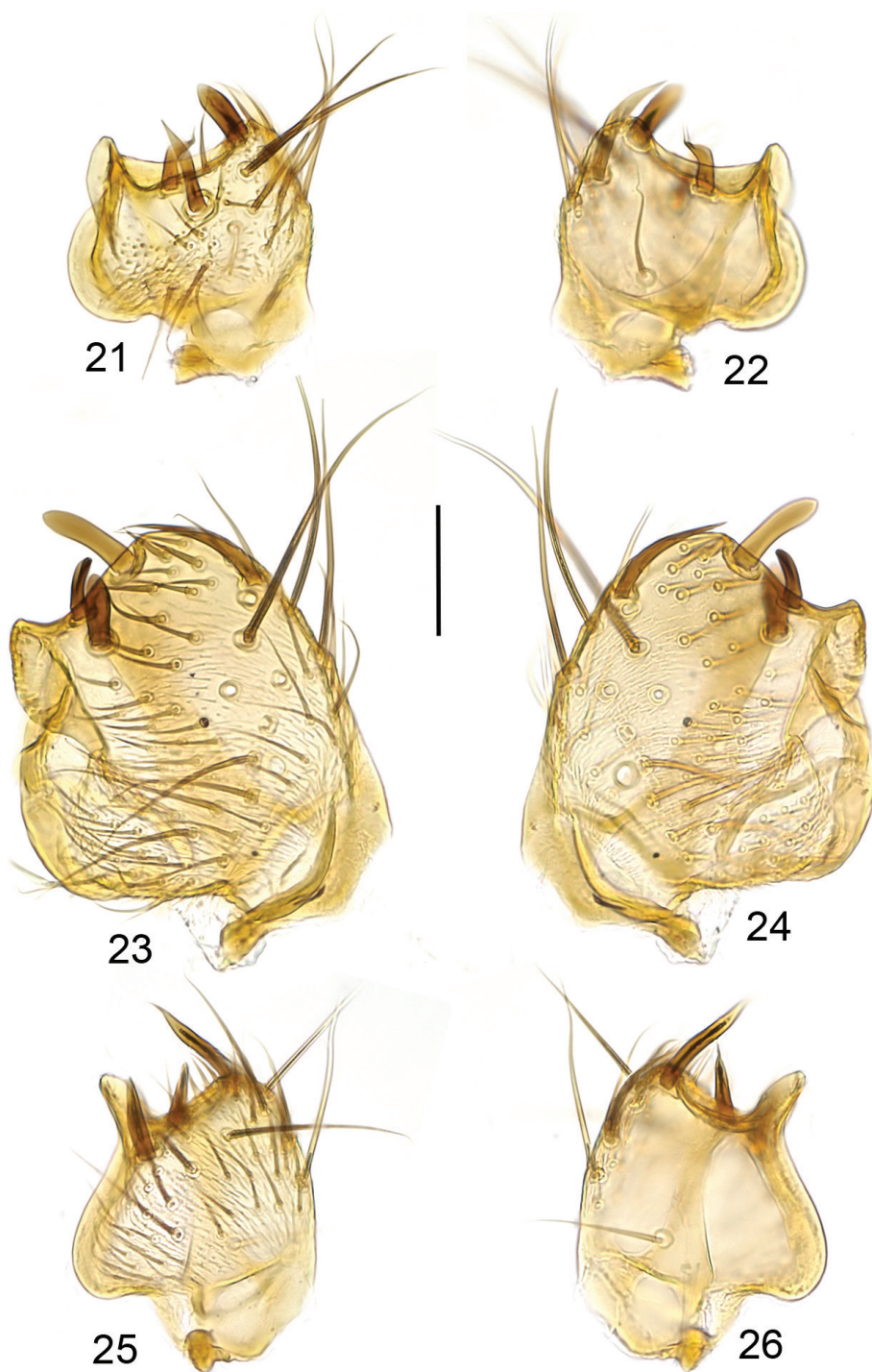


**Figures 6–14.** Dorsal branch of gonostylus. **6** *M. britannica* **7** *M. idonea* **8** *M. evanida* **9** *M. ichneumonea* **10** *M. ruficollis* **11** *M. sepulta* **12** *M. strobli* **13** *M. suffusala* **14** *M. uninotata*. Scale bar = 0.1 mm. Abbreviations: ba = basal angle; bm = basal margin; lm = lateral margin; pm = posterior margin; mb = medial bristle; dpp= distal posterior process; ppp = proximal posterior process.



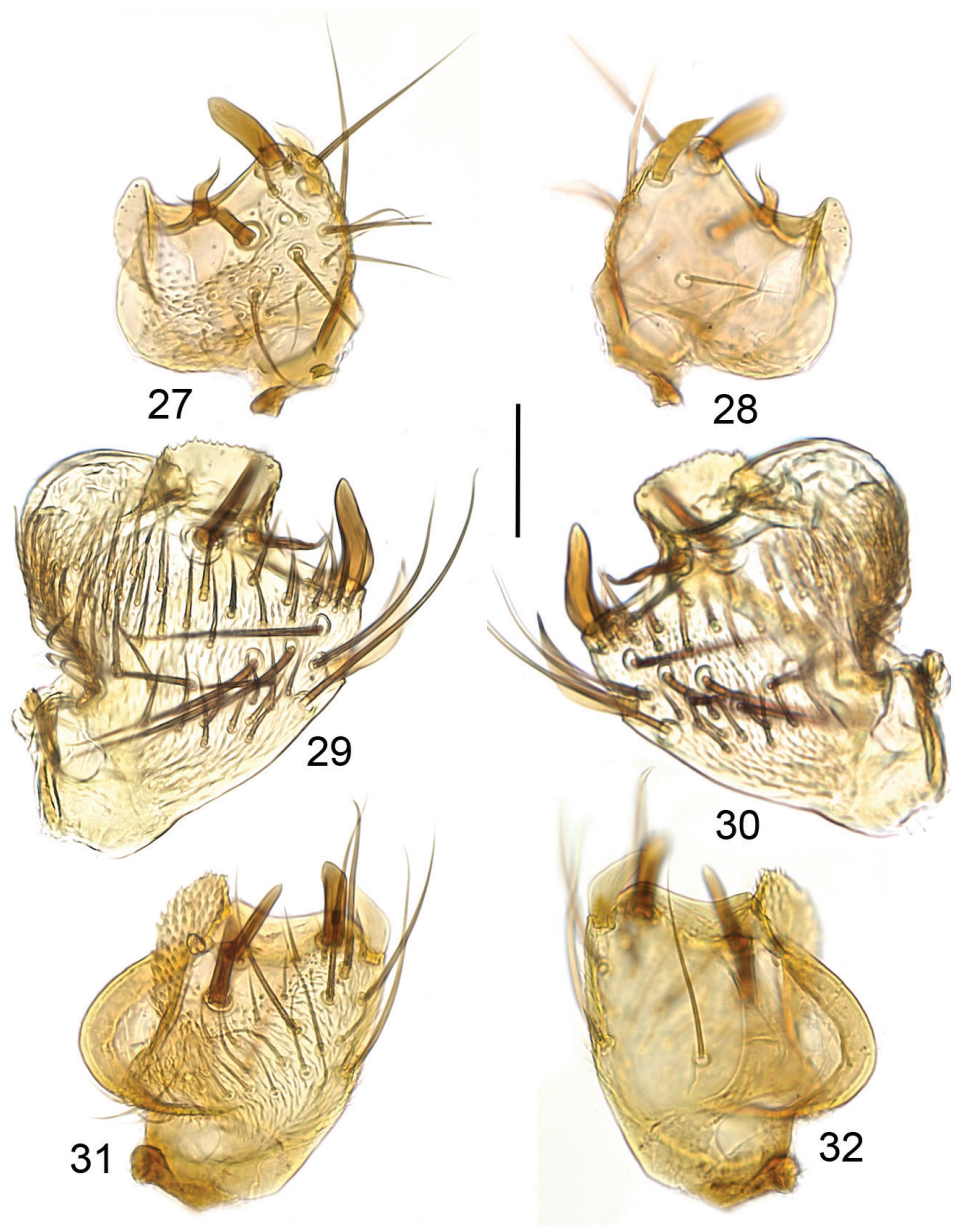


**Figures 15–20.** Ventral branch of gonostylus, ventral view (15, 17, 19) and internal view (16, 18, 20). 15, 16 *M. britannica* 17, 18 *M. idonea* 19, 20 *M. ichneumonea*. Scale bar = 0.05 mm. Abbreviations: pp = posterior process; sp = posterior spines on the ventral branch of gonostylus.

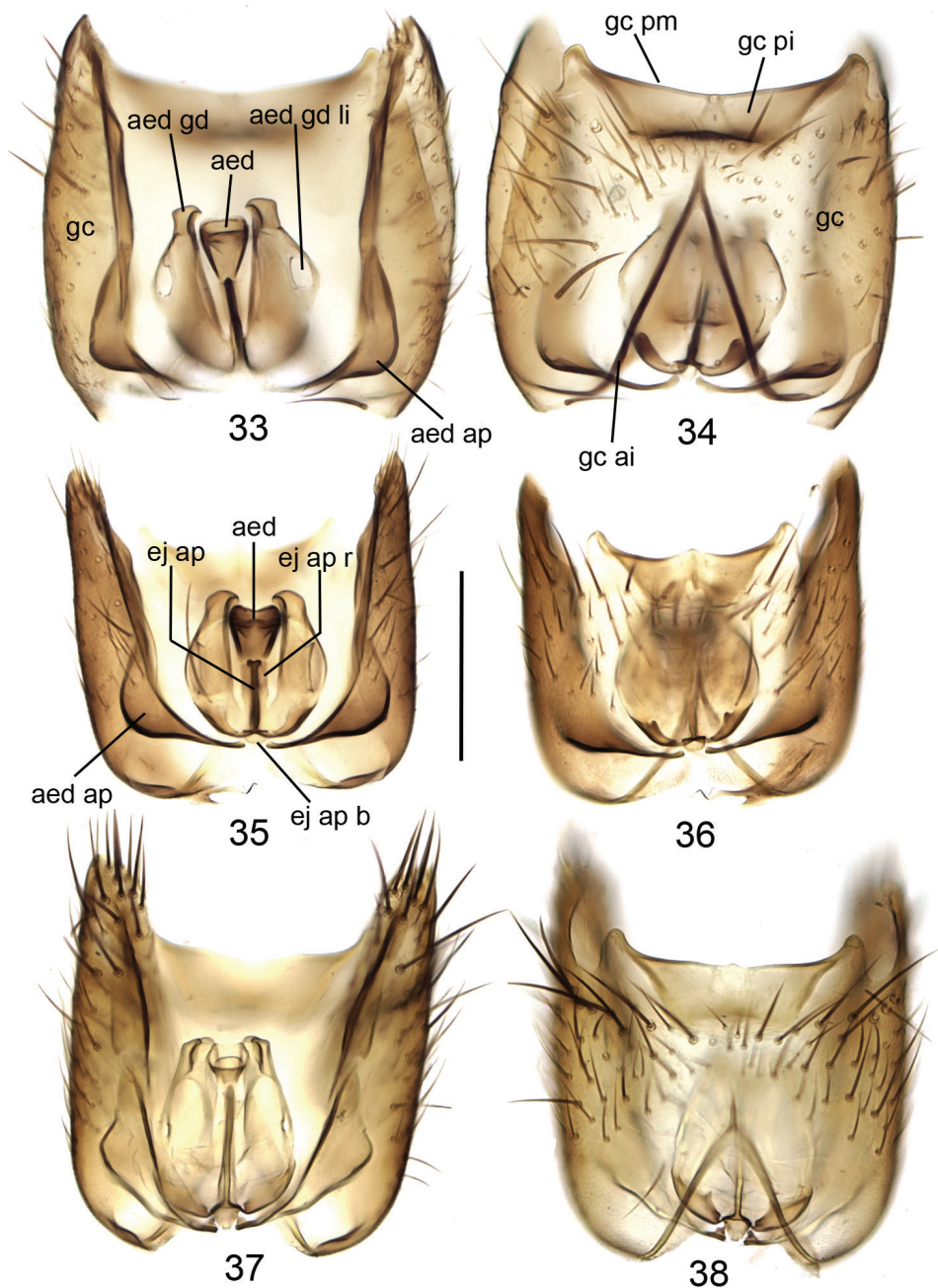


**Figures 21–26.** Ventral branch of gonostylus, ventral view (21, 23, 25) and internal view (22, 24, 26). 21, 22 *M. evanida* 23, 24 *M. ruficollis* 25, 26 *M. sepulta*. Scale bar = 0.05 mm.

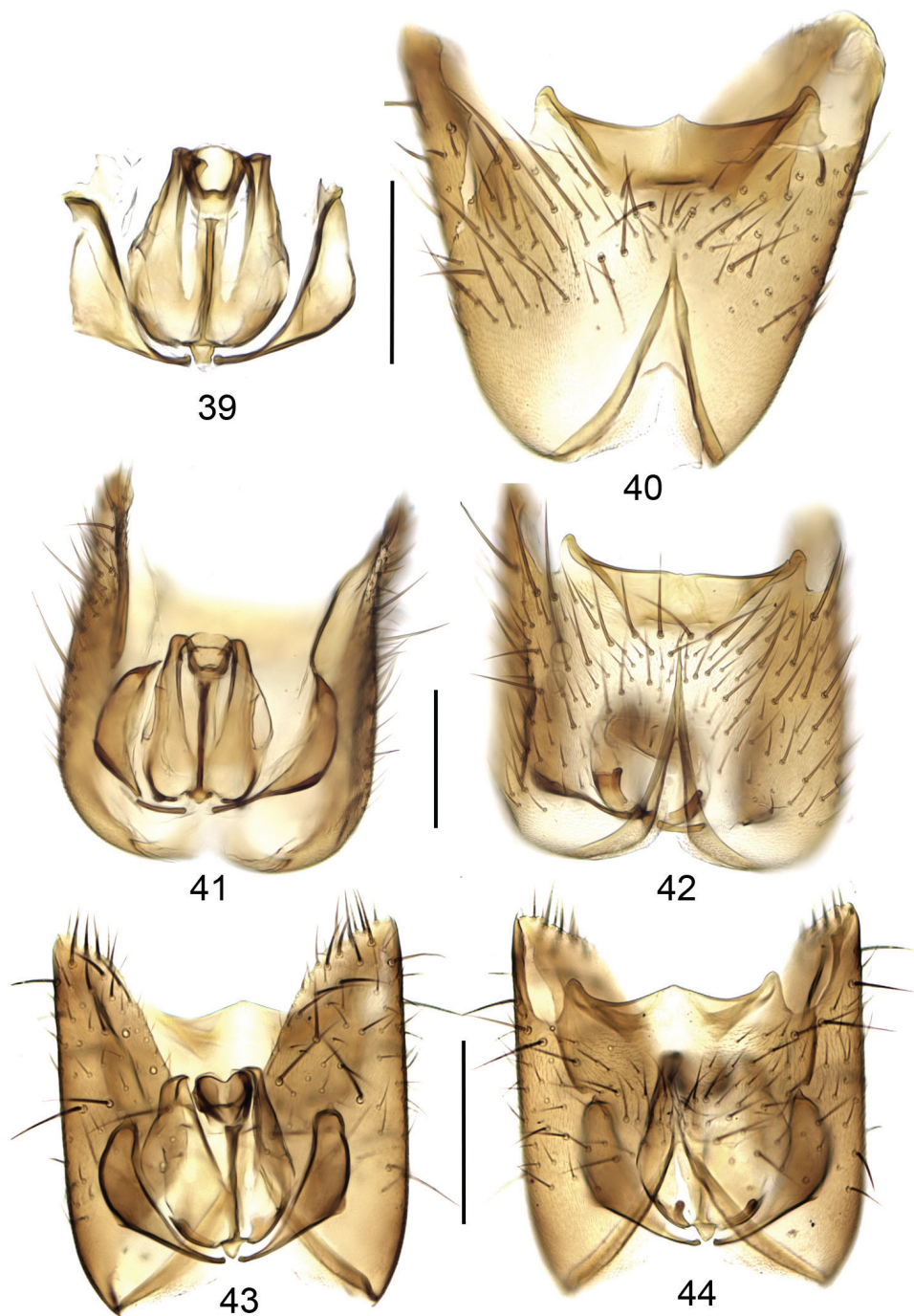




**Figures 27–32.** Ventral branch of gonostylus, ventral view (27, 29, 31) and internal view (28, 30, 32). 27, 28 *M. strobli* 29, 30 *M. suffusala* 31, 32 *M. uninotata*. Scale bar = 0.05 mm.

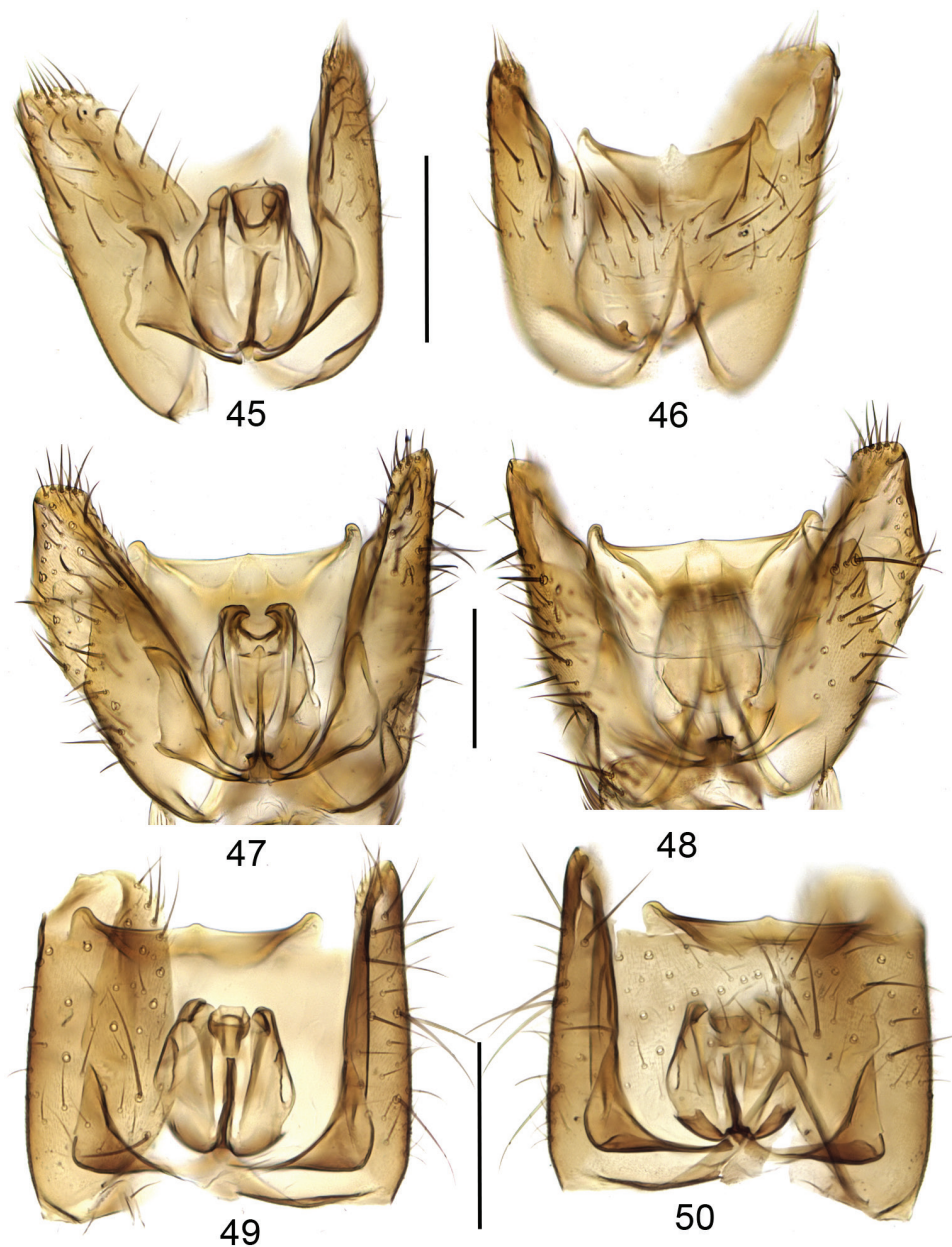


**Figures 33–38.** Gonocoxites with aedeagal complex, dorsal view (33, 35, 37) and ventral view (34, 36, 38). 33, 34 *M. britannica* 35, 36 *M. idonea* 37, 38 *M. ichneumonea*. Scale bar = 0.2 mm. Abbreviations: aed = aedeagus; aed ap = aedeagal apodeme; aed gd = aedeagal guide; aed gd li = lateral impression on the aedeagal guide; ej ap = ejaculatory apodeme; ej ap b = base of ejaculatory apodeme; ej tb r = rim of ejaculatory apodeme; gc = gonocoxite; gc ai = anterior impression of gonocoxite; gc pi = posterior impression of gonocoxite; gc pm = posterior margin of gonocoxite.



**Figures 39–44.** Gonocoxites with/and aedeagal complex, dorsal view (39, 41, 43) and ventral view (40, 42, 44). 39, 40 *M. evanida* 41, 42 *M. ruficollis* 43, 44 *M. sepulta*. Scale bar = 0.2 mm.





**Figures 45–50.** Gonocoxites with aedeagal complex, dorsal view (45, 47, 49) and ventral view (46, 48, 50). 45, 46 *M. strobli* 47, 48 *M. suffusala* 49, 50 *M. uninotata*. Scale bar = 0.2 mm.

**Intraspecific variation.** The wide and compressed posterior impression of the gonocoxite, the distinct warts on the posterior process of the ventral branch of gonostylus and almost equal posterior processes of the dorsal branch of the gonostylus al-

low the species to be safely distinguished. In Finnish material, spine 2 on the ventral branch of the gonostylus is more massive than described by Laštovka (1972). In some specimens the base of the ejaculatory apodeme and the lateral margin of the dorsal branch of the gonostylus resemble those of *M. ichneumonea*. Phylogenetic analysis based on molecular data revealed three different clades (Fig. 4) that cannot be distinguished based on morphology (see also Discussion).

**Hosts and distribution.** *Mycetophila uninotata* has been reared from species of *Collybia*, *Cortinarius* and *Lactarius* (Yakovlev 1994). Widely distributed in Central and Northern Europe but seems to be absent in the Mediterranean (Chandler 2013). The distribution gap between Central and Eastern Europe (e.g. absence in Poland, Belarus, Ukraine) can be explained by insufficient collecting.

## Discussion

This study represents the first evaluation of morphology-based species delimitation of fungus gnats by applying DNA sequence data. Results of the analyses, based on molecular data obtained for seven out of the nine European species from the *M. ruficollis* group, mostly supported the morphological species delimitation outlined by Laštovka (1972) and Laštovka and Kidd (1975). The genetic distance between members of different species, calculated from the COI barcoding region exceeded 2.9%, with intraspecific distance remaining below 1%. Our results are thus in accordance with inter- and intraspecific variation in different insect orders, documented to be on average over 2% and under 1%, respectively (e.g. Hebert et al. 2004, 2010). Only in *M. uninotata* the intraspecific distance was 2.3%, whereas three strongly supported clades were distinguished within this species in the phylogenetic trees. This evidence suggests that the current circumscription of *M. uninotata* includes two to three cryptic species. However, we were not able to find any morphological differences distinguishing these clades, each of which includes geographically distant material. More samples are needed to evaluate the genetic heterogeneity within *M. uninotata*.

The barcoding region of COI provided a clear barcoding gap for the distinction of all species, except for those in the described subclade of *M. ichneumonea* and *M. uninotata* that seems to include several recently differentiated species. In general, both the inter- and intraspecific variation remained lower than observed in other groups of insects (Angélica et al. 2014, Wang et al. 2012, Schwarzfeld and Sperling 2014). The comparison of the three regions sequenced from two genes revealed that the variation in the barcoding region of COI was slightly higher than in the following stretch of comparable length. The latter has been widely used in taxonomic studies on fungus gnats, focusing on higher taxonomic levels (Rindal et al. 2009, Ševčík et al. 2013). Here we showed its applicability also for species discrimination. By contrast, ITS2 rDNA that has become widely used in delimitation of insect taxa (e.g. Rokas et al. 2002, Schwarzfeld and Sperling 2014, Haarto and Ståhls 2014), included much less variation than each of the COI regions. Analyses of ITS2 rDNA data also resulted in

lower phylogenetic resolution with some of the species remaining unresolved. Outperformance of COI over ITS2 has been observed also in Ichneumonidae (Schwarzfeld and Sperling 2014) and Mycetophilidae (Kurina et al. 2015).

Identification of gnats in the *M. ruficollis* group on the basis of morphological characters is complicated due to considerable intraspecific, yet only limited interspecific variation, mostly observed only upon examination of male genitalia. It was found to be most difficult to distinguish *M. strobli* and *M. uninotata* from *M. ichneumonea*. Also Laštovka (1972) mentioned that *M. uninotata* is most similar to *M. ichneumonea*. The phylogenetic analyses revealed close relationship of *M. uninotata* and *M. ichneumonea*, with lowest distance between the COI barcodes of their members. The difficulties in identification are obviously caused by high variation within the current concept of *M. uninotata*, suggested here to comprise cryptic species. COI data revealed *M. strobli* as more distant, but it appeared indistinguishable from *M. ichneumonea* based on ITS2 sequences. The morphologically distinct members of geographically separated populations, observed in some species, were not distinguished in molecular analyses. For example, some specimens of *M. idonea* from Georgia and Poland differed morphologically from their conspecifics but appeared homogeneous in the sequence data. Regarding geographic ranges, five out of the nine European species (viz. *M. evanida*, *M. ichneumonea*, *M. strobli*, *M. ruficollis* and *M. uninotata*) are widely distributed in the region while the rest of them (viz. *M. britannica*, *M. idonea*, *M. sepulta* and *M. suffusala*) have more scattered or endemic distribution patterns. Our study adds five new country records (viz. *M. evanida* from Estonia, *M. strobli* from Norway, and *M. ichneumonea*, *M. idonea* and *M. ruficollis* from Georgia) which widen the known distribution ranges, yet not changing the known patterns.

Our results suggest that several of the species in the *M. ruficollis* group have distinct host ranges. Thus far the larval stages of all European species in the group, except for *M. suffusala*, had been reported to feed from fruit bodies of a variety of mushrooms (Hackman and Meinander 1979, Yakovlev 1994, Chandler and Ribeiro 1995, Chandler 2010, Ševčík 2010), without preference for any fungal taxa. However, our data support the tendency apparent from literature records suggesting that gnats in the *M. ruficollis* group do not or only occasionally consume fruit bodies of the Boletales. Our results do not agree with the suggestion by Laštovka (1972) that polytypic *M. ichneumonea* consists of two to three subspecies. However, taken the difficulties in distinguishing this species from the *M. uninotata* complex, as reported here, it is possible that previous authors have had a mixture of species under consideration.

Combining morphological and molecular characters for identification of fungus gnats reared from identified fungal fruit bodies provides unique information of host use (Pöldmaa et al. 2015). Most of the fungus gnats from the *M. ruficollis* group reared during recent years in Estonia from more than 680 fruit bodies represented *M. ichneumonea*. While not of frequent occurrence, *M. ichneumonea* was reared mostly from fruit bodies of two phylogenetically distant genera: *Lactarius* and *Cortinarius*. Such ‘disjunct host range’ is typical for several phytophagous insects (e.g. Bernays and Chapman 1994, Janz and Nylin 1997) but has so far not been described for mycetophages. Molecular



data confirmed the conspecificity of adults reared from these two host genera with no host-related variation observed in the ITS2 or COI sequences. Other species, obtained by rearing of adults, were generally feeding on hosts from other fungal genera. While larvae of *M. ruficollis* consumed mostly saprotrophic mushrooms, *M. idonea* was reared from a single fruit body of *Amanita*. *Mycetophila strobli* seemed to prefer species from the earliest diverged lineages in the genus *Russula*, recognised as the subgenus *Compactae* by most authors. Taken the extensive rearing experiments from diverse mushroom taxa collected from the pine-dominated boreal forests we consider the observed host use patterns to represent fungus gnats' specialisation in this habitat. Apparently, different fungi can serve as (preferred) hosts of members of the *M. ruficollis* group in other forest types.

Identification of closely related fungus gnats, as reported here in the *M. ruficollis* group, relies to large extent on a few morphological characters, mostly those of male terminalia. Blurred by intraspecific variation and the lack of such features in females, unambiguous identification is often impossible. Molecular data overcomes these obstacles and should be considered in species delimitation of fungus gnats. For that purpose, we advocate the use of the barcoding region of COI. Special value should be given to sequencing adults reared from identified fungi as these enable to elucidate host as well as geographic range of individual species of fungus gnats.

## Acknowledgements

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# Four new species of *Epicephala* Meyrick, 1880 (Lepidoptera, Gracillariidae) associated with two species of *Glochidion* (Phyllanthaceae) from Hainan Island in China

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## Abstract

Four new *Epicephala* species that feed on the seeds of *Glochidion sphaerogynum* (Phyllanthaceae) from Yinggeling Mountain Nature Reserves in Hainan Province of China are described: *E. domina* **sp. n.**, *E. impolliniferens* **sp. n.**, *E. angustisaccula* **sp. n.** and *E. camurella* **sp. n.** The latter two species are also associated with *Glochidion wrightii*. Photographs of adults and genital structures are provided.

## Keywords

Lepidoptera, Gracillariidae, *Epicephala*, Phyllanthaceae, *Glochidion*, new species, China

## Introduction

The genus *Epicephala* Meyrick, 1880 consists of 49 described species worldwide, mainly distributed in the Old World, with 15 species occurring in the Australian Region, 28 in the Oriental Region, one in the Palearctic Region, and six in the Afrotropical Region (Vári 1961; Kuznetsov 1979; Nielsen et al. 1996; De Prins and De Prins 2005, 2014; Zhang et al. 2012b; Li and Yang 2015). In China, thirteen species have been

recorded prior to this study (Meyrick 1935; Kendrick 2005; Hu et al. 2011a, b; Zhang et al. 2012b; Li and Yang 2015; Wang and Li 2015; Yang and Li 2015). But there are still a large number of undescribed species of *Epicephala*, especially in tropical areas (Kawakita et al. 2004; Kawakita and Kato 2006, 2009; Hembry et al. 2012, 2013).

In the course of studying the coevolutionary relationships between *Epicephala* moths and *Glochidion* plants in Yinggeling Mountain Nature Reserves of Hainan Province, we identified four new *Epicephala* species. *Epicephala domina* sp. n. and *E. impolliniferens* sp. n. are associated with *G. sphaerogynum* (Müll. Arg.) Kurz; *E. angustisaccula* sp. n. and *E. camurella* sp. n. are associated with both *G. sphaerogynum* (Figs 1–4) and *G. wrightii* Benth.

Larvae of *Epicephala* species feed on seeds in the fruit of their host-plants in the family Phyllanthaceae (Euphorbiaceae *sensu lato*). Some *Epicephala* species have been known to be highly species-specific with their host-plants within Phyllanthaceae (Kawakita 2010, Hu et al. 2011b, Zhang et al. 2012c), and have presumably co-evolved with their hosts. In most cases, within pollinating *Epicephala* and their hosts, a single species of *Epicephala* and a single species of Phyllanthaceae are uniquely associated at a single site, producing so-called “one-to-one” patterns of interaction (Kawakita and Kato 2006; Zhang et al. 2012b). However, in some cases, more complex patterns of specialization, such as one-to-two (Kawakita and Kato 2006; Zhang et al. 2012a), one-to-three (Li and Yang 2015) and two-to-two (Zhang et al. 2012c) patterns, have occurred. We find one-to-four and one-to-two relationships, which will be described further in a separate paper. The present paper just aims at describing the four new *Epicephala* species associated with the two *Glochidion* species from Hainan Island in China.

## Material and methods

Specimens examined in this study were mainly reared from fruits of host-plants, which were gained during a field study from 2009 to 2014 in Yinggeling Mountain Nature Reserves (18°59'30"–19°04'20"N, 109°28'00"–109°35'30"E) in midwestern Hainan Province, China (Fig. 1), and only some were collected on flowers or leaves of two *Glochidion* plants in the late evening. Genitalia dissection and mounting methods follow Li and Zheng (1996). Photos of the host-plant *G. sphaerogynum* were taken in the field using a Canon Power Shot G10 digital camera. Photos of adult specimens were taken with a Leica M250A stereo microscope. Illustrations of the genitalia were prepared by using Leica DM750 microscope, and refined in Photoshop® CS4 software.

The type specimens and vouchers of host plants are deposited in the Insect Collection, College of Life Sciences, Nankai University (NKUM), Tianjin, China and some paratypes are deposited in the Department of Life Sciences, Division of Terrestrial Invertebrates, Natural History Museum, London, UK (BMNH).



**Figures 1–4.** Habitats and common host plant of four *Epicephala* species in Yinggeling Mountain Nature Reserves, Hainan Province, China. **1** general habitat **2–4** morphological features of *Glochidion sphaerogynum*: **2** an individual tree **3** branches and leaves **4** male flowers and young fruits.

## Taxonomic account

### *Epicephala domina* Li, sp. n.

<http://zoobank.org/0161B207-D186-47ED-97B6-7FC3518C07E1>

Figs 5, 9, 11, 15

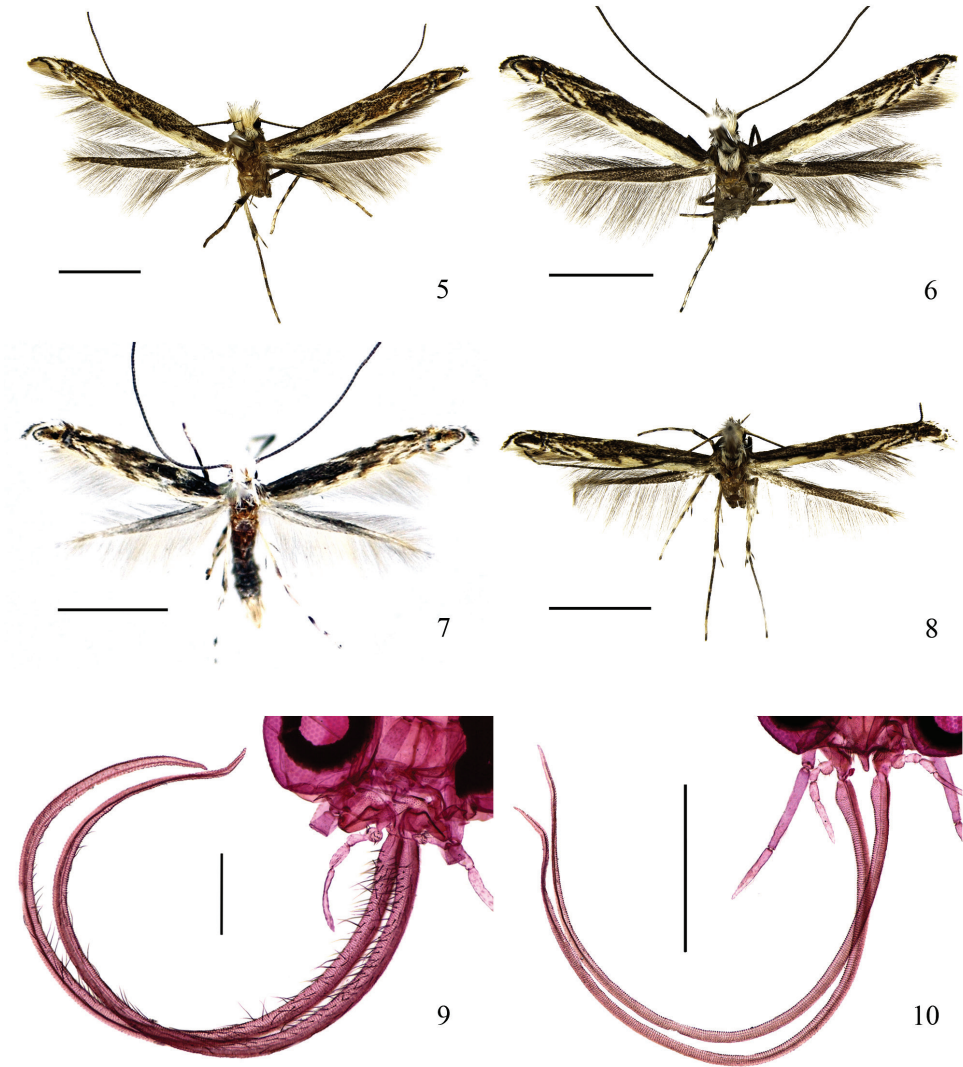
**Description.** Adult (Fig. 5). Forewing expanse 7.5–11.0 mm. Head creamy white tinged with pale yellow, lateral sides with long brown scales. Labial palpus white or grayish white, distal half of second and third palpomeres dark brown on outer surface, inner surface of third palpomere white to gray. Antenna grayish brown. Thorax white. Tegula yellowish brown. Forewing grayish brown to deep brown; three pairs white striae from both costal and dorsal margins at 2/5, 3/5 and 4/5 extending obliquely outward to middle as well as to end and outside of cell, third dorsal striae broader and more distinct; dorsal margin with a broad white band extending from base to tornal area; a narrow silvery-white fascia with metallic reflection from costal 6/7 to dorsal margin; distal 1/7 yellowish brown, with a central black dot, with a triangular white dot near costa and a white streak along dorsal margin; cilia basally black, medially grayish white, distally black from distal 1/7 of costal margin along termen to tornus, gray along dorsal margin. Hindwing grayish brown; cilia brown along costal margin and grayish or yellowish brown along dorsal margin.

**Male genitalia** (Fig. 11). Tegumen broadly triangular, narrowed and sclerotized laterally. Tuba analis elongate, gradually narrowed toward apex, exceeding caudal margin of tegumen apically. Costa sub-rectangular, longer than tegumen, distal 1/3 more or less broadened, apex obliquely rounded; dorsal margin straight; ventral margin with basal 3/4 slightly arched inward, with a rounded process at 3/4, with dense long setae on distal half. Saccus elongate oval, about 4/5 length of costa, acute-angled apically, dorsal margin more arched, distal part of dorsal and ventral margins heavily sclerotized. Transtilla broad at base, long triangular. Vinculum broad V-shaped, rounded on posterior margin; saccus club-shaped, shorter than vinculum, acute at apex. Phallus thin and straight, as long as valva; cornutus in a shape of a rolled plate, with minute spines.

**Female genitalia** (Fig. 15). Ovipositor small, bilobed apically, dentate laterally. Apophysis posterioris slightly longer than apophysis anterioris. Lamella postvaginalis large, as long as 8th abdominal segment, heavily sclerotized, deeply concave at middle on caudal margin to half length, forming two triangles with acute apex. Antrum cylindrical, strongly sclerotized, as long as ductus bursae. Ductus bursae about half length of apophysis anterioris, membranous, with broad longitudinal sclerotized parallel folds, extending from base to corpus bursae; ductus seminalis membranous, arising anterior of antrum. Corpus bursae oval, as long as ductus bursae, with reticulate patches medially; signum a stout tooth, placed at middle.

**Diagnosis.** This new species is similar to the majority of *Epicephala* species in forewing pattern by having a white dorsal margin, but can be separated from other species by its genitalia. The new species is more similar to *E. ancylopa* Meyrick, 1918, but can be distinguished from the latter in the male genitalia by the ventral margin of the





**Figures 5–10.** Adult morphology of *Epicephala* spp. **5–8** Adults: **5** *E. domina* sp. n., female paratype **6** *E. impolliniferens* sp. n., female paratype **7** *E. camurella* sp. n., male paratype **8** *E. angustisaccula* sp. n., male paratype **9–10** Morphology of female proboscis: **9** *E. domina* sp. n., female paratype, head slide No. WZB14297 (genitalia slide No. WZB14295 for determination) **10** *E. impolliniferens* sp. n., female paratype, head slide No. WZB14298 (genitalia slide No. WZB14160 for determination). Scale bar: 2.0 mm (**5–6**); 0.5 mm (**9, 10**).

costa with a rounded process at  $3/4$ , the sacculus about  $4/5$  length of the costa and the acute apex; and in the female genitalia by the lamella postvaginalis as long as the 8th abdominal segment and the presence of signum. In *E. ancylopa* (Lectotype ♂, BMNH, examined; Syntypes: ♂, BMNH, examined, genitalia slide No. 32324, ♀, BMNH, examined, genitalia slide No. 32330, both dissected by Houhun Li), in the male geni-

talia the ventral margin of the costa has a rounded process at 2/3, the sacculus is about 3/4 length of the costa and bluntly rounded at apex; in the female genitalia the lamella postvaginalis is shorter than the 8th abdominal segment and the signum is absent.

**Type material.** 244♂, 430♀, with genitalia preparations of 244♂ and 93♀.

Holotype ♂ – **CHINA: Hainan Province:** Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 07.ii.2014, reared from host-plant *Glochidion sphaerogynum* by Zhibo Wang, genitalia slide no. WZB14337.

Paratypes – **CHINA: Hainan Province:** 4♀, Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 25.xii.2009–25.i.2010, leg. Bingbing Hu; 243♂, 426♀, same locality as holotype, 19.xii.2012–22.i.2013, 12.i.–19.ii.2014, reared or collected from *Glochidion sphaerogynum* by Zhibo Wang (2♂, 2♀, deposited in BMNH).

**Distribution.** China (Hainan).

**Biology.** Larvae feed on seeds in the fruits of *Glochidion sphaerogynum* (Müll.Arg.) Kurz (Phyllanthaceae).

**Etymology.** The specific name is derived from the Latin *dominus* (master, lord), in reference to its status as the dominant *Epicephala* species associated with *G. sphaerogynum*.

### *Epicephala impolliniferens* Li, sp. n.

<http://zoobank.org/EBB03B6E-C800-4492-A563-74E31E89EF54>

Figs 6, 10, 12, 16

**Description.** Adult (Fig. 6). Forewing expanse 7.0–10.5 mm. Head white to yellowish white mixed with brown scales. Labial palpus white, inner surface with scattered grayish brown scales; distal 1/2 to 2/3 of second palpomere brown to dark brown on outer surface, third palpomere dark brown on outer surface except tip. Antenna grayish brown to dark brown, each flagellomere paler at base. Thorax white. Tegula dark brown. Forewing brown to dark brown; three pairs white striae from both costal and dorsal margins at 2/5, 2/3 and 3/4 extending obliquely outward to middle as well as to end and outside of cell, second dorsal stria longest and third costal stria shortest; dorsal margin with a broad white band extending from base to tornus; a silvery-white fascia with metallic reflection from costal 6/7 to dorsal margin, gently arched outward; distal 1/7 yellowish brown, with a central black dot, with an indistinct white dot at costa and a white streak along dorsal margin; cilia basally black, medially white, distally black from distal part of costal margin to apex, white from termen to tornus, gray along dorsal margin. Hindwing yellowish white (especially at base) to brown; cilia gray.

**Male genitalia** (Fig. 12). Tegumen broadly oval, sclerotized laterally. Costa as long as tegumen, slightly broadened at base, rounded at apex, with long dense setae ventrally, distal 4/5 nearly parallel dorso-ventrally; dorsal margin straight; ventral margin with lobed process obliquely outward. Sacculus elongate oval, about 3/4 length of costa, acute distally. Transtilla broad at base, narrowed triangularly, curved ventrad distally, acute apically. Vinculum V-shaped, rounded on posterior margin; saccus broad digitiform, as long as vinculum, slightly narrowed at base, rounded at apex.



Phallus thick and straight, slightly longer than valva, gently thinned from base to apex; cornuti consisting of four to six spines, usually one or two large, compactly grouped into a bundle.

**Female genitalia** (Fig. 16). Ovipositor small, triangular, dentate laterally, acute apically. Apophysis posterioris obviously thick and strong, 1.5 times longer than apophysis anterioris. Lamella postvaginalis small, rounded, about twice as wide as ostium bursae. Antrum sclerotized, short, as long as lamella postvaginalis. Ductus bursae about same length of apophysis anterioris, membranous, with longitudinal parallel folds, compactly grouped into a broad, heavily sclerotized band extending from base to corpus bursae; ductus seminalis membranous, arising anterior of ductus bursae. Corpus bursae oval, small, about half length of ductus bursae, with reticulate patches medially; signum triangular, placed at middle.

**Diagnosis.** This species is similar to *E. domina* sp. n. in appearance, but can be separated from the latter by the female proboscis without tip-dilated sensory setae (Fig. 10); in the male genitalia by the apex-rounded costa with a lobed process on ventral margin medially, the sacculus with apex elongate-acute, the phallus with four to six cornuti compactly grouped into a bundle; in the female genitalia by the apically acute ovipositor, the small rounded lamella postvaginalis, the antrum as long as the lamella postvaginalis, the ductus bursae about same length of apophysis anterioris. In *E. domina* sp. n., the female proboscis possesses a large number of tip-dilated sensory setae as most species in the genus (Fig. 9), which can hold numerous pollen grains for pollination; in the male genitalia the costa has an obliquely rounded apex and a rounded protuberance at 3/4 on ventral margin, the sacculus is shortly acute at apex, the cornutus is a rolled plate; in the female genitalia the ovipositor is bilobed at apex, the lamella postvaginalis consists of two triangles with acute apex; the antrum is as long as the ductus bursae, and the ductus bursae is about half length of the apophysis anterioris.

**Remarks.** *Epicephala impolliniferens* sp. n. is the first species of non-pollinating *Epicephala* associated with *Glochidion*, and the second named species within the genus (following *E. relictella* Kuznetsov, 1979) in which the female proboscis lacks the tip-dilated sensory setae on its surface for carrying pollens. Species of the genus *Epicephala* are noteworthy for their obligate pollination habits, which involve mutualistic relationship with trees of Phyllanthaceae. However, both *E. impolliniferens* sp. n. and *E. relictella* Kuznetsov are not associated with pollination in biology referring to the morphology of the female proboscis. *Epicephala relictella* feeds on the seeds of *Flueggea suffruticosa* (Pall.) Baill. (Hu et al. 2011b). Kawakita and Kato (2009) reported several undescribed *Epicephala* species that do not pollinate their hosts. We have confirmed one of them not belonging to the genus *Epicephala* (unpublished data), and the status of the other undescribed species needs to be determined.

**Type material.** 48♂, 64♀, with genitalia preparations of 48♂ and 46♀.

Holotype ♂ – **CHINA: Hainan Province:** Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 11.i.2013, reared from host-plant *Glochidion sphaerogynum* by Zhibo Wang, genitalia slide no. WZB14178.

Paratypes – **CHINA: Hainan Province:** 3♂, 5♀, Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 12.vi.2010, 18–26.ix.2010, leg. Bingbing Hu; 45♂, 58♀, same locality as holotype, 19.xii.2012–24.i.2013, 12.i.–20.ii.2014, reared or collected from *Glochidion sphaerogynum* by Zhibo Wang (2♂, 2♀, deposited in BMNH).

**Distribution.** China (Hainan).

**Biology.** Larvae feed on seeds in the fruits of *Glochidion sphaerogynum* (Müll. Arg.) Kurz (Phyllanthaceae).

**Etymology.** The specific name is derived from the Latin *im-* (= not), *pollinicus* (= pollen) and *ferre* (= to carry, to bear), in reference to the non-pollinating habit.

***Epicephala camurella* Li, sp. n.**

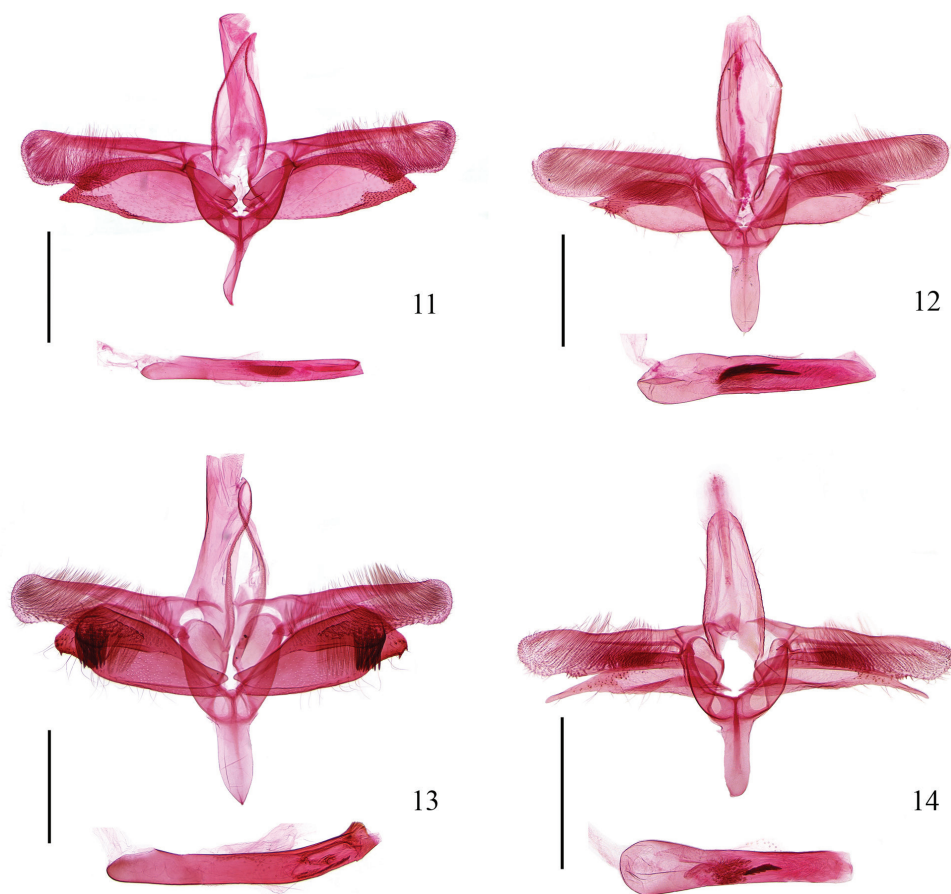
<http://zoobank.org/55573693-A511-43CD-8250-E553797D20B6>

Figs 7, 13, 17

**Description.** Adult (Fig. 7). Forewing expanse 7.0–10.0 mm. Head white, mixed with brown scales. Labial palpus white, second and third palpomeres dark brown on outer surface, second palpomere scattered with grayish brown scales on inner surface. Antenna grayish brown to dark brown. Thorax white. Tegula brown, with a few grayish white or brown mottled white scales distally. Forewing grayish brown to brown, sometimes tinged with ochreous scales; three pairs white striae from both costal and dorsal margins at 1/3, 3/5 and 4/5 extending obliquely outward to middle and end of cell as well as to outside of cell, second dorsal stria longest and extending to 6/7; dorsal margin with a broad white band from base to tornus; a silvery-white fascia with metallic reflection from costal 6/7 to dorsal margin, nearly straight; distal 1/7 ochreous, with a central black dot, with a white dot at costa and a broad white streak along dorsal margin; cilia white from distal part of costal margin along termen to tornus except black at base and apex, grayish white to gray along dorsal margin. Hindwing gray; cilia grayish white to gray.

**Male genitalia** (Fig. 13). Tegumen elongately oval, sclerotized laterally. Tuba analis long and broad, more or less sclerotized, apically far exceeding caudal margin of tegumen. Costa as long as tegumen, subrectangular, slightly narrowed before rounded apex, with long dense setae ventrally; dorsal margin nearly straight; ventral margin sinuate, with a small protuberance at 2/5, with a large roundly protuberance at 3/5, bearing long strong spines distally. Saccus broad, subtriangular, about 3/4 length of costa, narrowed at base, widened to about middle, distal half parallel except slightly concave ventrally before apex; apex truncate obliquely, with sparse short spines ventrally. Transtilla broad at base, elongate triangular, acute apically. Vinculum V-shaped, rounded on posterior margin; saccus broad digitiform, shorter than vinculum, apex obtusely acute. Phallus long and strong, about 1.5 times longer than valva, gently curved at distal 1/3, with pieces of irregular sclerites in distal 1/3; cornutus absent.

**Female genitalia** (Fig. 17). Ovipositor small, triangular, dentate laterally, acute apically. Apophysis posterioris 1.8 times longer than apophysis anterioris. Lamella postvaginalis developed, heavily sclerotized, deeply concave at middle caudally, form-



**Figures 11–14.** Male genitalia of *Epicephala* spp. **11** *E. domina* sp. n., holotype, genitalia slide No. WZB14337 **12** *E. impolliniferens* sp. n., paratype, genitalia slide No. WZB14278 **13** *E. camurella* sp. n., holotype, genitalia slide No. WZB14043 **14** *E. angustisaccula* sp. n., paratype, genitalia slide No. WZB14001. Scale bar = 0.5 mm.

ing two rhombic plates originated from caudal margin of ostium bursae, outer margin serrate, apex acute. Lamella antevaginalis a pair of sclerotized and curved carinae. Antrum sclerotized, thick and strong, as long as 8th abdominal segment. Ductus bursae membranous, about same length as antrum, with longitudinal sclerotized folds extending from base to  $2/3$ ; ductus seminalis membranous, arising anterior of ductus bursae. Corpus bursae oval, small, about  $2/3$  length of ductus bursae, with reticulate patches medially; signum triangular, small, placed at posterior  $1/3$ .

**Diagnosis.** This new species is similar to *E. frenata* Meyrick, 1908, but differs from the latter in the shape of sacculus and phallus in male genitalia as well as in the shape of sterigmatic sclerotizations in female genitalia. In the male genitalia of the new species, the apex of the sacculus is truncate obliquely and the phallus is gently curved at distal  $1/3$ ; in the female genitalia, the caudal margin of the lamella postvaginalis

is deeply concave medially and the lamella antevaginalis is a pair of sclerotized and curved carinae. In the male genitalia of *E. frenata* (Syntype♂, BMNH, examined, genitalia slide No. 32303, dissected by Houhun Li; syntype♀, BMNH, examined, genitalia slide No. 32304, dissected by Houhun Li), the apex of the sacculus is broadly rounded and the phallus is straight; in the female genitalia, the caudal margin of the lamella postvaginalis is slightly concave and the lamella antevaginalis is absent.

**Type material.** 20♂, 42♀, with genitalia preparations of 20♂ and 42♀.

**Holotype** ♂ – **CHINA: Hainan Province:** Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 29.xii.2012, reared from host-plant *Glochidion wrightii* by Zhibo Wang, genitalia slide no. WZB14043.

**Paratypes** – **CHINA: Hainan Province:** 12♂, 25♀, Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 06.i.–08.vi.2010, reared or collected from *Glochidion wrightii* by Bingbing Hu; 2♂, same locality as holotype except the dates 11–12.iv.2011, reared from *Glochidion wrightii* by Jing Zhang; 5♂, 15♀, same locality as holotype except the dates 28.xii.2012–24.i.2013, reared from *Glochidion wrightii* by Zhibo Wang; 2♀, same locality except the date 12.i.2013, reared from *Glochidion sphaerogynum* by Zhibo Wang (1♂, 1♀, deposited in BMNH).

**Distribution.** China (Hainan).

**Biology.** *Glochidion wrightii* is the primary host-plant and *G. sphaerogynum* (Phyllanthaceae) is secondary. Larvae feed on seeds in the fruit.

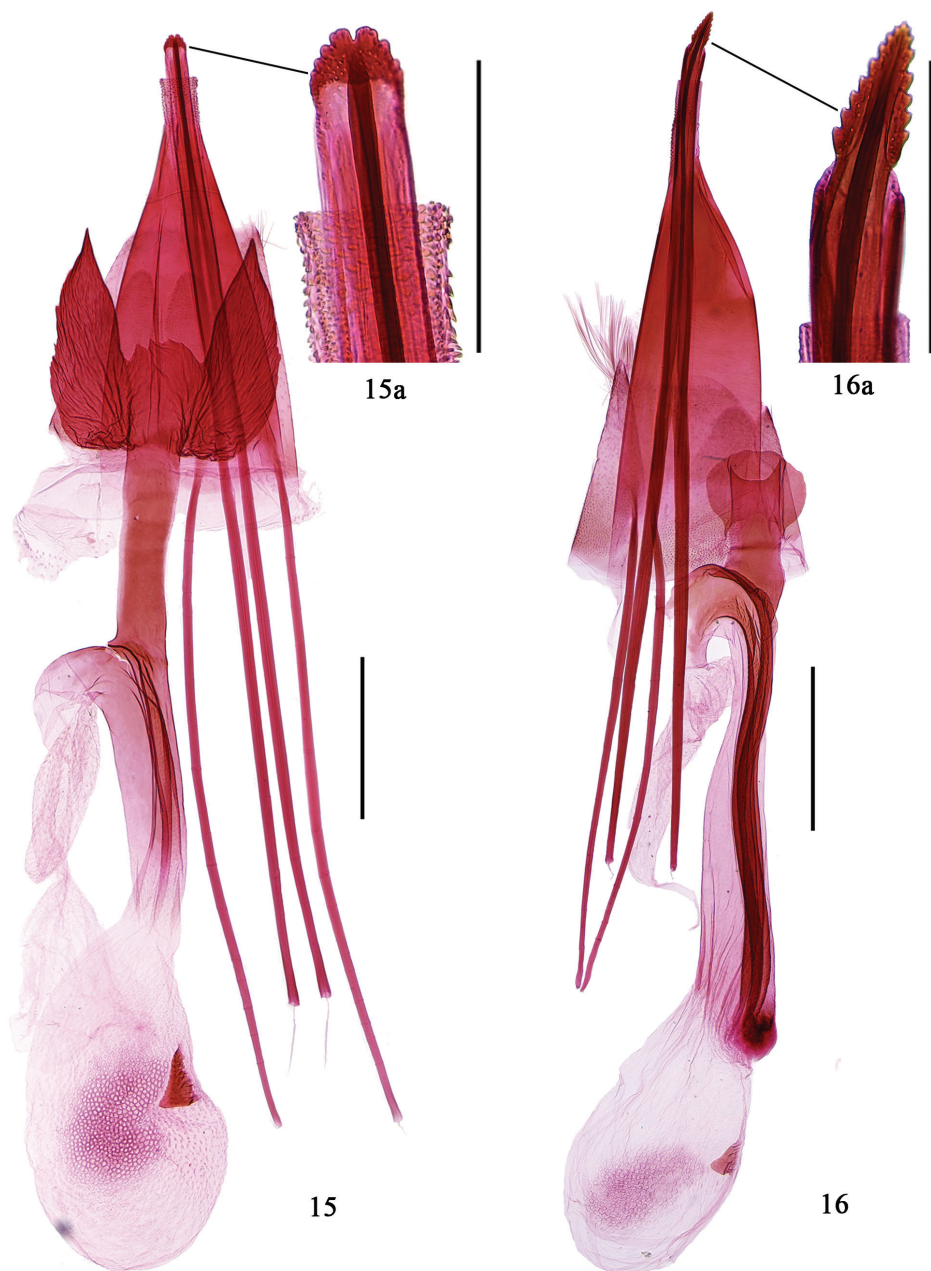
**Etymology.** The specific name is derived from the Latin *camur* (curved) and post-fix *-ella*, in reference to the lamella antevaginalis being a pair of sclerotized and curved carinae in the female genitalia.

### ***Epicephala angustisaccula* Li, sp. n.**

<http://zoobank.org/C083610A-8261-4A76-8E1F-D1F7012B2620>

Figs 8, 14, 18

**Description.** Adult (Fig. 8). Forewing expanse 7.0–8.5 mm. Head grayish white to white, laterally mixed with brown scales. Labial palpus grayish white, grayish brown on outer surface of second palpomere, basal 2/3 of third palpomere brown. Antenna grayish brown. Thorax dirty white to snowy white; tegula grayish white to brown. Forewing grayish brown to deep brown; costal margin with three parallel white striae obliquely extending outward from basal 1/3, 1/2 and 3/4 respectively, first and third striae broad and short, reaching 1/3 of wing width, second stria narrow and long, reaching midwing; a broad creamy white band extending from base to tornus along dorsal margin, its upper margin extended to a broad, ill-defined white stria at 2/5, reaching below fold dorsally, second white stria from 2/3 obliquely outward to meet second costal stria at midwing, third stria from beyond second one and parallel with it to midwing, sometimes meeting third costal stria; a silvery fascia with metallic reflection from costal 5/6 to dorsal margin, slightly arched outward medially; distal 1/6 yellowish brown, with a central black dot, with a small white dot at costa and a white



**Figures 15–16.** Female genitalia of *Epicephala* spp. **15** *E. domina* sp. n., paratype, genitalia slide No. WZB14295 **16** *E. impolliniferens* sp. n., paratype, genitalia slide No. WZB14249. Scale bar: 0.5 mm (**15**, **16**); 0.1 mm (**15a**, **16a**).



streak along dorsal margin; cilia white from distal part of costal margin along termen to tornus except black at base and apex, gray along dorsal margin. Hindwing gray to deep gray, sometimes basal 1/3 densely covered with rough black scales; cilia gray.

**Male genitalia** (Fig. 14). Tegumen elongate oval, sclerotized laterally. Costa longer than tegumen, nearly parallel dorso-ventrally, rounded at apex, with long dense setae ventrally; dorsal margin slightly sinuate; ventral margin slightly protruded with strong short setae at 3/4, then concave inward before apex. Sacculus elongate triangular, about 4/5 length of costa, slightly curved ventrad, tapered distally. Transtilla triangular, curved ventrad distally, acute apically. Vinculum broad U-shaped, rounded on posterior margin; saccus broad digitiform, as long as vinculum, apex rounded. Phallus straight, longer than valva, expanded in basal 1/3; cornuti with four spines compactly grouped into a bundle.

**Female genitalia** (Fig. 18). Ovipositor small and short, triangular, dentate laterally, acute apically. Apophysis posterioris 1.6 times longer than apophysis anterioris. Lamella postvaginalis well developed, heavily sclerotized, composed of two narrow, widely spaced rectangular plates derived from caudal margin of ostium bursae, about half length of 8th abdominal segment, caudal margin serrated. Ostium bursae sclerotized, broad. Antrum heavily sclerotized, broad, as long as 8th abdominal segment, with large rounded appendix protruding near ductus bursae on right side. Ductus bursae slightly longer than antrum, sclerotized, expanded, broader than corpus bursae; ductus seminalis arising from base of ductus bursae. Corpus bursae elongate oval, as long as ductus bursae; signum triangular, small, placed at posterior 1/3.

**Diagnosis.** This species is similar to *E. domina* sp. n. in appearance and genitalia, but can be separated from the latter in the male genitalia by the subtriangular sacculus and the dilated basally phallus; in the female genitalia by the apically acute ovipositor and the lamella postvaginalis being shorter than 8th abdominal segment. In *E. domina* sp. n., in the male genitalia the sacculus is broad-oval and the phallus is not dilated basally; in the female genitalia the ovipositor is bilobed apically and the lamella postvaginalis is as long as 8th abdominal segment.

**Type material.** 5♂, 1♀, with genitalia preparations of 5♂ and 1♀.

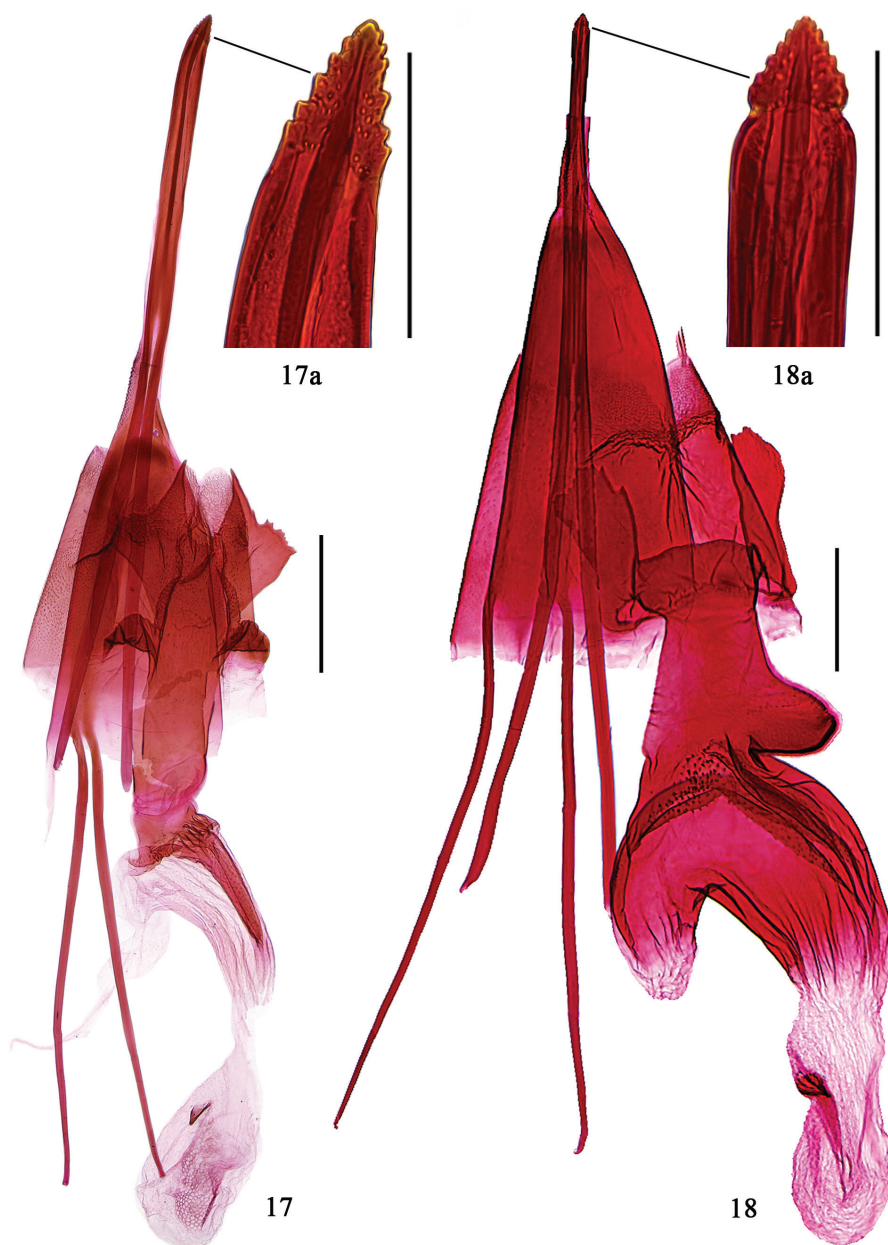
Holotype ♂ – **CHINA: Hainan Province:** Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 18.i.2014, reared from the host-plant *Glochidion sphaerogynum* by Zhibo Wang, genitalia slide no. WZB14001.

Paratypes – **CHINA: Hainan Province:** 1♂, Yinggeling Mountain Nature Reserves (19°01'N, 109°33'E), 450 m, 02.x.2010, reared from *Glochidion sphaerogynum* by Bingbing Hu; 3♂, same locality as holotype except the dates 29.x.2013 and 18–25.i.2014, reared or collected from *Glochidion sphaerogynum* by Zhibo Wang; 1♀, same locality as holotype except the date 15.v.2010, reared from *Glochidion wrightii* by Bingbing Hu; 1♂, same locality as holotype except the date 24.i.2014, reared from *Glochidion wrightii* by Zhibo Wang.

**Distribution.** China (Hainan).

**Biology.** Larvae feed on seeds in the fruits of *Glochidion sphaerogynum* (Müll.Arg.) Kurz and *G. wrightii* Benth. (Phyllanthaceae).





**Figures 17–18.** Female genitalia of *Epicephala* spp. **17** *E. camurella* sp. n., paratype, genitalia slide No. WZB14253 **18** *E. angustisaccula* sp. n., paratype, genitalia slide No. WZB14412. Scale bar: 0.5 mm (**17, 18**); 0.1 mm (**17a, 18a**).

**Etymology.** The specific name is derived from the Latin *angustus* (narrow) and *sacculus*, in reference to the distally narrowed sacculus in the male genitalia.

## Acknowledgements

The corresponding author would like to express his cordial thanks to Kevin Tuck for the loan of types and the allowance to examine some related species during his visit to Natural History Museum, London (BMNH). We also give our thanks to the workers at Yinggeling Mountain Nature Reserves for their generous help during our fieldwork. We thank the two reviewers and the editor Dr. Erik van Nieukerken for their comments. This research was supported by the National Natural Science Foundation of China (No. 31272356 and No. 30930014).

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# The genus *Scapheremaeus* (Acari, Oribatida, Cymbaeremaeidae) in the oribatid mite fauna of New Zealand, with description of two new species

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<http://zoobank.org/03EB8C1E-01F6-4E4A-88A8-D81EF7EAE503>

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## Abstract

Two new species of oribatid mites of the genus *Scapheremaeus* (Oribatida, Cymbaeremaeidae), *S. gibbus* **sp. n.** and *S. luxtoni* **sp. n.**, are described from New Zealand. *Scapheremaeus gibbus* **sp. n.** is morphologically most similar to *S. humeratus* Balogh & Mahunka, 1967, but differs from the latter by the number of notogastral, genital and adanal setae, morphology of bothridial setae, position of adanal lyrifissures and absence of humeral processes. *Scapheremaeus luxtoni* **sp. n.** is morphologically most similar to *S. yamashitai* Aoki, 1970, but differs from the latter by the morphology of notogastral and rostral setae, morphology of leg solenidia  $\varphi_2$  and development of humeral processes. The species *Scapheremaeus zephyrus* Colloff, 2010 is recorded for the first time in New Zealand. An identification key to the known New Zealand species of *Scapheremaeus* is provided.

## Keywords

Oribatid mites, *Scapheremaeus*, new species, new record, key, New Zealand



## Introduction

*Scapheremaeus* is a large genus of oribatid mites (Acari, Oribatida, Cymbaeremacidae), which was proposed by Berlese (1910) with *Eremaeus patella* Berlese, 1886 as type species. At present, the genus comprises more than 110 species and has a cosmopolitan distribution (except the Antarctic region) (Subías 2004, updated 2015; Ermilov and Anichkin 2015). The generic characters of *Scapheremaeus* are summarized by Colloff (2009). The identification keys to species from some regions and countries have been presented by Sitnikova (1975), Rios and Palacios-Vargas (1998), Balogh and Balogh (2002), Colloff (2010), Norton et al. (2010), and Ermilov and Anichkin (2015). The information about juvenile instars is summarized by Norton and Ermilov (2014), with some new data added by Ermilov et al. (2015).

During studies of oribatid mites from New Zealand, we discovered two new species of *Scapheremaeus*, *S. gibbus* sp. n. and *S. luxtoni* sp. n., and also found a known species, *S. zephyrus* Colloff, 2010, which was previously recorded only in Australia. The primary aim of our paper is to describe these species.

Three other species of *Scapheremaeus* are known from New Zealand (Hammer 1966): *S. emarginatus* Hammer, 1966, *S. insularis* Hammer, 1966 and *S. patella* (Berlese, 1886). The second aim of our paper is to provide an identification key for all known species of this genus in New Zealand.

## Materials and methods

The collection locality and habitat for each new species are given in the “*Material examined*” sections. Additionally, two specimens (female and male) of *S. zephyrus* were collected from: New Zealand, South Island, Central Otago, Old Man’s Range, 45°18'58"S, 169°11'45"E, 1646 m a.s.l., in soil and debris under *Dracophyllum muscoides* cushion, 17 February 2014 (M. Minor).

Specimens were mounted in lactic acid on temporary cavity slides for measurement and illustration. The body length was measured in lateral view, from the tip of the rostrum to the posterior edge of the ventral plate. Notogastral width refers to the maximum width in dorsal aspect. Lengths of body setae were measured in lateral aspect. All body measurements are presented in micrometers. Formulas for leg setation are given in parentheses according to the sequence trochanter–femur–genu–tibia–tarsus (femulus included). Formulas for leg solenidia are given in square brackets according to the sequence genu–tibia–tarsus.

General terminology used in this paper follows that of Grandjean (summarized by Norton and Behan-Pelletier 2009; Colloff 2009).

Drawings were made with a camera lucida using a Carl Zeiss transmission light microscope “Axioskop-2 Plus”. Images were obtained with an AxioCam ICc3 camera using a Carl Zeiss transmission light microscope “Axio Lab.A1”.

## Descriptions

### *Scapheremaeus gibbus* sp. n.

<http://zoobank.org/A7609D9D-BC17-4683-91C9-F76EB06C79BA>

Figs 1–22

**Diagnosis.** Body size: 270–307 × 131–147. Body surface areolate-reticulate. Costulae reduced, terminated by tubercles. Transcostula not developed. Rostral setae thin, directed medially. Lamellar setae minute. Bothridial setae globular. Humeral processes and circumdorsal scissure absent. Thirteen pairs of short, simple notogastral setae. Anterior tectum of ventral plate strongly developed. Palp femora with one seta. Five pairs of genital setae. Lyrifissures *iad* in transverse position. Monodactylous. Femora I and II with extremely large ventral expansions.

**Description.** *Measurements.* Body length: 299 (holotype: female), 270–307 (seven paratypes: four females and three males); notogaster width: 147 (holotype), 143–151 (seven paratypes).

*Integument.* Body color light yellow-brownish. Body surface with areolate-reticulate sculpturing.

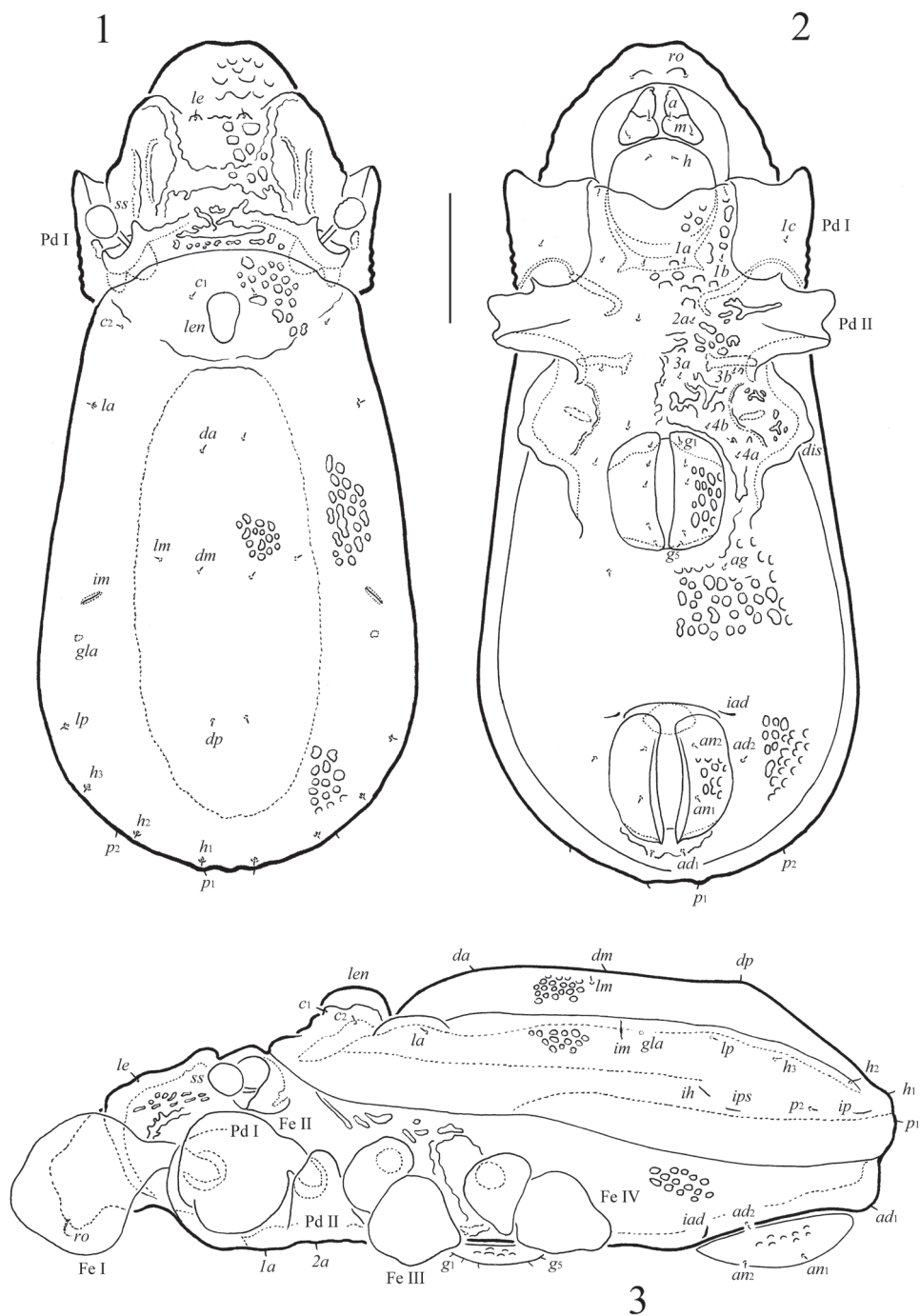
*Prodorsum.* Rostrum broadly rounded. Costulae reduced, terminated by tubercles, bearing lamellar setae. Transcostula absent. Rostral setae (*ro*, 10) thin, smooth, directed medially, inserted on transverse fold. Lamellar setae minute (*le*, 4), thin, straight, inserted nearer to bothridia than rostral setae. Interlamellar and exobothridial setae and their alveoli absent. Bothridial setae (*ss*, 22–24) globular, pigmented, with short stalk (6–8) and longer (16) head, having longitudinal ridges.

*Notogaster.* Normal in form, not flattened. Anterior margin slightly convex medially. Lenticulus (*len*) distinct. Humeral regions without processes. Centrodorsal zone forming longitudinal elongate hump-like structure. Circumdorsal scissure absent. Thirteen pairs of simple notogastral setae, located on small tubercles. Centro-dorsal part with four pairs of setae (*da*, *dm*, *lm*, *dp*). All lyrifissures (*im*, *ip*, *ih*, *ips*; except *ia*) well visible. Opisthonotal gland openings (*gla*) located posteriorly to *im*.

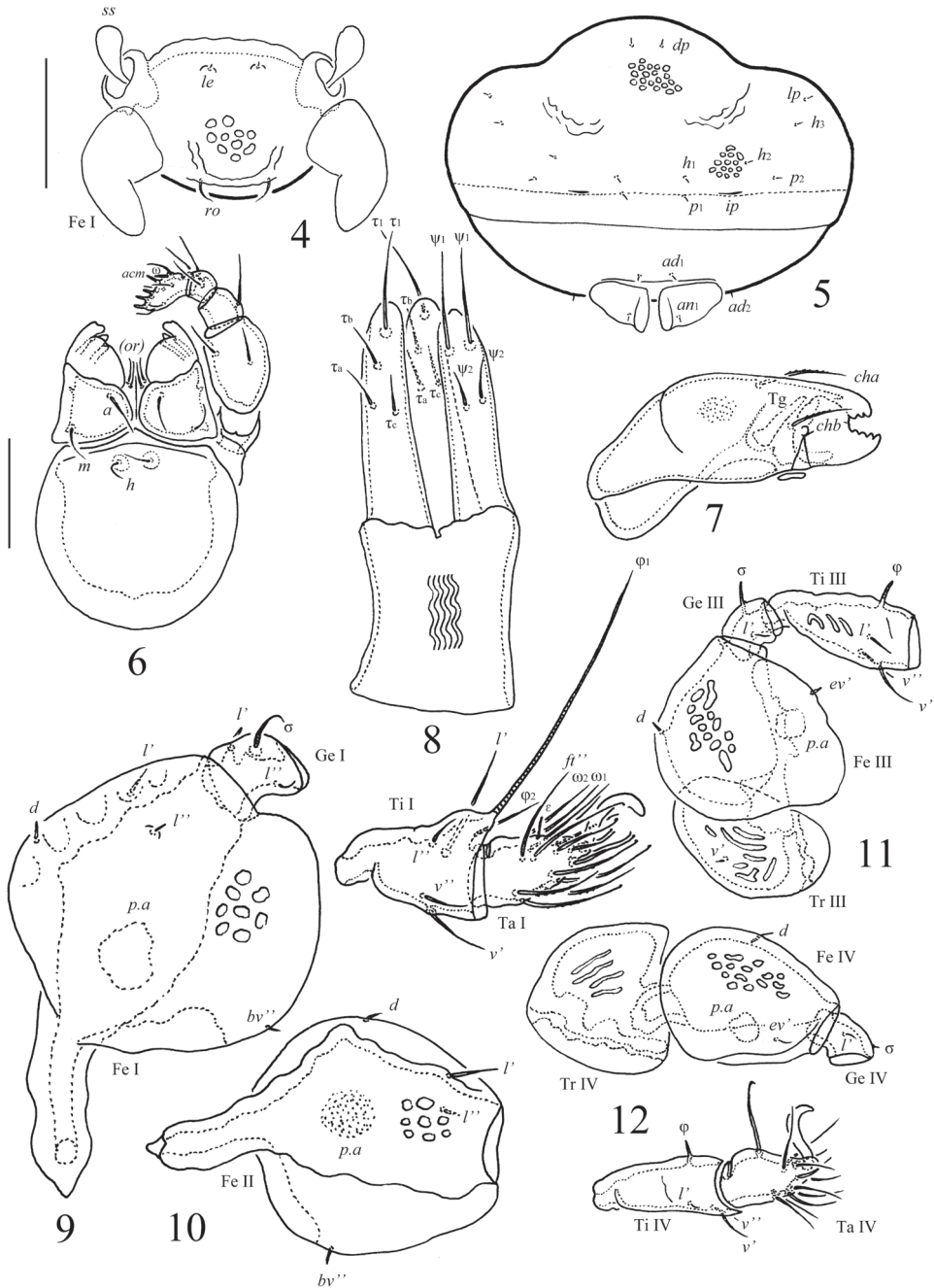
*Gnathosoma.* Subcapitulum longer than wide (53–57 × 32–36). Subcapitular setae thin, smooth; *a* and *m* (both 10) longer than *h* (6) and adoral setae (*or*<sub>1</sub>, *or*<sub>2</sub>, 4–6). Setae *a* slightly thicker than other. Palps (41–45) with setation 0–1–1–3–9(+ω). Solenidion free, not attached to eupathidium (*acm*). Chelicerae (53–57) with two simple, barbed setae; *cha* (16–18) longer than *chb* (12). Trägårdh's organ long, tapered.

*Epimeral and lateral podosomal regions.* Anterior tectum strongly developed. Pedotecta I large, concave in dorsal view and scale-like in lateral view. Pedotecta II elongated, bifurcate distally in ventral view and broadly triangular in lateral view. Apodemes 1, 2, sejugal and 3 distinctly developed. Epimeral setal formula 3–1–2–2. Epimeral setae short (4), thin, smooth. Discidia (*dis*) roundly triangular.

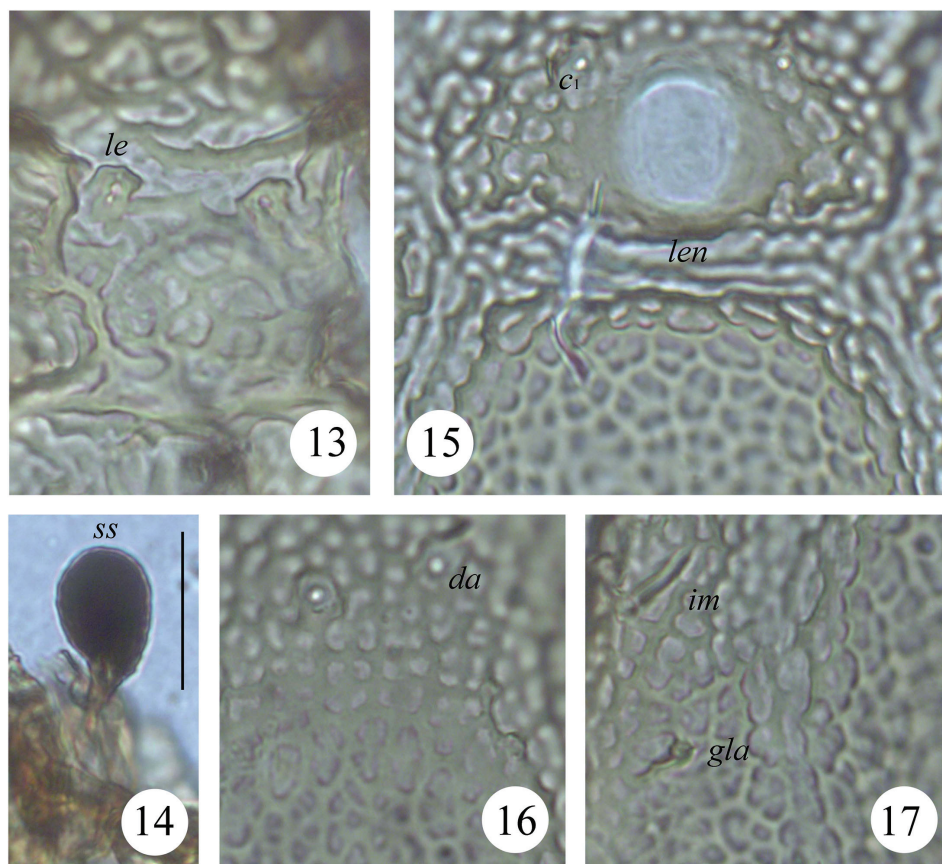
*Anogenital region.* Five pairs of genital (*g*<sub>1</sub>–*g*<sub>5</sub>), one pair of aggenital (*ag*), two pairs of anal (*an*<sub>1</sub>, *an*<sub>2</sub>) and two pairs of adanal (*ad*<sub>1</sub>, *ad*<sub>2</sub>) setae similar in length (4), thin, smooth, inserted on small tubercles. Lyrifissures *iad* in transverse position. Ovipositor



**Figures 1–3.** *Scapheremaeus gibbus* sp. n., adult: **1** dorsal view **2** ventral view (legs not shown) **3** lateral view (gnathosoma and legs except basal parts not shown). Scale bar 50  $\mu$ m.



**Figures 4–12.** *Scapheremaeus gibbus* sp. n., adult: **4** frontal view of prodorsum (legs I except basal parts not shown) **5** posterior view **6** subcapitulum and palp **7** chelicera, antiaxial view **8** ovipositor **9** leg I, without trochanter, right, antiaxial view **10** femur of leg II, left, paraxial view **11** leg III, without tarsus, left, antiaxial view **12** leg IV, left, antiaxial view. Scale bars 50  $\mu$ m (**4**, **5**), 20  $\mu$ m (**6–12**).



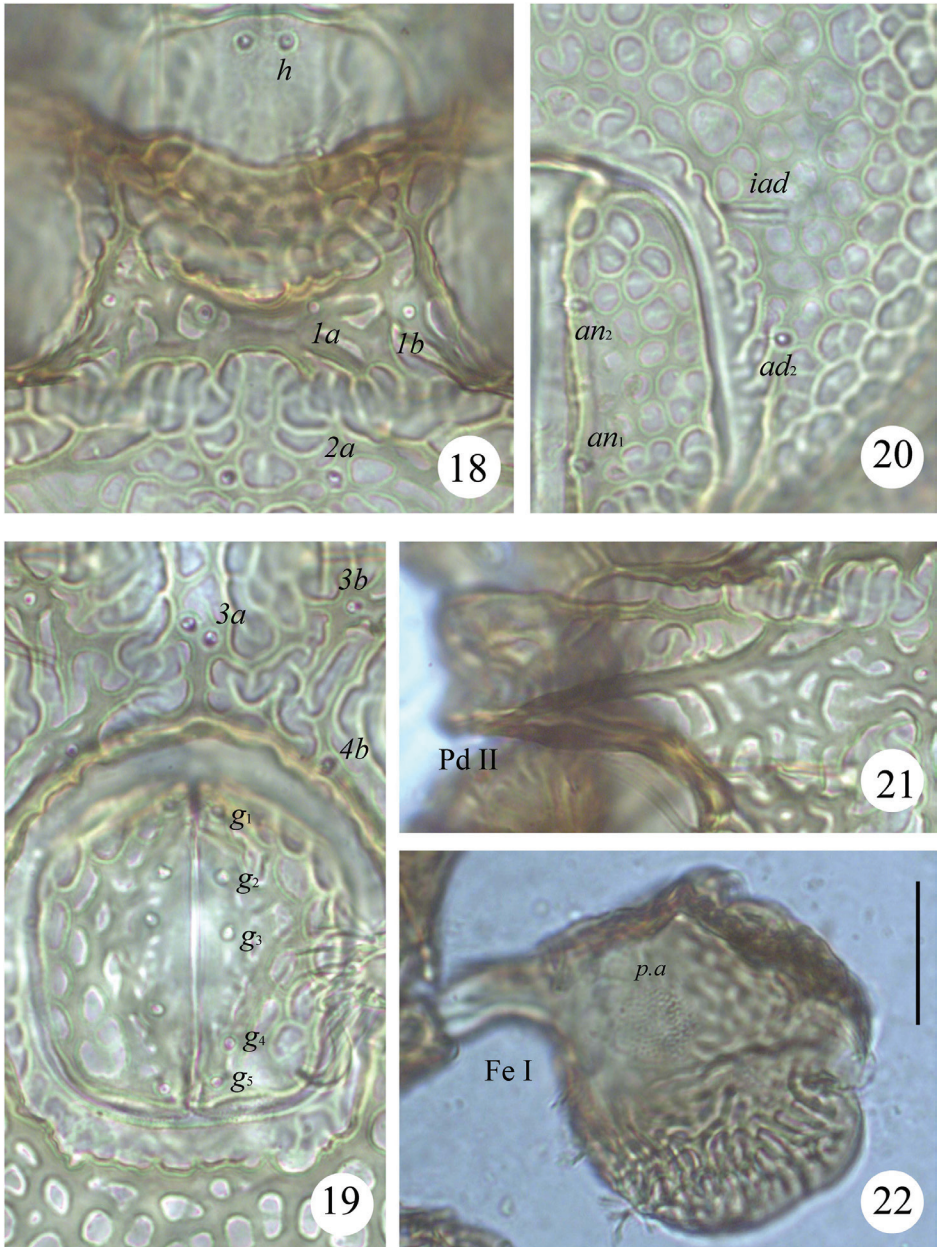
**Figures 13–17.** *Scapheremaeus gibbus* sp. n., dissected adult, microscope images: **13** lamellar setae and ornamentation in centro-dorsal part of prodorsum **14** bothridial seta **15** lenticulus and sculpture on anterior part of notogaster **16** sculpture on centro-dorsal part of notogaster **17** sculpturing in dorso-lateral part of notogaster. Scale bar 20  $\mu$ m.

elongated ( $68\text{--}77 \times 32\text{--}36$ ), lobes ( $36\text{--}41$ ) longer than length of distal section (beyond middle fold;  $32\text{--}36$ ). Each of three lobes with four straight, smooth setae,  $\psi_1 \approx \tau_1$  (20) longer than  $\psi_2 \approx \tau_a \approx \tau_b \approx \tau_c$  (8–10). Coronal setae and their alveoli absent.

**Legs.** Monodactylous. Femora I and II with extremely large ventral expansions. Porose areas (*p.a*) slightly visible, oval. Formulas of leg setation and solenidia: I (0–4–2–4–16) [1–2–2], II (0–4–2–3–15) [1–1–1], III (1–2–1–3–14) [1–1–0], IV (0–2–1–3–12) [1–1–0]; homology of setae and solenidia as indicated in Table 1. Famuli ( $\epsilon$ ) short, slightly dilated distally. Solenidia simple,  $\sigma$  on genua IV minute. Setae *l* on tibiae I setiform, not modified.

**Material examined.** Holotype (female) and seven paratypes (four females and three males): New Zealand, South Island, Central Otago, Old Man's Range,  $45^{\circ}18'58''\text{S}$ ,  $169^{\circ}11'45''\text{E}$ , 1646 m a.s.l., in soil and debris under *Dracophyllum muscoides* cushion, 17 February 2014, collected by M. Minor.





**Figures 18–22.** *Scapheremaeus gibbus* sp. n., dissected adult, microscope images: **18** sculpturing in anterior part of epimeral region **19** genital plates **20** left anal plate and sculpturing in adanal part of ventral plate **21** pedotecta II **22** femur I, left, paraxial view. Scale bar 20  $\mu$ m.

**Type deposition.** The holotype and two paratypes are deposited in the New Zealand National Arthropod Collection, Auckland, New Zealand; two paratypes are deposited in the collection of the Senckenberg Institution, Frankfurt, Germany; three

**Table 1.** Leg setation and solenidia of adult *Scapheremaeus gibbus* sp. n. (same data for *S. luxtoni* sp. n.).

Leg	Trochanter	Femur	Genu	Tibia	Tarsus
I	–	<i>d</i> , ( <i>l</i> ), <i>bv</i> "	( <i>l</i> ), $\sigma$	( <i>l</i> ), ( <i>v</i> ), $\varphi_1$ , $\varphi_2$	( <i>ft</i> ), ( <i>tc</i> ), ( <i>it</i> ), ( <i>p</i> ), ( <i>u</i> ), ( <i>a</i> ), <i>s</i> , ( <i>pv</i> ), $\varepsilon$ , $\omega_1$ , $\omega_2$
II	–	<i>d</i> , ( <i>l</i> ), <i>bv</i> "	( <i>l</i> ), $\sigma$	<i>l'</i> , ( <i>v</i> ), $\varphi$	( <i>ft</i> ), ( <i>tc</i> ), ( <i>it</i> ), ( <i>p</i> ), ( <i>u</i> ), ( <i>a</i> ), <i>s</i> , ( <i>pv</i> ), $\omega$
III	<i>v'</i>	<i>d</i> , <i>ev'</i>	<i>l'</i> , $\sigma$	<i>l'</i> , ( <i>v</i> ), $\varphi$	( <i>ft</i> ), ( <i>tc</i> ), <i>it</i> ", ( <i>p</i> ), ( <i>u</i> ), ( <i>a</i> ), <i>s</i> , ( <i>pv</i> )
IV	–	<i>d</i> , <i>ev'</i>	<i>l'</i> , $\sigma$	<i>l'</i> , ( <i>v</i> ), $\varphi$	<i>ft</i> ", ( <i>tc</i> ), ( <i>p</i> ), ( <i>u</i> ), ( <i>a</i> ), <i>s</i> , ( <i>pv</i> )

Note: Roman letters refer to normal setae, Greek letters to solenidia (except  $\varepsilon$  = famulus). Single prime (') marks setae on the anterior and double prime (") setae on the posterior side of a given leg segment. Parentheses refer to a pair of setae.

paratypes are deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

**Etymology.** The specific name *gibbus* refers to the clearly convex centrodorsal notogastral region, forming longitudinal elongate hump-like structure.

**Remarks.** The new species is most similar to *S. humeratus* Balogh & Mahunka, 1967 from Congo (see Balogh and Mahunka 1967) in having small body size, monodactylous legs, simple notogastral setae, areolate body surface, and absence of circumdorsal furrow. However, it differs from the latter by the presence of 13 pairs of notogastral setae (versus 11), globular bothridial setae (versus fusiform), five pairs of genital setae (versus six), two pairs of adanal setae (versus three), transverse position of adanal lyrifissures (versus longitudinal) and absence of humeral processes (versus well developed).

***Scapheremaeus luxtoni* sp. n.**

<http://zoobank.org/8F13864C-3F7C-44A9-B831-2C4892BB7F89>

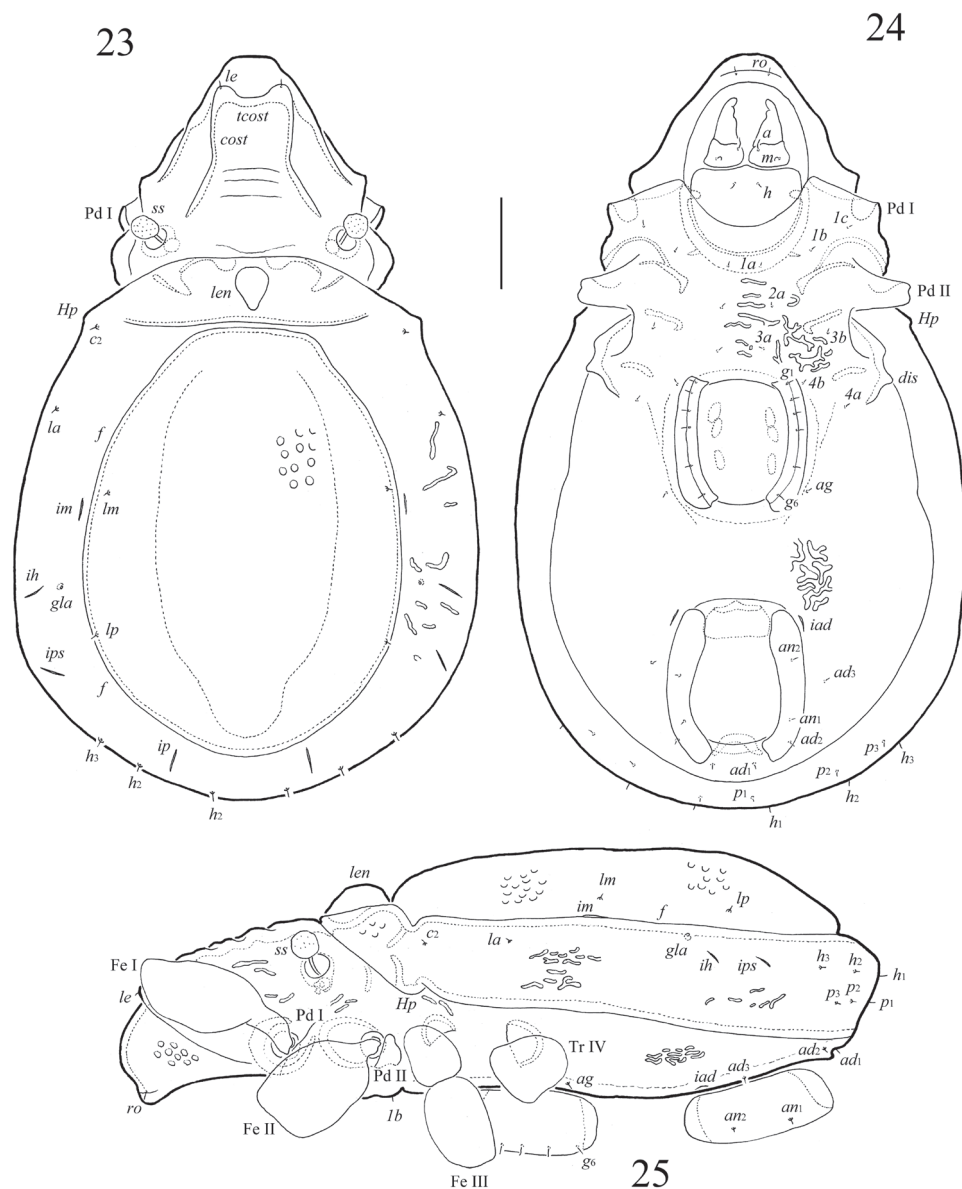
Figs 23–47

**Diagnosis.** Body size: 381–415 × 199–232. Centro-dorsal part of notogaster areolate. Dorso-lateral parts of notogaster and ventral plate tuberculate. Costulae and transcostula strong. Rostral setae thin, straight. Lamellar setae minute. Bothridial setae globular. Humeral processes small, rounded. Circumdorsal scissure present. Ten pairs of short, simple notogastral setae. Palp femora with two setae. Six pairs of genital setae. Lyrifissures *iad* longitudinally oriented. Tridactylous.

**Description.** *Measurements.* Body length: 381 (holotype: male), 381–415 (three paratypes: all females); notogaster width: 199 (holotype), 232 (same for three paratypes).

*Integument.* Body color light yellow-brownish. Anterior part of prodorsum and centro-dorsal part of notogaster with areolate sculpturing. Dorso-lateral parts of notogaster and ventral plate with elongated ridge-like tubercles.

*Prodorsum.* Rostrum broadly rounded. Costulae (*cos*) distinct, forming slightly visible X-structure, terminated by large tubercles, which connected by thick transcostula (*tcos*). Rostral setae (6) thin, straight, inserted on transverse fold. Lamellar setae (4)



**Figures 23–25.** *Scapheremaeus luxtoni* sp. n., adult: **23** dorsal view **24** ventral view (legs not shown) **25** lateral view (gnathosoma and legs except basal parts not shown). Scale bar 50  $\mu$ m.

minute, inserted nearer to rostral setae than to bothridia. Interlamellar and exobothridial setae and their alveoli absent. Bothridial setae (22–24) globular, pigmented, with short stalk (6) and longer (16–18) head, having longitudinal ridges.

*Notogaster* flattened. Anterior margin straight. Lenticulus distinct. Humeral processes (*Hp*) slightly developed, tubercle-like in dorsal view and rounded in lateral view.

Centrodorsal zone with longitudinal elongate hump-like structure. Circumdorsal scissure (*f*) present. Ten pairs of simple notogastral setae, located on small tubercles. Centro-dorsal part with two pairs of setae (*lm*, *lm*), both inserted near to scissure. All lyrifissures (except *ia*) well visible. Opisthonotal gland openings located medially to *ih*.

*Gnathosoma*. Subcapitulum longer than wide (82–90 × 61–69). Subcapitular setae thin, smooth; *a* and adoral setae (all 10) longer than *m* and *h* (both 6–8). Setae *a* slightly thicker than other. Palps (53–61) with setation 0–2–1–3–9(+ $\omega$ ). Solenidion free, not attached to eupathidium. Chelicerae (82–90) with two simple, barbed setae (both 16–20). Trägårdh's organ long, tapered.

*Epimeral and lateral podosomal regions*. Anterior tectum slightly developed. Pedotecta I of medium size, concave in dorsal view and scale-like in lateral view. Pedotecta II elongated, bifurcate distally in ventral view and broadly triangular in lateral view. Apodemes 1, 2, sejugal and 3 distinctly developed. Epimeral setal formula 3–1–2–2. Epimeral setae short (4), thin, smooth. Discidia roundly triangular.

*Anogenital region*. Six pairs of genital, one pair of aggenital, two pairs of anal and three pairs of adanal setae similar in length (4), thin, smooth, inserted on small tubercles. Lyrifissures *iad* longitudinally oriented. Ovipositor elongated (52–56 × 41–45), lobes (32–36) longer than length of distal section (beyond middle fold; 20). Each of three lobes with four straight, smooth setae,  $\psi_1 \approx \tau_1$  (24–28) longer than  $\psi_2 \approx \tau_a \approx \tau_b \approx \tau_c$  (16). Coronal setae and their alveoli absent.

*Legs*. Tridactylous. Porose areas slightly visible, oval. Formulas of leg setation and solenidia as in *S. gibbus* sp. n.; homology of setae and solenidia as indicated in Table 1. Famuli short, slightly dilated distally. Solenidia (except simple  $\omega$  on tarsi and  $\varphi_1$ , and thin  $\sigma$  on genua I) dilated distally. Setae *l* on tibiae I setiform, not modified.

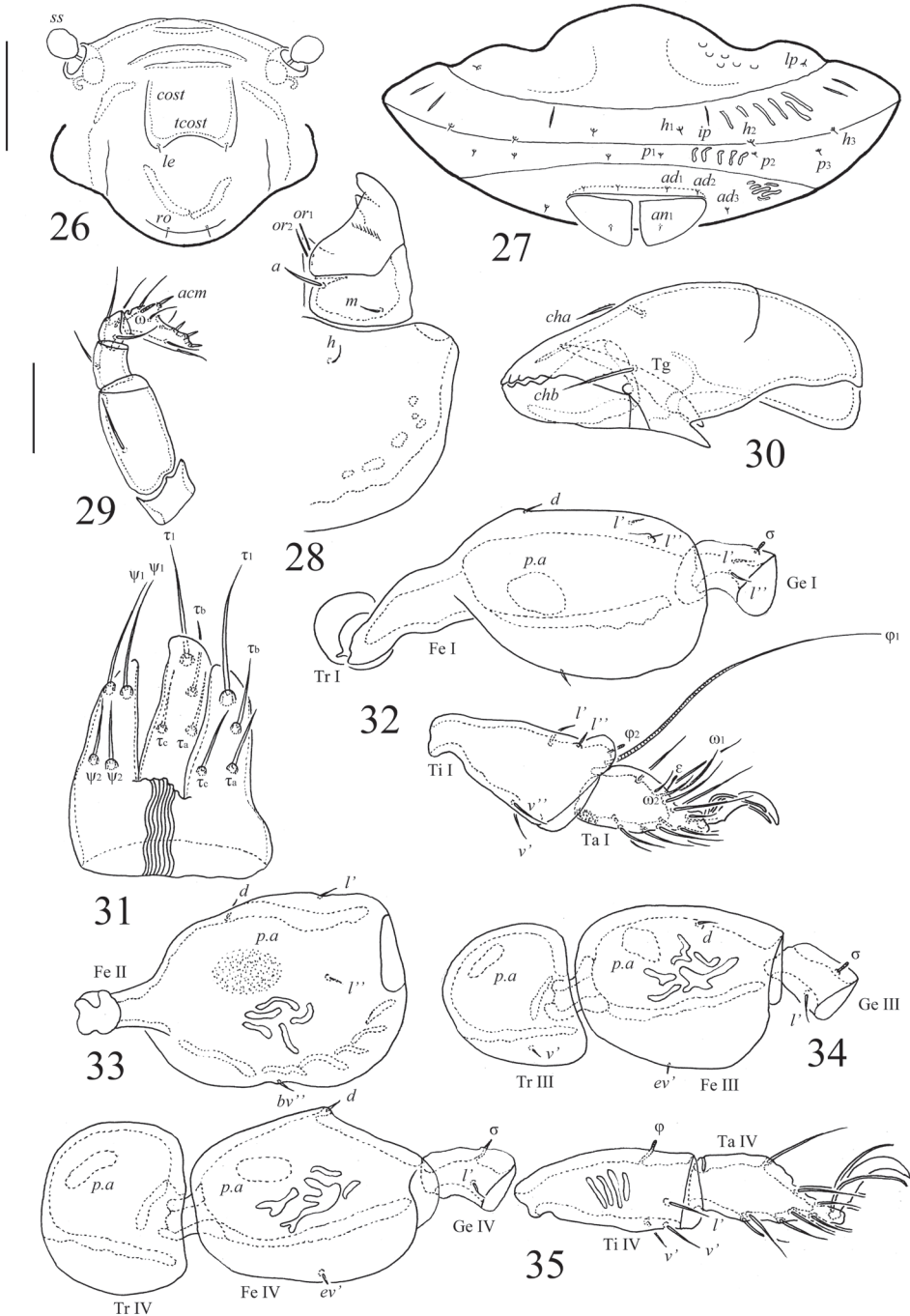
**Material examined.** Holotype (male) and three paratypes (all females): New Zealand, South Island, Central Otago, Pisa Range, 44°52'19"S, 169°10'30"E, 1880 m a.s.l., in soil and debris under *Dracophyllum muscoides* cushion and in the soil outside of *D. muscoides* cushion, 18 February 2014, collected by M. Minor.

**Type deposition.** The holotype and one paratype are deposited in the New Zealand National Arthropod Collection, Auckland, New Zealand; one paratype is deposited in the collection of the Senckenberg Institution, Frankfurt, Germany; one paratype is deposited in the collection of the Tyumen State University Museum of Zoology, Tyumen, Russia.

**Etymology.** The specific name is dedicated to the well-known acarologist Malcolm Luxton, for his extensive contributions to our knowledge of New Zealand oribatid mite fauna.

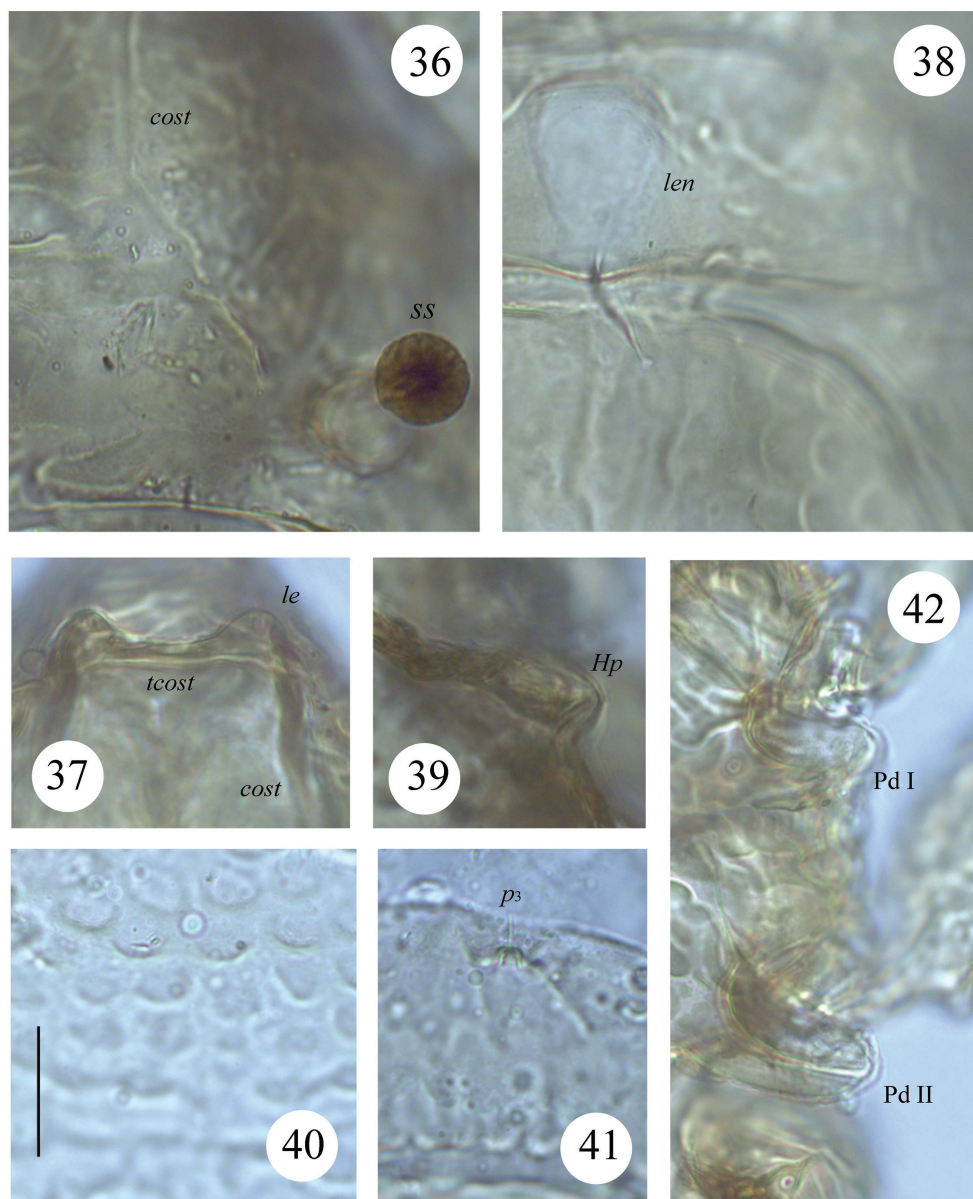
**Remarks.** The new species is similar to *S. yamashitai* Aoki, 1970 from Japan (see Aoki 1970; Fujikawa 2002) in having circumdorsal furrow, tridactylous legs, costulae and transcostula, ten pairs of minute notogastral setae and areolate centrodorsal region of notogaster. However, it differs from the latter by the presence of thin notogastral setae (versus thickened), straight rostral setae (versus curved medially), short and dilated distally leg solenidia  $\varphi_2$  (versus long and simple) and slightly developed humeral processes (versus well developed).





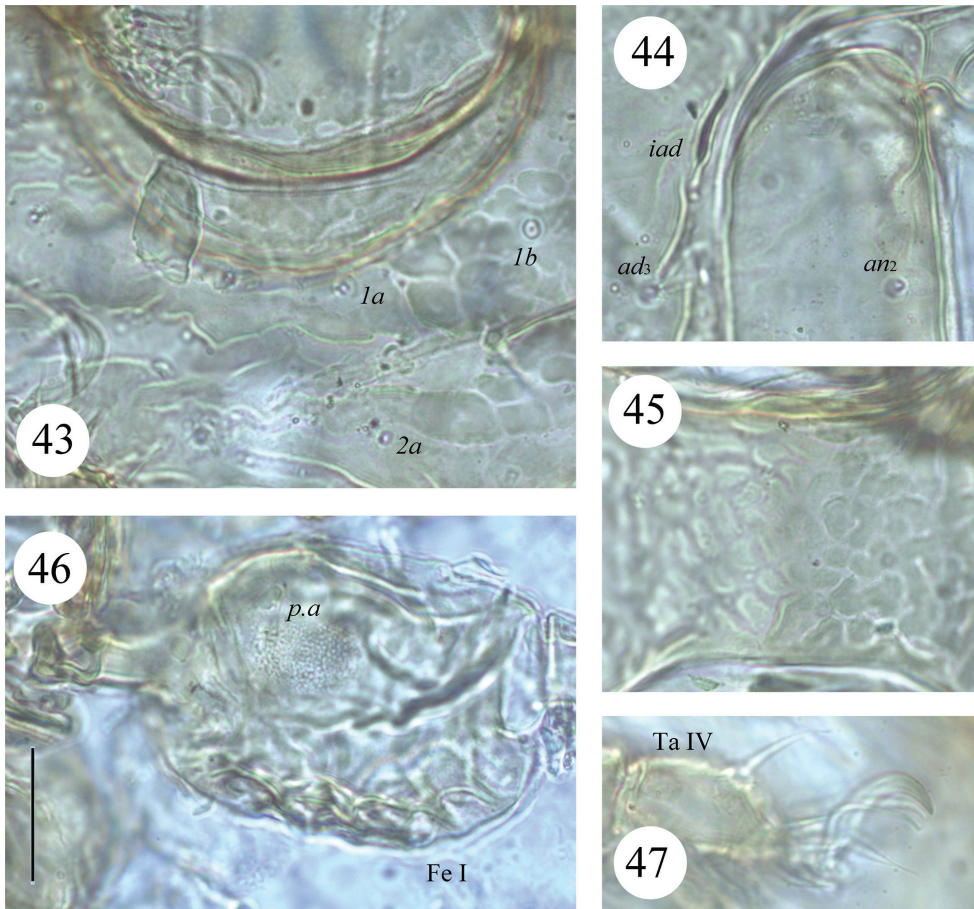
**Figures 26–35.** *Scapheremaeus luxtoni* sp. n., adult: **26** frontal view of prodorsum **27** posterior view **28** subcapitulum **29** palp **30** chelicera, antiaxial view **31** ovipositor **32** leg I, right, antiaxial view **33** femur of leg II, left, paraxial view **34** trochanter, femur and genu of leg III, left, antiaxial view **35** leg IV, left, antiaxial view. Scale bars 50  $\mu$ m (**26**, **27**), 20  $\mu$ m (**28–35**).





**Figures 36–42.** *Scapheremaeus luxtoni* sp. n., dissected adult, microscope images: **36** bothridial seta and sculpture of latero-basal part of prodorsum **37** costulae and transcostula **38** lenticulus and sculpture on latero-anterior part of notogaster **39** humeral process, right, dorsal view **40** sculpture on centro-dorsal part of notogaster **41** notogastral seta  $p_3$  **42** pedotecta I and II. Scale bar 20  $\mu$ m.

Also, in having circumdorsal furrow, tridactylous legs, costulae, minute notogastral setae, straight rostral setae and areolate centrodorsal region of notogaster, *S. luxtoni* sp. n. is similar to *S. zephyrus* Colloff, 2010 from Australia (see Colloff 2010) and



**Figures 43–47.** *Scapheremaeus luxtoni* sp. n., dissected adult, microscope images: **43** sculpture on anterior part of epimeral region **44** anterior part of right anal plate **45** sculpture between genital and anal apertures **46** femur I, left, paraxial view **47** tarsus IV, left, antiaxial view. Scale bar 20 µm.

New Zealand (our data). However, it differs from the latter by the presence of large tubercle-like distal parts of costulae (versus small), strong transcostula (versus absent), ten pairs of thin notogastral setae (versus nine pairs and thickened) and three pairs of adanal setae (versus two pairs).

**Key to species *Scapheremaeus* from New Zealand**

- 1 Notogastral circumdorsal scissure absent; costulae reduced, represented by tubercle-like cusps; legs monodactylous .....**2**
- Notogastral circumdorsal scissure present; costulae well developed; legs tri-dactylous .....**3**

- 2 Notogaster with 13 pairs of setae; notogastral setae simple; leg femora I, II with extremely large ventral expansions; body size: 270–307 × 131–147 ..... *S. gibbus* sp. n.
- Notogaster with 10 pairs of setae; notogastral setae dilated distally; leg femora I, II without extremely large expansions; body length: 330 ..... *S. emarginatus* Hammer, 1966
- 3 Costular cusps elongate conical; notogastral setae dilated distally; body length: 420 ..... *S. insularis* Hammer, 1966
- Costular cusps tubercle-like, not elongated; notogastral setae simple or slightly thickened ..... 4
- 4 Notogaster with 14 pairs of setae; centro-dorsal notogastral setae (*da*, *dm*, *dp*) developed; body size: 360–495 × 284 ..... *S. patella* (Berlese, 1886)<sup>1</sup>
- Notogaster with 9–10 pairs of setae; centro-dorsal notogastral setae (*da*, *dm*, *dp*) not developed ..... 5
- 5 Notogaster with 9 pairs of setae (*p*<sub>3</sub> not developed); two pairs of adanal setae; transcostula absent; body size: 384–391 × 202–211 ..... *S. zephyrus* Colloff, 2010
- Notogaster with 10 pairs of setae (*p*<sub>3</sub> developed); two pairs of adanal setae; transcostula present; body size: 381–415 × 199–232 ..... *S. luxtoni* sp. n.

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# A new Chinese species of *Eostrobilops* Pilsbry, 1927 with a checklist of *Eostrobilops* and *Enteroplax* Gude, 1897 species (Gastropoda, Pulmonata, Strobilopsidae)

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## Abstract

*Eostrobilops humicolus* Páll-Gergely & Hunyadi, **sp. n.** is described from Guangxi Province, China. It is characterized by the combination of a small shell (diameter: 2.3–2.4 mm), strongly ribbed dorsal surface, an infraparietal lamella not reaching the callus, and long basal folds. The new species is found approximately 500 and 800 km from the two nearest species *E. infrequens* (northern Vietnam), and *E. diodontina* (Hunan, China), respectively. A checklist of extant *Eostrobilops* Pilsbry, 1927 and *Enteroplax* Gude, 1899 species is provided. *Enteroplax yaeyamensis* Habe & Chinen, 1974, *Enteroplax kanjiokuboi* Minato & Tada, 1992 and *Enteroplax taiwanica* Minato & Tada, 1992 are moved to the genus *Eostrobilops* because of the lack of an elevated parietal callus and a peripheral thread. A map showing all *Eostrobilops* records is provided.

## Keywords

Revision, taxonomy, systematics, land snail

## Introduction

The family Strobilopsidae is mainly defined on a conchological basis; the shell is trochiform, dome-shaped or discoidal, umbilicate and consists of 4.5–6 slowly increasing whorls. The aperture is oblique, peristome more or less thickened and expanded; the

ends of the lips are connected by a parietal callus. The main characteristic feature of the family is the armature consisting of two or three parietal lamellae and several deeply-placed basal folds, all growing continuously from an early neanic state (Pilsbry 1927). Only four species belonging to two genera have been examined anatomically (Baker in Pilsbry 1935, 1948, Minato 1975, Matsumura and Minato 1998). None of these works revealed anatomical characters that would distinguish the Strobilopsidae from other orthurethran families, such as the Pupillidae and Valloniidae (Manganelli et al. 1989). Strobilopsid DNA sequences were published in two works, but neither of them focussed explicitly on the systematic position of the Strobilopsidae. In the phylogenetic tree of Tongkerd et al. (2004), which focussed on the family Hypselostomatidae, *Strobilops labyrinthica* (Say, 1817) nested within the valloniid clade. The closest taxon to *Strobilops* is *Zoogenetes harpa* (Say, 1824) and two samples of *Vallonia costata* (O. F. Müller, 1774) formed the sister clade of the *Strobilops-Zoogenetes* clade. The samples of *Pupilla* (Pupillidae) and *Vertigo* (Vertiginidae) were more distantly related to *Strobilops* than to the members of the Valloniidae. In the phylogenetic tree of Wade et al. (2006), which provided an overview of the phylogenetic relationships between most pulmonate groups, *Eostrobilops nipponica* (Pilsbry, 1908) clustered with *Lauria*, *Pyramidula* and *Orcula*. In this analysis, *Vallonia* was only included in the larger, orthurethran clade. These data show that Strobilopsidae is an orthurethran family, but its relationships with other families still require clarification.

Living Strobilopsidae occur in America, from northern Mexico to the northern part of South America, and East Asia, from North Korea and south-eastern Russia to southern Borneo (Pilsbry 1927, Kuroda and Miyanaga 1939, Solem 1968, Miller and Christensen 1980, Schileyko 1984, Vermeulen 1992a,b). Fossil strobilopsids have been reported from Europe, North and South America as well as China (as reviewed by: Wenz 1923, Pilsbry 1927–1935, Manganelli et al. 1989). The oldest fossils that have properly been assigned to the Strobilopsidae dated to the Middle Eocene of Europe. In the New World, fossils no older than from the Late Pliocene can be ascribed to the family. The assignment of Upper Cretaceous-Lower Tertiary Chinese and South American fossils to the Strobilopsidae is speculative only (Manganelli et al. 1989).

Pilsbry (1948) proposed that the family Strobilopsidae had radiated from Asia into Europe and the New World. By contrast, Ferreira and Dos Santos Coelho (1970) believed that South America has been the centre of origin of the family. Solem (1979, 1981) and Manganelli et al. (1989), however, stated that the radiation from a European centre was much easier to explain, especially if the Cretaceous fossils, which are not certainly strobilopsids, were ignored. Solem (1979, 1981) mentioned the Strobilopsidae as one of the most interesting cases of “moved” families, i.e. recent families that live far away from the main stock of their fossil records.

East Asia is inhabited by two recent strobilopsid genera: *Enteroplax* Gude, 1899 and *Eostrobilops* Pilsbry, 1927, which differ from each other in the morphology of the parietal callus, the edge of the body whorl and the parietal lamellae. Herein, we describe a new species of *Eostrobilops* from the Chinese province of Guangxi and provide a critically revised checklist of *Eostrobilops* and *Enteroplax* species.

## Material and methods

The nomenclature for the armature follows that of Pilsbry (1927). Scanning electron microscopy was undertaken on uncoated shells under a low vacuum SEM (Miniscope TM-1000, Hitachi High-Technologies, Tokyo). We counted shell whorls (to the nearest quarter of a whorl) following Kerney and Cameron (1979).

**Comparative material.** *Eostrobilops hirasei*, Korea, Quelpart (= Cheju Island), det. Zilch (?), NHMUK 1909.2.20.112.114.; *Eostrobilops nipponica* (labelled as *matsushimae*), Japan, Uzen, NHMUK 1912.6.28.19–20, NHMUK 1912.6.29.32–34; *Eostrobilops coreana*, 朝鮮京城府北溪山 (probably Cho-Sen Kei-Joh-Fu, Hoku-Kei-Zan), Sakurai collection, NSMT/2; *Eostrobilops kanjiokuboi*, 中華民國 (台灣) 南投縣信義鄉東埔樂々溫泉, Lo lo uen chuan, Tung-pu, Hsin-i shiang, Nan tou hsien, Taiwan, NSMT 69652/1 paratype; *Eostrobilops diodontina*, China, Tchen-k'eu, leg. Farges, excoll Musée Heude, 03.01.1946, MCZ, 167133 (photos of a syntype were received from Jochen Gerber). We could not examine most *Eostrobilops* types during our visit to the National Museum of Nature and Science, Tsukuba, Japan (11–13 March, 2015), because they were on loan. The comparisons of *Eostrobilops humicolus* sp. n. with *E. infrequens* and *E. triptychus* were based on the original descriptions of these species.

## Abbreviations

HA	Collection András Hunyadi, Budapest, Hungary
HNHM	Hungarian Natural History Museum, Budapest, Hungary
MCZ	Museum of Comparative Zoology, Massachusetts, USA
NHMUK	The Natural History Museum, London, United Kingdom
NSMT	National Museum of Nature and Science, Tsukuba, Japan

## Results

### Taxonomic description

### Family Strobilopsidae

### Genus *Eostrobilops* Pilsbry, 1927

1927 *Eostrobilops* Pilsbry (as a section of *Strobilops*), Manual of Conchology, Second Series, 28: 42.

**Type species.** *Strobilops hirasei* Pilsbry, 1908, by original designation.

***Eostrobilops humicolus* Páll-Gergely & Hunyadi, sp. n.**

<http://zoobank.org/AE9A1A96-A8F8-4E94-B5E7-45A6452ADC20>

**Material.** China, Guangxi (广西), Hechi Shi (河池市), Tiane Xian (天峨县), Qimu Xiang (岂暮乡), road junction toward Lahaoyan (拉号岩), cliff overlooking a memorial, 600 m, 24°51.130'N, 107°11.670'E, leg. Hunyadi, 12.09.2013., HNHM 99419 (holotype, Figure 1A–C), HNHM 99420 (paratype, Figure 1D–E and 2), HA/5 paratypes.

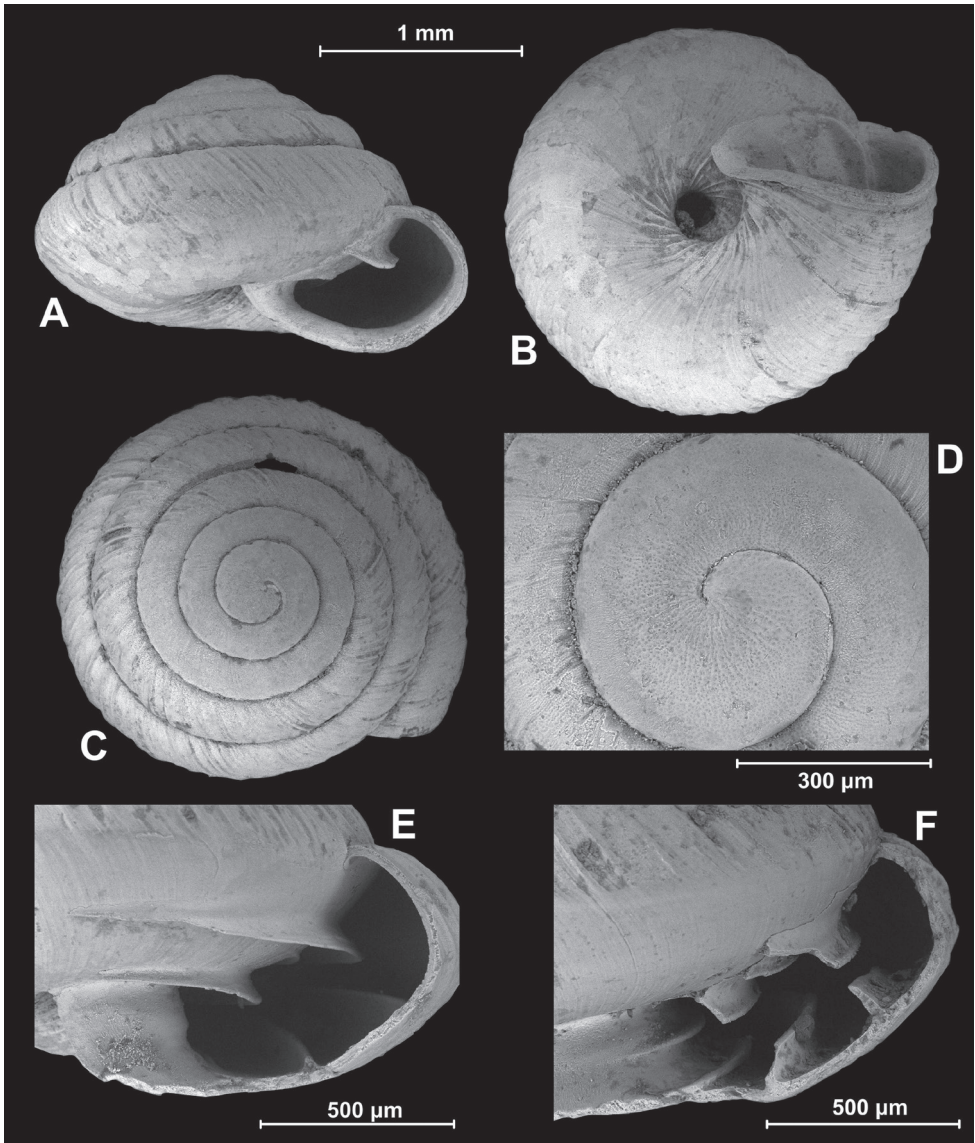
**Diagnosis.** A small *Eostrobilops* species with a strongly ribbed dorsal surface, an infraparietal lamella not reaching the callus, and long basal folds.

**Description.** Shell small, light brown (only one shell in type material had original colour); trochiform, rather domed above, almost flat ventrally (umbilical side), periphery rounded, very slightly keeled, suture rather deep; 4.75 ( $n = 3$ ) slowly expanding whorls, protoconch approximately 1.5 whorls; virtually smooth but with weak microscopic pits arranged in irregular radial lines; teleoconch irregularly ribbed; ribs strong dorsally, very weak ventrally (except for inside the umbilicus); spiral structure entirely lacking; umbilicus narrow; inner margin of the whorls exposed in umbilicus irregularly crenulated; aperture semilunar and oblique; peristome slightly thickened and slightly reflexed; parietal callus weak; parietal and infraparietal lamellae well-developed, although the infraparietal does not extend to the peristome; a low interparietal lamella deeply situated, not visible from aperture; three long basal folds and one short columellar lamella; basal folds visible in one specimen with a fresh, translucent shell only (Figs 1D–E, 2). However, this specimen was broken and the fold length can only be estimated (ca. a minimum of a quarter whorl).

**Differential diagnosis.** Both *E. hirasei* and *E. nipponica* are much larger than the new species, they have a more rounded body whorl, wider umbilicus, and weaker dorsal sculpture. *Eostrobilops coreana* is larger and flatter than *E. humicolus* sp. n., has a weaker dorsal sculpture, both of its parietal lamellae extend to the peristome, and has a shorter basal folds. *Eostrobilops kanjiokuboi* is similar to *E. humicolus* sp. n. by having a strongly ribbed dorsal surface and long basal folds, but differs in the larger size, wider umbilicus, and the serrated lamellae (not serrated in *E. humicolus* sp. n.); the infraparietal lamella extends to the callus (not in *E. humicolus* sp. n.), and has a long palatal fold, which is lacking in the new species. *Eostrobilops diodontina* is slightly larger ( $D = 2.88$ ,  $H = 1.75$  mm), has more elevated parietal and infraparietal lamellae, and both lamellae attain the peristome. Moreover, *E. diodontina* has shorter basal folds. *Eostrobilops infrequens* has a more elevated spire than *E. humicolus* sp. n., a stronger callus, a narrower umbilicus and shorter basal folds. The spire of *E. triptychus* is higher, it has more angulate periphery, thicker peristome and shorter basal folds.

**Measurements** (in mm):  $D = 2.3–2.4$ ,  $H = 1.45–1.5$  ( $n = 2$ ).

**Etymology.** From Latin ('humicolus' = soil-dwelling), in reference to the fact that this species was found in soil samples.



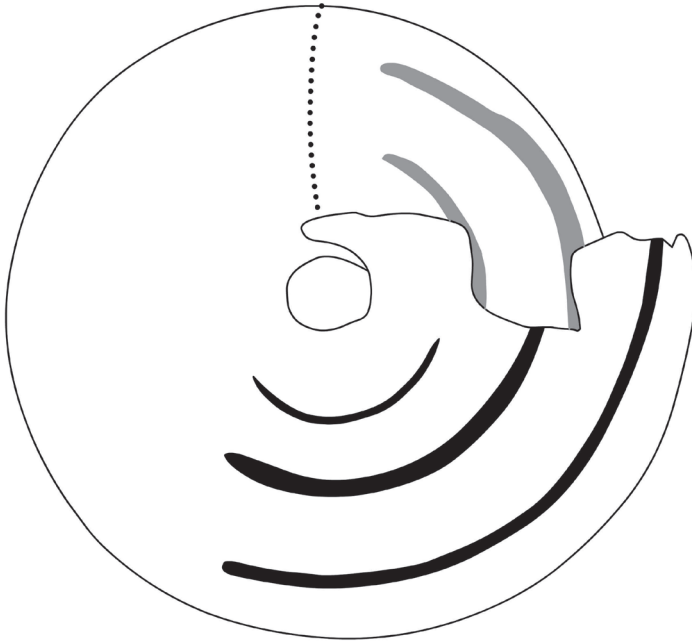
**Figure 1.** SEM images of *Eostrobilops humicolus* sp. n. **A–C** holotype **D–E** paratype1 **F** paratype2.

**Ecology.** No living specimens have been found. The empty shells were collected from a soil sample. *Eostrobilops humicolus* sp. n. probably lives under stones and dead leaves on the soil.

**Type locality.** China, Guangxi (广西), Hechi Shi (河池市), Tiane Xian (天峨县), Qimu Xiang (岂暮乡), road junction toward Lahaoyan (拉号岩), cliff overlooking a memorial, 600 m, 24°51.130'N, 107°11.670'E.

**Distribution.** Known from the type locality only.





**Figure 2.** Drawing showing the lamellae and folds of *Eostrobilops humicolus* sp. n. (paratype specimen, same as on Fig. 1D–E). Black lines: basal folds; grey lines: parietal and infraparietal lamellae. Dotted line indicates the supposed position of the parietal callus.

### Remarks on *Eostrobilops* species

Solem (1968) named three differences between the two genera: (1) *Enteroplax* has a raised parietal callus, lacking in *Eostrobilops*, (2) *Enteroplax* has a “peripheral thread”, lacking in *Eostrobilops* and (3) *Eostrobilops* has superior serrated nodes on the parietal lamellae, missing in *Enteroplax*. The morphology of the callus and the periphery seem to separate the two genera well, because the raised callus is always associated with the peripheral thread and the weak callus is a characteristic feature of species which lack the peripheral thread. The serrated nodes on the lamellae, however, were not observed in at least three species (*Eostrobilops infrequens*, *E. humicolus* sp. n. and *E. triptychus*) which belong to *Eostrobilops* based on the other two characters. Therefore, the serrated node is excluded from the diagnosis of *Eostrobilops*. Future investigations may provide additional information on the utility of this character for the subdivision of *Eostrobilops*.

*Eostrobilops yaeyamensis*, *E. taiwanica* and *E. kanjiokuboi* have been described as *Enteroplax* species. However, they lack the thickened parietal callus, which is conspicuous in every *Enteroplax* species. Moreover, although they have a somewhat angular periphery, they lack a distinct “peripheral thread”, which is also characteristic for *Enteroplax*. Therefore, all these three species belong to the genus *Eostrobilops*, as already proposed by Vermeulen (1992a) for *E. yaeyamensis*.

No obvious teleoconch spiral lines are visible in the photo of the syntype of *Eostrobilops diodontina*, as noted in the original description (Heude 1885, Pilsbry 1927).

The palatal fold, which is approximately a quarter whorl in length and runs just above the keel in the paratype of *E. kanjiokuboi* (see Material and methods) was not mentioned in the original description.

Vermeulen (1992a) compared *E. yaeyamensis* with *E. triptychus*. He mentioned that *E. yaeyamensis* “occasionally shows an interparietalis” and “may have an interparietalis”. This information is probably erroneous, because neither the original description (Habe and Chinen 1974), nor Minato (1982) mentioned an interparietalis lamella. Moreover, Vermeulen (1992a) mentioned that *E. yaeyamensis* has two basal folds, whereas *E. triptychus* possesses three. However, the description and figure of Minato (1982) describes three basal folds, and interstitial basal plicae were mentioned in the original description of *E. yaeyamensis*.

### Checklist of *Enteroplax* and *Eostrobilops* species, and their distributions (see also Fig. 3)

*Enteroplax dumogensis* Vermeulen, 1992: Indonesia, North Sulawesi Island, Utara, Dumoga valley, Mount Mogogonipa (Vermeulen 1992b).

*Enteroplax misoolensis* (Adam & van Benthem Jutting, 1939): Indonesia, Misool Island, near Lilinta, Waima and Fakal (Adam and van Benthem Jutting 1939, Solem 1968).

*Enteroplax polyptychia* (Möllendorff, 1887): Philippine Islands, Cebu and Siquijor Islands (Solem 1968).

*Enteroplax quadrai* (Moellendorff, 1893): Philippine Islands, Luzon, Bohol, Mindanao islands (Moellendorff 1893, Solem 1968).

*Enteroplax trochospira* (Möllendorff, 1887): Philippine Islands, Cebu and Bohol islands; Indonesia: North Sulawesi (Solem 1968, Vermeulen 1992b).

*Eostrobilops coreana* (Pilsbry, 1927): North Korea: Pyong Yang; southeast Russia, National reserve “Kedrovaya pad” (Кедровая падь = “Cedar valley”) (Pilsbry 1927, Schileyko 1984).

*Eostrobilops coreana echo* (Kuroda & Miyanaga, 1939): North Korea, Soto-Kongō (outer Kumgang Mountains) (Kuroda and Miyanaga 1939).

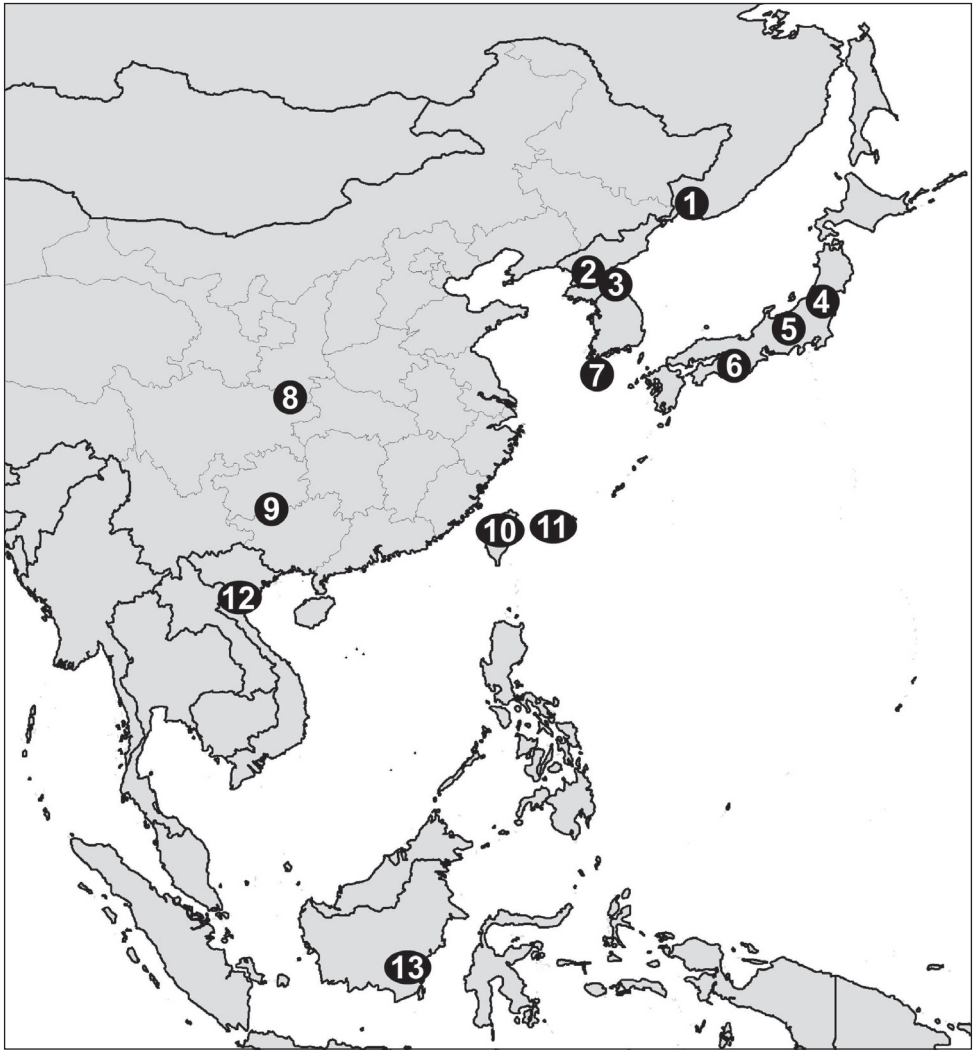
*Eostrobilops diodontina* (Heude, 1885): Tchen k’eu (=Chengkou, Chongqing Province, China) (Heude 1885, Solem 1968).

*Eostrobilops hirasei* (Pilsbry, 1908): South Korea, Quelpart Island (Pilsbry 1908).

*Eostrobilops infrequens* Maassen, 2006: Vietnam, Thanh Hoa Province, Pu Luong National Park (Maassen 2006).

*Eostrobilops kanjiokuboi* (Minato & Tada, 1992): Lo lo uen chuan, Tung-pu, Hsin-i shiang, Nan tou hsien, Taiwan (Minato and Tada 1992).

*Eostrobilops kongoensis* (Kuroda & Miyanaga, 1939): North Korea, near Tyō-anzi, Uti-Kongō (=Kumgang Mountains; Kuroda and Miyanaga 1939).



**Figure 3.** Distribution of *Eostrobilops* species. **1, 2** *Eostrobilops coreana* (Pilsbry, 1927) **3** *E. coreana echo* (Kuroda & Miyanaga, 1939) and *E. kongoensis* (Kuroda & Miyanaga, 1939) **4, 5** *Eostrobilops nipponica* (Pilsbry, 1927) **6** *E. nipponica reikoe* Matsumura & Minato, 1980 **7** *E. hirasei* (Pilsbry, 1908) **8** *E. diodontina* (Heude, 1885) **9** *E. humicolus* sp. n. **10** *E. kanjiokuboi* (Minato & Tada, 1992) and *E. taiwanica* (Minato & Tada, 1992) **11** *E. yaeyamensis* (Habe & Chinen, 1974) **12** *E. infrequens* Maassen, 2006 **13** *E. triptychus* Vermeulen, 1992.

*Eostrobilops humicolus* Páll-Gergely & Hunyadi, sp. n.: China, Guangxi, Hechi Shi, Tiane Xian, Qimu Xiang, cross towards Lahaoyan, 600 m, 24°51.130'N, 107°11.670'E.

*Eostrobilops nipponica* (Pilsbry, 1927): Japan, Yonezawa; Nagano Province (Pilsbry 1927, Minato 1975, 1982).

- Eostrobilops nipponica reikoe* Matsumura & Minato, 1980: Japan, Osaka Prefecture, Takatsuki-shi, Ibaragi-shi, Suita-shi and Minoo-shi (fourteen localities; Matsumura and Minato 1980).
- Eostrobilops taiwanica* (Minato & Tada, 1992): Meifeng, Lenai shiang, Nan tou hsien, Taiwan (Minato and Tada 1992).
- Eostrobilops triptychus* Vermeulen, 1992: Indonesia, Borneo, Kalimantan Selatan, northwestern part of the Meratus Mountains (Vermeulen 1992a).
- Eostrobilops yaeyamensis* (Habe & Chinen, 1974): Sonai, Irimote Island; Kabira, Ishigaki Island (both Yaeyama Group, Okinawa, Japan) (Habe and Chinen 1974, Vermeulen 1992a).

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# Three new species of eriophyoid mites (Acari, Eriophyoidea) from Xinjiang Uygur Autonomous Region, China

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## Abstract

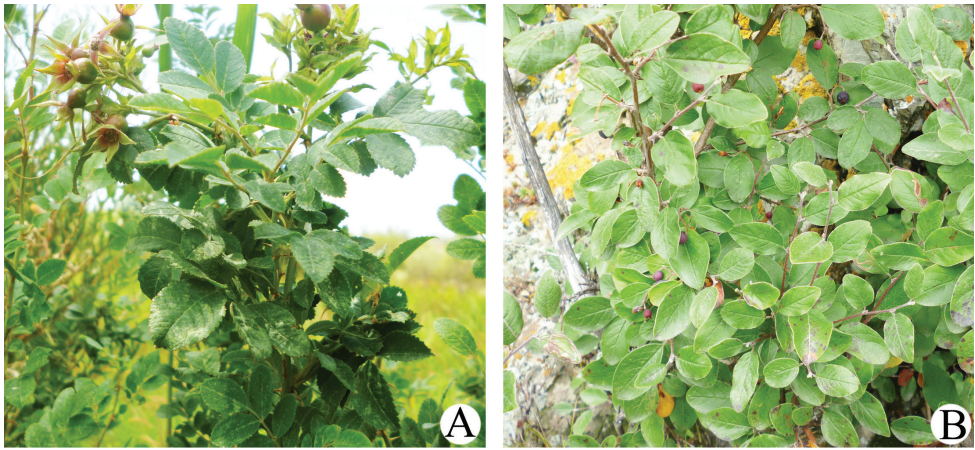
Three new species of eriophyoid mites from Xinjiang Uygur Autonomous Region, China, are described and illustrated. They are *Paracolomerus gonglius* **sp. n.** and *Phyllocoptruta beggerianae* **sp. n.** collected on *Rosa beggeriana* Schrenk ex Fisch. & C. A. Mey. (Rosaceae), and *Rhyncaphytoptus fuyuniensis* **sp. n.** collected on *Cotoneaster ignavus* E. L. Wolf (Rosaceae). All eriophyoid mites described here are vagrants on the undersurface of leaves and any apparent damage was not observed.

## Keywords

Taxonomy, Colomerini, Phyllocoptini, Rhyncaphytoptinae, Rosaceae

## Introduction

Eriophyoid mites (Acari: Eriophyoidea) have been recognized as important pests in agriculture and forestry all over the world (Lindquist et al. 1996). Their stylets are involved in piercing plant cells, injecting saliva into them and sucking cell sap (de Lillo et al. 2002). The saliva causes cytological, biochemical and physiological changes in the pierced plants (de Lillo and Monfreda 2004, Petanović and Kielkiewicz 2010a).



**Figure 1.** **A** *Rosa beggeriana* Schrenk ex Fisch. & C.A. Mey **B** *Cotoneaster ignavus* E.L. Wolf.

Eriophyoid mites induce plant malformations as galls, complex symptoms or vector pathogens disturbing the normal growth of plants (Petanović and Kielkiewicz 2010b). This is the case of *Colomerus vitis* (Pagenstecher), *Aceria pallida* Keifer and *Tegolophus zizyphagus* (Keifer) which induce erineae, galls or leaf edge curls and cause economic losses to grape, matrimony vine and jujube, respectively, in Xinjiang (Lu and Mao 1990, Zang 1998, Yang et al. 2012). However, about half eriophyoid mite species are vagrants on the surface of leaves and do not cause any apparent damage (Huang 2008, Skoracka et al. 2010, Petanović and Kielkiewicz 2010b). These mites, occurring in a large amount, may cause non-distortive changes and affect the normal growth of the plants (Oldfield 1996). Usually eriophyoids are tiny in size and hard to see with unaided eyes. Sometimes their symptoms can be confused with those due to viruses, nutrient deficiency and physiological disorders (Van Leeuwen et al. 2010). Therefore, it is necessary to study the systematic account of Eriophyoidea for having a further contribute in better understanding their significance in Agriculture.

Kuang (1995) first explored and reported the eriophyoid mite fauna in Xinjiang. After that, a number of field surveys were conducted in the same area and further 31 species were reported so far. Out of 31 species, 1 species belongs to the family Phytoseptidae, 2 species belong to the family Diptilomiopidae and 28 species belong to the family Eriophyidae (Table 1). The fact that more than 1000 species have been recognized from China (personal data of X.-F. Xue) suggests that many more areas need to be explored more carefully. For this purpose eriophyoid mites were collected by Ji-Wei Li from Tianshan Mountains, Altai Mountains, Farmlands and Gurbantunggut Desert of Xinjinag in 2013 and 2014.

In the present study, we describe 2 new species of the genera *Paracolomerus* and *Phyllocoptruta* collected on *Rosa beggeriana* (Fig. 1) and one new species of the genus *Rhyncaphytoptus* collected on *Cotoneaster ignavus* (Fig. 1), all from Xinjiang. Also, this is the first description of the genus *Paracolomerus* from Rosaceae.

**Table 1.** List of eriophyoid mites from Xinjiang Uygur Autonomous Region.

Family	Subfamily	Tribe	Species	Reference
Phytropidae	Nalepellinae	Nalepellini	<i>Setopius koriensis</i> Kuang & Hong, 1995	Xue et al. 2012a: 12.
	Cecidophyinae	Colomerini	<i>Colomerus vitis</i> (Pagenstecher, 1857)	Kuang 1995: 42–43.
Eriophyidae	Eriophyinae		<i>Paracolomerus gonglius</i> sp. n.	This study
		Eriophyini	<i>Eriophyes catacardiae</i> Keifer, 1962	Kuang 1995: 47.
		Aceriini	<i>Aceria bromi</i> Kuang & Zhang, 1992	Kuang 1995: 53–54.
			<i>Aceria changiensiensis</i> Kuang & Pang, 1997	Kuang and Pang 1997: 230–231.
			<i>Aceria dispar</i> (Nalepa, 1891)	Song et al. 2008: 13.
			<i>Aceria haloxylonis</i> Xue, Zhang, Li & Hong, 2012	Xue et al. 2012b: 203–208.
			<i>Aceria nimia</i> Hall, 1967	Kuang et al. 2005: 29.
			<i>Aceria pallida</i> Keifer, 1964	Hong et al. 2006: 230.
	Phyllocoptinae		<i>Aceria tamaricis</i> (Trotter, 1901)	Kuang et al. 2005: 33–34.
			<i>Aceria tosicella</i> Keifer, 1969	Kuang 1995: 64–65.
		Phyllocoptini	<i>Calepitrimerus alchemillae</i> (Liro, 1940)	Kuang et al. 2005: 58–59.
			<i>Epirimerus sabinae</i> Xue & Hong, 2005	Xue et al. 2012a: 31.
			<i>Phyllocoptes pyruvagrans</i> Kadono, 1985	Kuang et al. 2005: 68–69.
			<i>Phyllocoptiruta begerianae</i> sp. n.	This study
	Anthocoptini		<i>Proiectus tabulaeformis</i> Xue, Song, Amrine & Hong, 2007	Xue et al. 2012a: 39.
			<i>Aculodes shiheziensis</i> Kuang, Lu & Zhang, 2005	Kuang et al. 2005: 81–82.
			<i>Aculops alopecuroides</i> Kuang, 1998	Kuang 1998: 410–411.
			<i>Aculops salixis</i> Xue, Song & Hong, 2007	Xue et al. 2012a: 41–42.
			<i>Aculus amygdali</i> Xue & Hong, 2005	Xue et al. 2012a: 43.
			<i>Aculus schlechtendali</i> (Nalepa, 1890)	Kuang 1995: 120–121.
			<i>Aculus tetanothrix</i> (Nalepa, 1889)	Kuang 1995: 131–132.
			<i>Tegolophus zizyphagus</i> (Keifer, 1939)	Kuang 1995: 146–147.
			<i>Tetra cuihuai</i> Xue, Song & Hong, 2006	Xue et al. 2012a: 54.
			<i>Tetra nitariae</i> Li, Li, Zhang & Xue, 2014	Li et al. 2014: 348–351.
			<i>Tetra sativae</i> Li, Li, Zhang & Xue, 2014	Li et al. 2014: 339–343.



Family	Subfamily	Tribe	Species	Reference
			<i>Tetra shibeziensis</i> Wang & Lu, 2004	Wang and Lu 2004: 266–267.
			<i>Tetra tianchiensis</i> Li, Li, Zhang & Xue, 2014	Li et al. 2014: 335–339.
			<i>Tetra tianschanicae</i> Li, Li, Zhang & Xue, 2014	Li et al. 2014: 330–334.
			<i>Tetra viciae</i> Li, Li, Zhang & Xue, 2014	Li et al. 2014: 343–348.
Diptilomiopidae	Rhyncaphytopinae		<i>Rhyncaphytopus fuyunensis</i> sp. n.	This study
			<i>Rhyncaphytopus yilisi</i> Song, Xue & Hong, 2007	Song et al. 2007: 63–65.
			<i>Rhyncaphytopus ziziphi</i> Kuang, 2005	Kuang et al. 2005: 157–158.

## Materials and methods

Specimens of mites were collected from Xinjiang Uygur Autonomous Region, China. The morphological terminology used here follows Lindquist (1996). The generic classification of the eriophyoid mites is made according to Amrine et al. (2003), together with the comparison of genera erected after 2003. Specimens were cleared in Keifer's booster and slides were mounted using modified Berlese medium (Amrine and Manson 1996). The number of measured specimens (n) is given within parentheses in the description. All specimens were examined, measured, taken photos and drawn with the aid of an Olympus Bx61 microscope using phase contrast. The measurements and drawings were based on the methods outlined by de Lillo et al. (2010) and abbreviations follow Amrine et al. (2003). For each species, the holotype female measurement precedes the corresponding range for paratypes (given in parentheses). For males, only ranges are given. All measurements are given in micrometres and are lengths unless specified. Type specimens are deposited at the Department of Plant Protection, College of Agriculture, Shihezi University, Xinjiang Uygur Autonomous Region, China.

## Results

**Family Eriophyidae Nalepa, 1898**

**Subfamily Cecidophyinae Keifer, 1966**

**Tribe Colomerini Newkirk & Keifer, 1975**

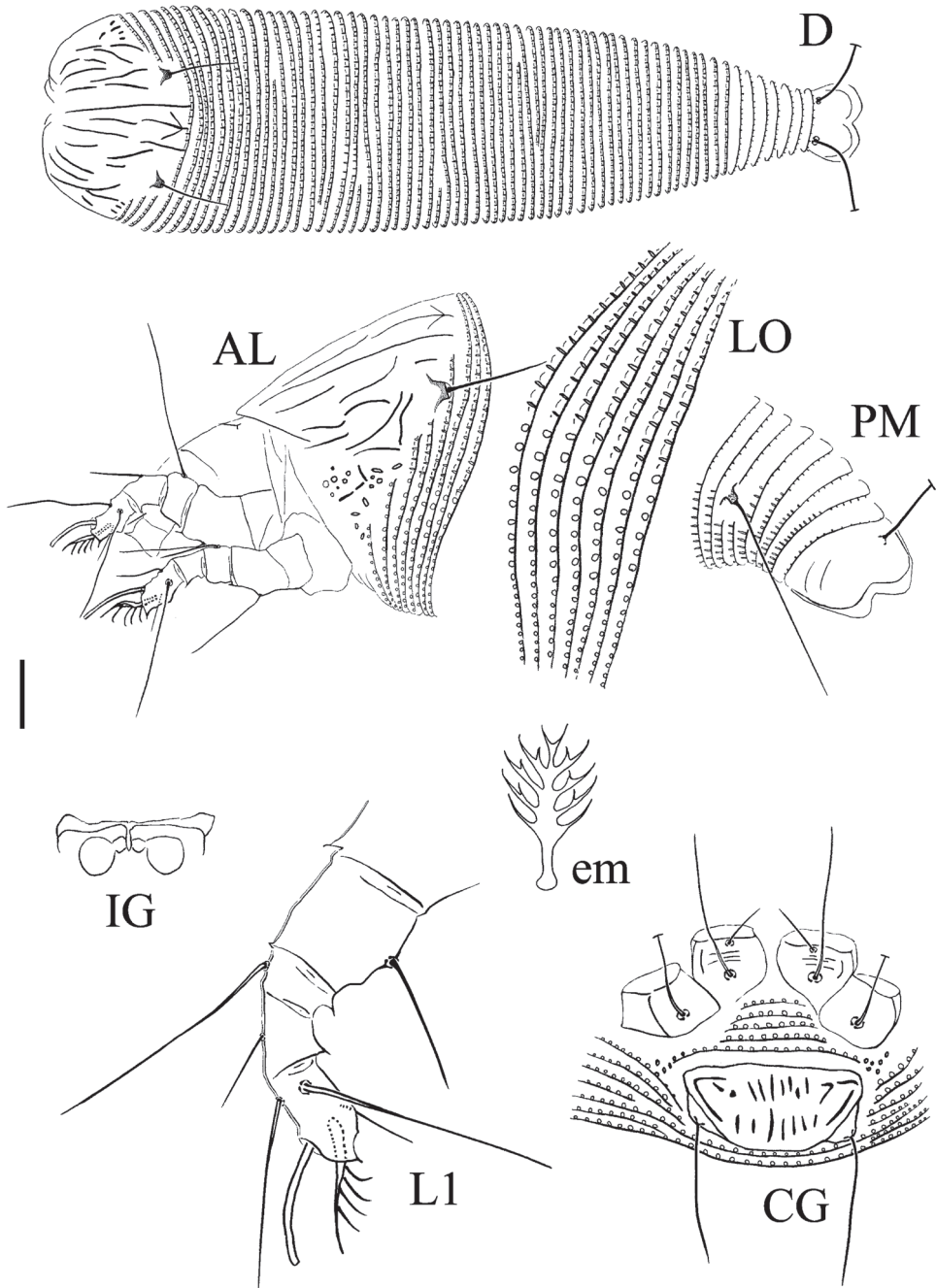
**Genus *Paracolomerus* Keifer, 1975**

***Paracolomerus gonglius* sp. n.**

<http://zoobank.org/36D41CF4-E10C-47F1-8635-3E2CC695A2CB>

Fig. 2

**Description.** FEMALE (n=6). Body vermiform, 187 (175–217, excluding gnathosoma), 50 (42–51) wide, 48 (40–47) thick; light yellow. **Gnathosoma** 24 (23–26), projecting obliquely down, pedipalp coxal setae (*ep*) 2 (2–3), dorsal pedipalp genual setae (*d*) 7 (6–8), unbranched, cheliceral stylets 21 (20–23). **Prodorsal shield** 30 (29–32), 37 (33–37) wide; median line almost complete, interrupted in the middle with short sloping lines on either side at the posterior end, admedian lines complete, submedian lines broken, with several short lines and granules on the lateral side; frontal shield lobe absent. Scapular tubercles near rear shield margin, 24 (23–24) apart, scapular setae (*sc*) 15 (14–15), projecting posterior. **Coxigenital region** with 5 (5–6) microtuberculated semiannuli. Coxal plates with several short lines, anterolateral setae on coxisternum I (*lb*) 7 (7–8), 11 (10–11) apart, proximal setae on coxisternum I (*la*) 25 (25–28), 13 (12–13) apart, proximal setae on coxisternum II (*2a*) 44 (39–44), 24 (23–24) apart, tubercles *lb* and *la* apart 5 (5–6), tubercles *la* and *2a* 8 (7–8) apart. Internal coxisternal apodeme absent. Legs with usual series of setae. **Leg I** 27 (26–28), femur 8 (7–8), basiventral femoral setae (*bv*) 12



**Figure 2.** Schematic drawings of *Paracolomerus gonglius* sp. n.: **AL** Lateral view of anterior body region **CG** Female coxigenital region **D** Dorsal view **em** Empodium **IG** Internal female genitalia **LO** Lateral view of annuli **L1** Leg I **PM** Lateral view of posterior opisthosoma. Scale bar: 15  $\mu$ m (**D**); 10  $\mu$ m (**AL**, **CG**, **IG**, **PM**); 7.5  $\mu$ m (**LO**); 5  $\mu$ m (**L1**); 2.5  $\mu$ m (**em**).

(12–14); genu 5 (4–5), antaxial genual setae ( $l'$ ) 25 (23–26); tibia 6 (6–7), paraxial tibial setae ( $l$ ) 7 (7–8), located in the middle; tarsus 7 (6–7), setae  $ft'$  15 (14–15), setae  $ft''$  22 (20–22), seta  $u'4$  (4–5); tarsal empodium ( $em$ ) 6 (5–6), simple, 5-rayed, tarsal solenidion ( $\omega$ ) 7 (7–8), rod-like. **Leg II** 25 (24–26), femur 7 (7–8), basiventral femoral setae ( $bv$ ) 13 (13–14); genu 5 (4–5), antaxial genual setae ( $l''$ ) 8 (8–10); tibia 5 (4–5); tarsus 7 (6–7), setae  $ft'$  6 (6–7), setae  $ft''$  23 (23–25), seta  $u'4$  (4–5); tarsal empodium ( $em$ ) 6 (5–6), simple, 5-rayed, tarsal solenidion ( $\omega$ ) 9 (9–10), rod-like. **Opisthosoma** dorsally arched, 64 (63–66) dorsal annuli, 63 (61–64) ventral annuli; microtubercles on the rear margin of the annuli, elliptical on the anterior part of dorsal annuli, linear and spiny on the posterior part of dorsal annuli and posterior part of ventral annuli, circular on the anterior part of ventral annuli. Setae  $c2$  25 (25–27) on ventral annulus 9 (8–9), 47 (40–49) apart; setae  $d$  65 (58–67) on ventral annulus 19 (18–21), 37 (34–37) apart; setae  $e$  12 (12–14) on ventral annulus 32 (30–32), 20 (20–21) apart; setae  $f$  29 (29–32) on 6th ventral annulus from rear, 21 (19–21) apart. Setae  $h1$  absent,  $h2$  71 (69–74). **Genital coverflap** 11 (11–12), 22 (21–22) wide, coverflap with two rows of ridges, the basal one with 12 (11–13) longitudinal ridges, the other one with 9 (8–10) longitudinal ridges, proximal setae on coxisternum III ( $3a$ ) 17 (17–20), 17 (17–18) apart.

MALE. Unknown.

**Type host plant.** *Rosa beggeriana* Schrenk ex Fisch. & C. A. Mey. (Rosaceae).

**Relation to the host plant.** Vagrant on leaves; no apparent damage was observed.

**Type locality.** Gongliu county, Xinjiang Uygur Autonomous Region, China (43°09'37"N, 81°36'34"E), elevation 1,396 m; 3 August 2014, coll. Ji-Wei Li.

**Type material.** Holotype: female (slide number SHZU *Paracolomerus* 1.1, marked Holotype). Paratypes: 6 females mounted on 6 separate microscope slides.

**Etymology.** The specific designation *gonglius* comes from the name of location, Gongliu, where the new species was collected.

**Differential diagnosis.** All traits are in accordance with the type species *Paracolomerus casimiroae* Keifer, 1975 of the genus *Paracolomerus* (opisthosomal annuli subequal, legs with usual series of setae, scapular tubercles on rear shield margin, scapular setae projected posteriorly) except for ventral surface ornamentation of coxa I (lines do not circle around tubercles  $1a$  and meet at sternum in *P. gonglius* sp. n.; lines originate at setae  $1b$ , circle distally around tubercles  $1a$  and meet at sternum, enclose most of the coxal surface in *P. casimiroae*).

This species is similar to *P. fopingacer* Xue, Song & Hong, 2011, from *Acer* sp. L. (Aceraceae), but can be differentiated from the latter by median line almost complete, with 5–6 short lines on the lateral sides of prodorsal shield (median line present for half, without short lines on the lateral sides in *P. fopingacer*), frontal shield lobe absent (frontal shield lobe acuminate in *P. fopingacer*) and 5-rayed empodium (6-rayed empodium in *P. fopingacer*).

**Remarks.** To date, only three species were reported from the genus *Paracolomerus*, *P. casimiroae* Keifer, 1975, *P. davidiae* Kuang & Hong, 1995 (in Kuang 1995) and *P. fopingacer*.

**Subfamily Phyllocoptinae Nalepa, 1892****Tribe Phyllocoptini Nalepa, 1892****Genus *Phyllocoptruta* Keifer, 1938*****Phyllocoptruta beggerianae* sp. n.**

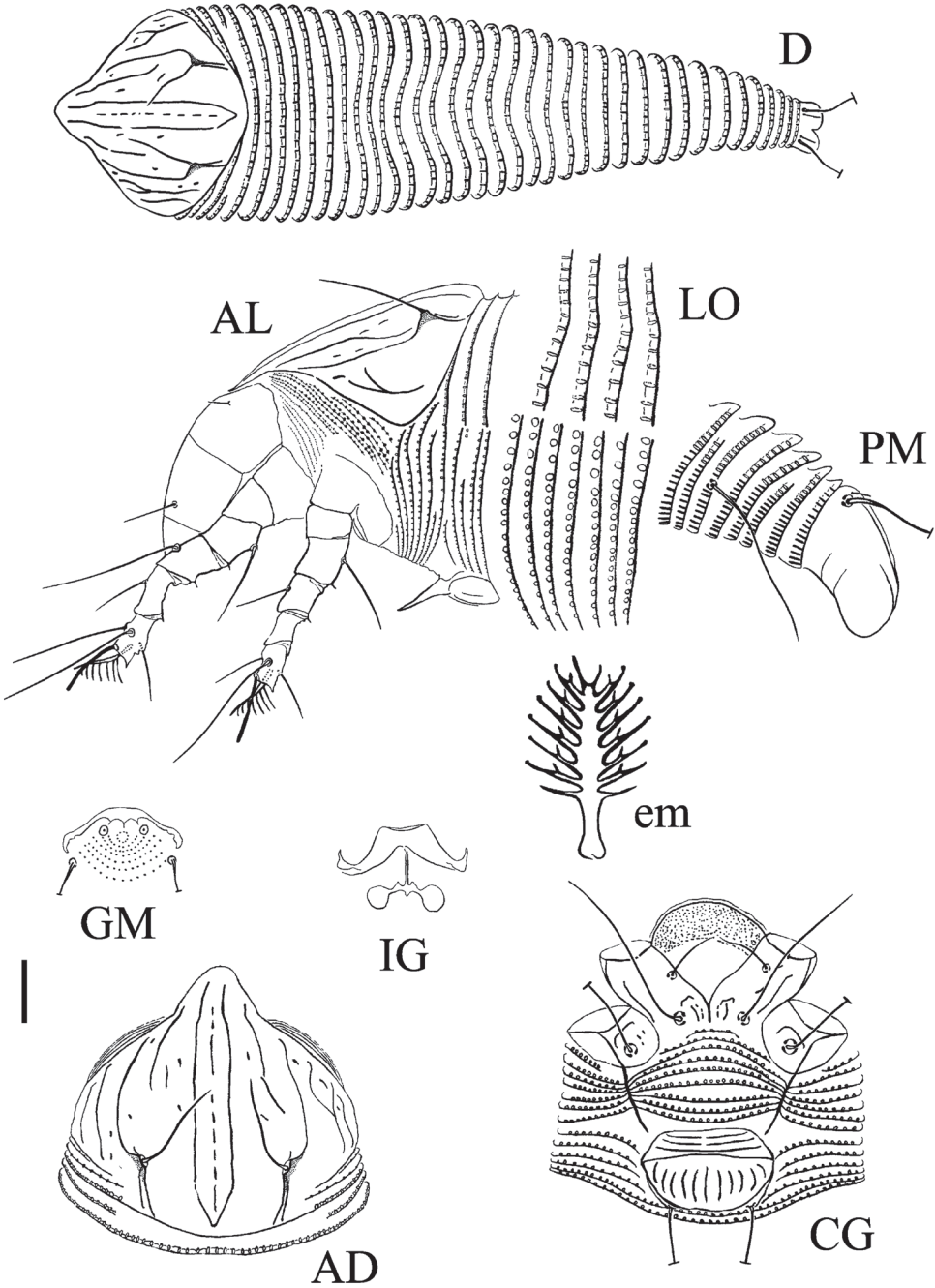
<http://zoobank.org/D33691AE-25DD-4A17-A854-E7C9925C96FF>

Fig. 3

**Description.** FEMALE (n=9). Body fusiform, 207 (182–207, excluding gnathosoma), 49 (46–51) wide, 43 (40–46) thick; white. **Gnathosoma** 28 (27–30), projecting obliquely down, pedipalp coxal setae (*ep*) 3 (2–3), dorsal pedipalp genual setae (*d*) 9 (8–9), unbranched, cheliceral stylets 26 (25–27). **Prodorsal shield** 42 (40–43), 43 (41–44) wide, median line formed by lined short lines, admedian lines complete and connected posteriorly, submedian lines present at the posterior half, with several short lines and granules; frontal shield lobe rounded, broad-based, 5 (4–5). Scapular tubercles ahead of rear shield margin, 23 (22–25) apart, scapular setae (*sc*) 16 (16–18), projecting forward and convergent. **Coxigenital region** with 9 (7–9) microtuberculated semiannuli. Coxal plates with several short lines and granules, anterolateral setae on coxisternum I (*1b*) 11 (10–11), 12 (11–12) apart, proximal setae on coxisternum I (*1a*) 27 (26–31), 10 (9–10) apart, proximal setae on coxisternum II (*2a*) 46 (42–46), 25 (23–25) apart, tubercles *1b* and *1a* apart 7 (6–7), tubercles *1a* and *2a* 9 (8–9) apart. Internal coxisternal apodeme 3 (3–4). Legs with usual series of setae. **Leg I** 36 (35–37), femur 10 (9–10), basiventral femoral setae (*bv*) 14 (13–15); genu 5 (4–5), antaxial genual setae (*l'*) 22 (22–25); tibia 8 (8–9), paraxial tibial setae (*l'*) 12 (10–12), located at 1/3 from dorsal base; tarsus 9 (8–9), setae *ft'* 19 (19–21), setae *ft''* 22 (22–25), seta *u'* 10 (9–10); tarsal empodium (*em*) 8 (7–8), simple, 8-rayed, tarsal solenidion ( $\omega$ ) 10 (9–10), rod-like. **Leg II** 29 (28–30), femur 8 (8–9), basiventral femoral setae (*bv*) 16 (15–16); genu 4 (4–5), antaxial genual setae (*l''*) 8 (6–8); tibia 5 (5–6); tarsus 8 (7–8), setae *ft'* 9 (8–10), setae *ft''* 23 (23–25), seta *u'* 9 (8–9); tarsal empodium (*em*) 8 (7–8), simple, 8-rayed, tarsal solenidion ( $\omega$ ) 10 (9–10), rod-like. **Opisthosoma** dorsally with a furrow in the middle; 35 (33–38) dorsal annuli, elliptical microtubercles on the rear margin; 74 (72–77) ventral annuli, microtubercles on the rear margin, circled on the anterior ventral annuli, and linear and spiny on the last posterior ventral annuli. Setae *c*2 28 (25–28) on ventral annulus 14 (12–14), 47 (45–48) apart; setae *d* 52 (48–52) on ventral annulus 28 (27–29), 35 (32–35) apart; setae *e* 33 (33–36) on ventral annulus 49 (47–51), 13 (12–14) apart; setae *f*28 (26–29) on 6th ventral annulus from rear, 16 (16–17) apart. Setae *h*1 4 (3–4), *h*2 77 (75–83). **Genital coverflap** 13 (11–13), 19 (18–20) wide, coverflap with 3 transverse lines basally, 11 (11–13) longitudinal ridges distally, proximal setae on coxisternum III (*3a*) 43 (41–44), 15 (14–15) apart.

MALE (n=2). Similar in shape and prodorsal shield arrangement to female, 155–169. Prodorsal shield 32–35, 28–31 wide; scapular setae *sc* 16–17, 21–23 apart. Opisthosoma dorsally with a furrow, 32–37 annuli, ventrally with 74–81 annuli, dor-





**Figure 3.** Schematic drawings of *Phyllocoptruta beggerianae* sp. n.: **AL** Lateral view of anterior body region **AD** Dorsal view of anterior body region **CG** Female coxigenital region **D** Dorsal view **em** Empodium **GM** Male genital region **IG** Internal female genitalia **LO** Lateral view of annuli **PM** Lateral view of posterior opisthosoma. Scale bar: 15  $\mu\text{m}$  (**D**); 10  $\mu\text{m}$  (**AD**, **AL**, **CG**, **IG**, **GM**, **PM**); 7.5  $\mu\text{m}$  (**LO**); 2.5  $\mu\text{m}$  (**em**).

sal and ventral microtubercles are similar to females. Male genitalia 17–18 wide, setae 3a 18–20, 14–15 apart.

**Type host plant.** *Rosa beggeriana* Schrenk ex Fisch. & C. A. Mey. (Rosaceae).

**Relation to the host plant.** Vagrant on leaves; no apparent damage was observed.

**Type locality.** Xinyuan county, Xinjiang Uygur Autonomous Region, China (43°36'29"N, 82°17'56"E), elevation 758 m; 29 July 2014, coll. Ji-Wei Li.

**Type material.** Holotype: female (slide number SHZU *Phyllocoptruta* 1.1, marked Holotype). Paratypes: 16 females and 2 males mounted on 18 separate microscope slides.

**Etymology.** The specific designation *beggerianae* comes from the epithet of the host plant, *beggeriana*.

**Differential diagnosis.** This species is similar to *Phyllocoptruta clematoclethra* Xue, Song & Hong, 2010, from *Clematoclethra* sp. Maxim. (Actinidiaceae), but can be differentiated from the latter by admedian lines connected posteriorly (admedian lines separate in *P. clematoclethra*), scapular tubercles ahead of rear shield margin, scapular setae 16–18 (scapular tubercles on rear shield margin, scapular setae 3–4 in *P. clematoclethra*), female genital coverflap with 3 transverse basal lines (coverflap without transverse lines in *P. clematoclethra*) and 8-rayed empodium (5-rayed empodium in *P. clematoclethra*).

## Family Diptilomiopidae Keifer, 1944

### Subfamily Rhyncaphytopinae Roivainen, 1953

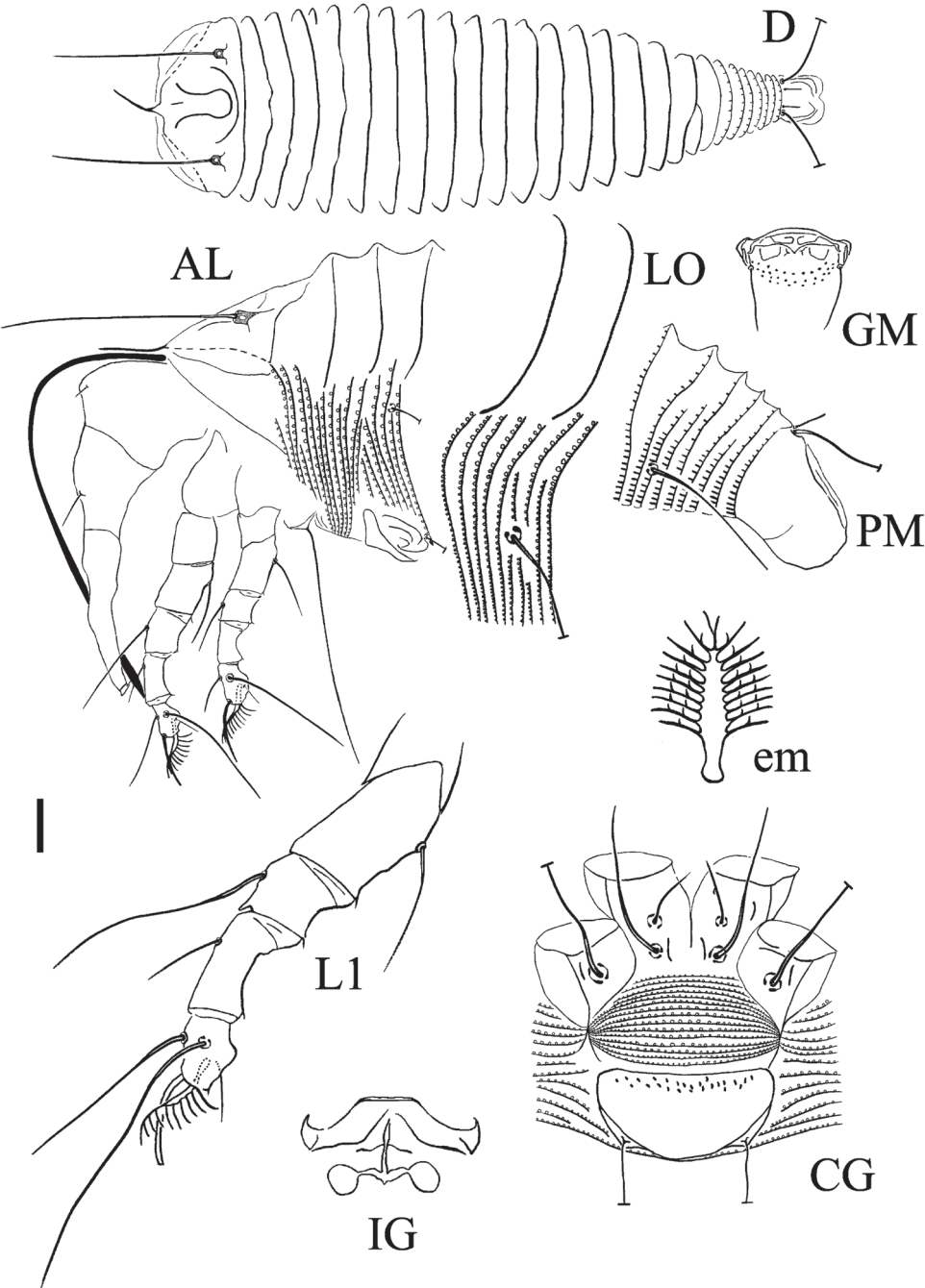
#### Genus *Rhyncaphytoptus* Keifer, 1939

#### *Rhyncaphytoptus fuyuniensis* sp. n.

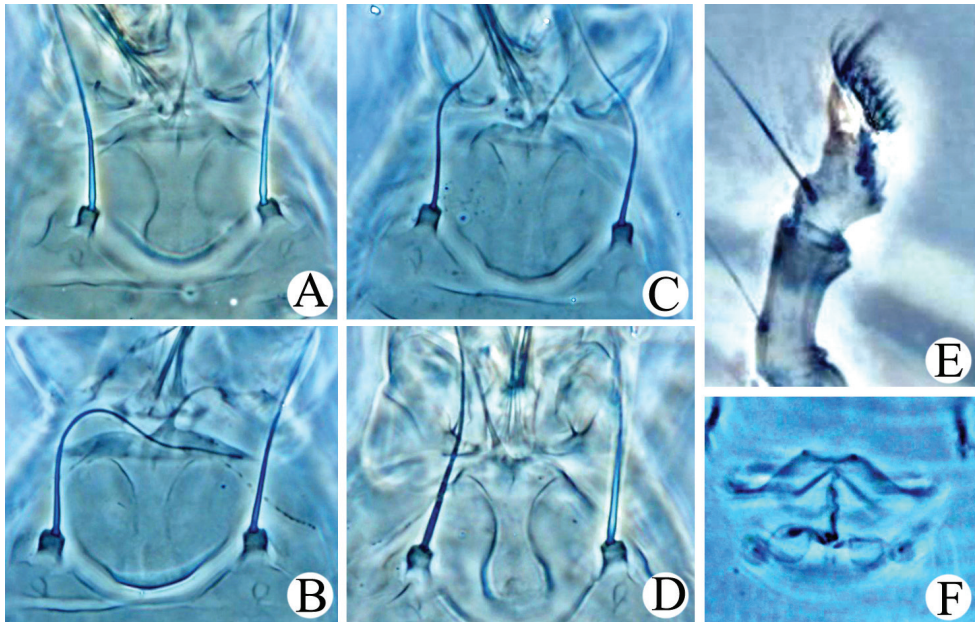
<http://zoobank.org/CE644BB3-52C6-43E6-AAC7-06BC248177F7>

Figs 4–5

**Description.** FEMALE (n=8). Body fusiform, 256 (216–267, excluding gnathosoma), 60 (55–64) wide, 58 (54–62) thick; light yellow. Gnathosoma 61 (55–64), projecting downwards, pedipalp coxal setae (*ep*) 3 (2–3), dorsal pedipalp genual setae (*d*) 7 (6–7), unbranched, cheliceral stylets 83 (76–88). **Prodorsal shield** 29 (28–30) excluding the thin anterior process length from frontal lobe, 47 (46–49) wide, sub-triangular in anterior shape; long and flexible frontal lobe ending with a thin anterior process, the process extends for 14 (13–15). Median line very short, on 1/5 anterior part of prodorsal shield; admedian lines complete and connected at base with transverse lines, forming a vase-shaped outline; semicircled line between the scapular tubercles. Scapular tubercles ahead of rear shield margin, 30 (28–31) apart, scapular setae (*sc*) 47 (46–50), projecting forward. **Coxigenital region** with 15 (14–16) microtuberculated semiannuli. Coxal plates with 1–3 short lines, anterolateral setae on coxisternum I (*Ib*) 10 (10–12), 10 (10–11) apart, proximal setae on coxisternum I (*Ia*) 32 (29–33), 10 (10–11) apart, proximal setae on coxisternum II (*2a*) 45 (42–47), 29 (28–30) apart, tubercles *Ib* and *Ia* apart 7 (6–7), tubercles *Ia* and *2a* 10 (9–11) apart. Internal coxisternal apodeme 7 (6–7). Legs with



**Figure 4.** Schematic drawings of *Rhyncaphytoptus fuyuniensis* sp. n.: **AL** Lateral view of anterior body region **CG** Female coxigenital region **D** Dorsal view **em** Empodium **GM** Male genital region **IG** Internal female genitalia **LO** Lateral view of annuli and setae **LI** Leg I **PM** Lateral view of posterior opisthosoma. Scale bar: 15  $\mu$ m (**D**); 10  $\mu$ m (**AL**, **CG**, **IG**, **GM**, **PM**); 7.5  $\mu$ m (**LO**); 5  $\mu$ m (**LI**); 2.5  $\mu$ m (**em**).



**Figure 5.** *Rhyncaphytoptus fuyuniensis* sp. n.: **A–D** Frontal lobe **E** Tibia and tarsus of Leg I **F** Female internal genitalia.

usual series of setae. **Leg I** 42 (41–43), femur 13 (12–14), basiventral femoral setae (*bv*) 13 (13–15); genu 7 (6–7), antaxial genual setae (*l''*) 23 (23–25); tibia 10 (10–11), paraxial tibial setae (*l'*) 7 (7–8), located at 1/3 from dorsal base; tarsus 8 (7–8), setae *ft'* 20 (18–20), setae *ft''* 26 (23–26), seta *u'* 5 (4–5); tarsal empodium (*em*) 8 (7–8), simple, 10-rayed, tarsal solenidion ( $\omega$ ) 8 (8–9), rod-like. **Leg II** 39 (38–40), femur 13 (12–13), basiventral femoral setae (*bv*) 14 (14–16); genu 6 (5–6), antaxial genual setae (*l''*) 9 (9–11); tibia 8 (7–9); tarsus 8 (8–9), setae *ft'* 10 (9–11), setae *ft''* 30 (26–30), seta *u'* 5 (4–5); tarsal empodium (*em*) 8 (7–8), simple, 10-rayed, tarsal solenidion ( $\omega$ ) 10 (10–11), rod-like. **Opisthosoma** dorsally arched, 25 (20–25) dorsal annuli, 92 (90–104) microtuberculated ventral annuli; the anterior dorsal annuli smooth (for about 5/6 of them), the anterior ventral annuli with circled microtubercles (for about 2/3 of them), the posterior part of dorsal and ventral annuli with linear and spiny microtubercles. Setae *c* 2 13 (12–14) on ventral annulus 19 (17–21), 59 (53–61) apart; setae *d* 51 (46–51) on ventral annulus 37 (36–42), 45 (41–45) apart; setae *e* 26 (26–29) on ventral annulus 55 (53–64), 25 (24–26) apart; setae *f* 30 (27–30) on 7th ventral annulus from rear, 21 (20–22) apart. Setae *h* 1 3 (3–4), *h* 2 75 (70–79). **Genital coverflap** 15 (14–16), 30 (28–30) wide, coverflap with many granules basally, proximal setae on coxisternum III (*3a*) 14 (13–14), 20 (19–21) apart.

**MALE** (n=5). Similar in shape and prodorsal shield arrangement to female, 202–243. Prodorsal shield 22–25 without the frontal lobe length, 45–48 wide; scapular setae *sc* 40–46, 27–30 apart. Opisthosoma dorsally with 19–21 annuli; ventrally with 78–85 annuli, dorsal and ventral microtubercles are similar to females. Male genitalia 21–22 wide, setae *3a* 12–13, 18–20 apart.

**Type host plant.** *Cotoneaster ignavus* E. L. Wolf (Rosaceae).

**Relation to the host plant.** Vagrant on leaves; no apparent damage was observed.

**Type locality.** Fuyun county, Xinjiang Uygur Autonomous Region, China (47°17'39"N, 89°58'26"E), elevation 1,359 m; 15 August 2014, coll. Ji-Wei Li.

**Type material.** Holotype: female (slide number SHZU *Rhyncaphytoptus* 7.1, marked Holotype). Paratypes: 12 females and 15 males mounted on 27 separate microscope slides.

**Etymology.** The specific designation *fuyuniensis* comes from the name of location, Fuyun, where the new species was collected.

**Differential diagnosis.** This species is similar to *Rhyncaphytoptus buxifoliae* Song, Xue & Hong, 2009, from *Cotoneaster buxifolius* Lindl. (Rosaceae), but can be differentiated from the latter by median line very short, on 1/5 anterior part of prodorsal shield (prodorsal shield with incomplete median line on posterior 1/2 in *R. buxifoliae*), scapular tubercles small (scapular tubercles 5–13 long in *R. buxifoliae*) and with a long and flexible frontal lobe (lack a distinct, long frontal lobe in *R. buxifoliae*).

## Acknowledgements

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# Four new species and additional records of *Domene* and *Lathrobium* from the Dayao Mountains, southern China

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## Abstract

Material of the paederine genera *Domene* Fauvel, 1873 and *Lathrobium* Gravenhorst, 1802 from the Dayao Mountains, southern China, is examined. Eight species are identified, three of them described previously and five undescribed. Four species are described and illustrated for the first time: *Domene hei* Peng & Li, **sp. n.**, *Lathrobium jinxiuense* Peng & Li, **sp. n.**, *L. kuan* Peng & Li, **sp. n.** and *L. leii* Peng & Li, **sp. n.** One probably undescribed species of *Lathrobium* remains unnamed.

## Keywords

Coleoptera, Staphylinidae, Paederinae, *Domene*, *Lathrobium*, new species, additional records, Dayao Mountains, China

## Introduction

Sixteen species of Lathrobiina have been reported from Guangxi (Assing 2012a, b, 2013a, 2014a, b; Feldmann et al. 2014; Li et al. 2013; Li et al. 2013a, b; Lü and Li 2014; Peng et al. 2012, 2013a, 2014a). One species of *Lobrathium* Mulsant & Rey, 1878 and two micropterous species of *Lathrobium* were previously recorded from Dayao Mountains: *Lobrathium fuscoguttatum* Li, Dai & Li, 2013 (Yinshan Station),

*Lathrobium shengtangshanense* Peng & Li, 2012 (Shengtang Shan) and *L. dayaoshanense* Peng & Li, 2012 (Dayaoshan Nature Reserve), but no species of *Domene* have been reported from this mountain range.

Covering an area of 249.07 km<sup>2</sup>, the Dayao Mountains are situated in central Guangxi, southern China. The highest peak is the Shengtang Shan at 1,979 m. According to Deng (1984) two vertical zones of forest vegetation can be distinguished: an evergreen broad-leaved forest zone at elevations below 1,300 m and a mixed broad-leaved and coniferous forest zone at altitudes above 1,300 m.

In 2011 and 2014, Jia-Yao Hu, Zi-Wei Yin, Xiao-Bin Song, Yi-Ming Yu, Zhu-Qi Yan and the first author made two collecting trips to Dayao Mountains, where they collected numerous *Domene* and *Lathrobium* specimens. Eight species were identified, four of which are described for the first time.

## Material and methods

The following abbreviations are used in the text, with all measurements in millimeters:

Body length (BL) from the anterior margin of the mandibles (in resting position) to the abdominal apex; length of forebody (FL) from the anterior margin of the mandibles to the posterior margin of the elytra; head length (HL) from the anterior margin of the frons to the posterior margin of the head; head width (HW): maximum width of head; length of antenna (AnL); length of pronotum (PL) along midline; maximum width of pronotum (PW); elytral length (EL) at the suture from the apex of the scutellum to the posterior margin of the elytra (at the sutural angles); length of aedeagus (AL) from the apex of the ventral process to the base of the aedeagal capsule.

The type material is deposited in the Insect Collection of Shanghai Normal University, Shanghai, China (SNUC).

## Results

### *Domene (Macromene) hei* Peng & Li, sp. n.

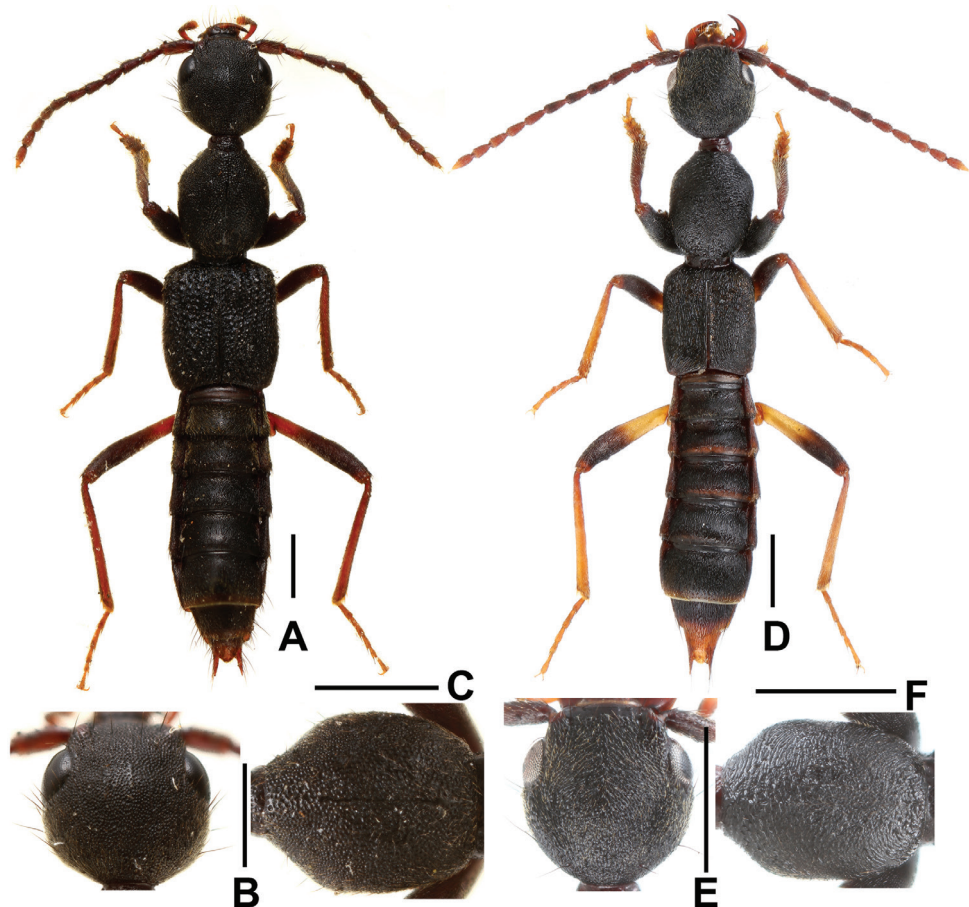
<http://zoobank.org/0452270A-1DEF-406F-8520-97BEB541A7AA>

Figs 1A–C, 2

**Type material.** HOLOTYPE: ♂, labelled ‘China: Guangxi Prov., Jinxiu Hsien, Shengtang Shan, 23°57'37"N, 110°06'46"E, 1300 m, 21.VII.2014, Peng, Song, Yan & Yu leg.’ (SNUC). PARATYPES: 2 ♀♀, same data, but ‘23°59'N, 110°06'E, 1200–1400 m, 25.VII.2011, Peng, Hu & Yin leg.’ (SNUC).

**Description.** Measurements (in mm) and ratios: BL 10.23–10.66, FL 5.67–5.84, HL 1.54–1.57, HW 1.44–1.51, AnL 3.56–3.67, PL 1.67–1.74, PW 1.39–1.44, EL 1.41–1.48, AL 1.07, HL/HW 1.04–1.07, HW/PW 1.04–1.06, HL/PL 0.90–0.92, PL/PW 1.20–1.22, EL/PL 0.84–0.86.





**Figure 1.** *Domene hei* (A–C) and *D. chenae* (D–F). A, D habitus B, E head C, F pronotum. Scale bars: 1.0 mm.

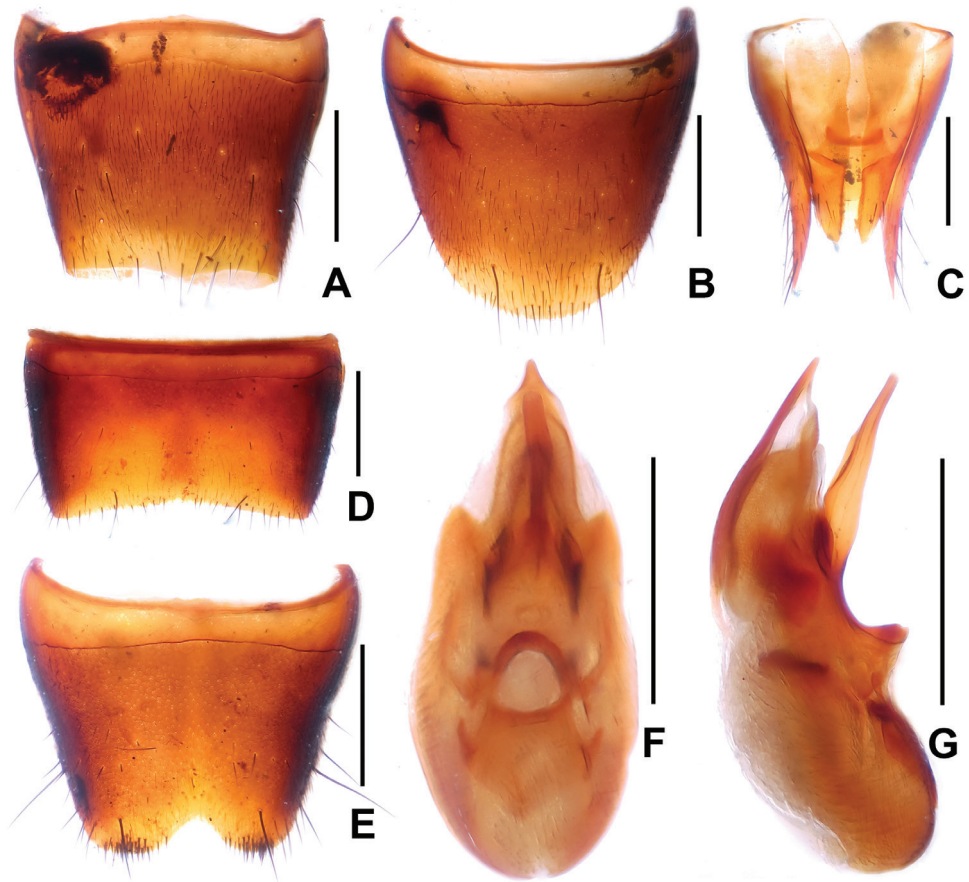
Habitus as in Fig. 1A. Body black; legs blackish brown to brown; antennae dark brown to brown.

Head (Fig. 1B) orbicular, widest behind eyes; punctuation coarse, umbilicate and dense, interstices forming narrow ridges; antenna slender.

Pronotum (Fig. 1C) somewhat narrower than head, widest in the middle; lateral margins convex in dorsal view; punctuation similar to that of head; midline with rudiment of a fine glossy line.

Each elytron with more or less irregular longitudinal narrowly elevated ridges; suture elevated in posterior two thirds; macropunctuation coarse and partly somewhat seriate; interstices with irregular micropunctuation. Hind wings reduced. Protarsomeres I–IV distinctly dilated.

Abdomen with fine and dense punctuation on tergites III–VIII; posterior margin of tergite VIII weakly convex (Fig. 2A); interstices with distinct microreticulation; posterior margin of tergite VII with palisade fringe.



**Figure 2.** *Domene hei*. **A** female tergite VIII **B** female sternite VIII **C** female tergites IX–X. **D** male sternite VII **E** male sternite VIII **F** aedeagus in ventral view **G** aedeagus in lateral view. Scale bars: 0.5 mm.

Male. Sternites III–VI unmodified; sternite VII (Fig. 2D) without modified pubescence, posterior margin broadly concave; sternite VIII (Fig. 2E) with narrow and shallow postero-median impression, posterior excision small, on either side of posterior excision with cluster of short dark setae; aedeagus as in Figs 2F, G; ventral process slender; dorsal plate with long sclerotized apical portion and short basal portion.

Female. Posterior margin of sternite VIII (Fig. 2B) broadly convex; genital segments (Fig. 2C) with a slender sclerotized structure.

**Comparative notes.** Based on more or less irregular longitudinal elevations of elytra, the derived morphology of the aedeagus and particularly on the shapes and chaetotaxy of the male sternites VIII, *D. hei* belongs to the *D. scabripennis* species group. It is distinguished from other species of this group by the coloration of legs, the fine glossy line on the pronotum, the shape of the ventral process of the aedeagus and the slender sclerotized structure in the female genital segments. For illustrations of

the species of the *D. scabripennis* species group see Assing (in press a) and Assing and Feldmann (2014).

**Etymology.** The species is dedicated to Wei-Jun He, specialist of Phasmatodea, who supported us on our field trips.

**Distribution and natural history.** The type locality is situated in the Shengtang Shan to the southwest of Jinxiu, central Guangxi. The specimens were sifted from leaf litter in a rhododendron forest at altitudes of 1,200–1,400 m, together with *Lathrobium shengtangshanense* Peng & Li, 2012.

***Domene (Macromene) chenae* Peng & Li, 2014**

Figs 1D–F, 3

**Material studied.** China: Guangxi: 1 ♂, Jinxiu Hsien, Shengtang Shan, 23°57'37"N, 110°06'46"E, 1300 m, 20.VII.2014, Peng, Song, Yan & Yu leg. (SNUC); 2 ♀♀, Jinxiu Hsien, Shengtang Shan, 23°59'N, 110°06'E, 1200–1400 m, 25.VII.2011, Peng, Hu & Yin leg. (SNUC).

**Comment.** *Domene chenae* was previously known only from Anjiangping in Guangxi. The above material indicates that this species is subject to considerable intraspecific variation. According to the description and illustrations provided by Feldmann et al. (2014), the distal halves of metafemora are brown (blackish brown in the material listed above, Fig. 1D) and the ventral process of aedeagus is slender (stouter in the material listed above, Figs 3F–G). For illustrations of the material from Shengtang Shan and of the type material of *D. chenae* see Figs 1D–F, 3 and Feldmann et al. (2014), respectively.

***Lathrobium jinxiuense* Peng & Li, sp. n.**

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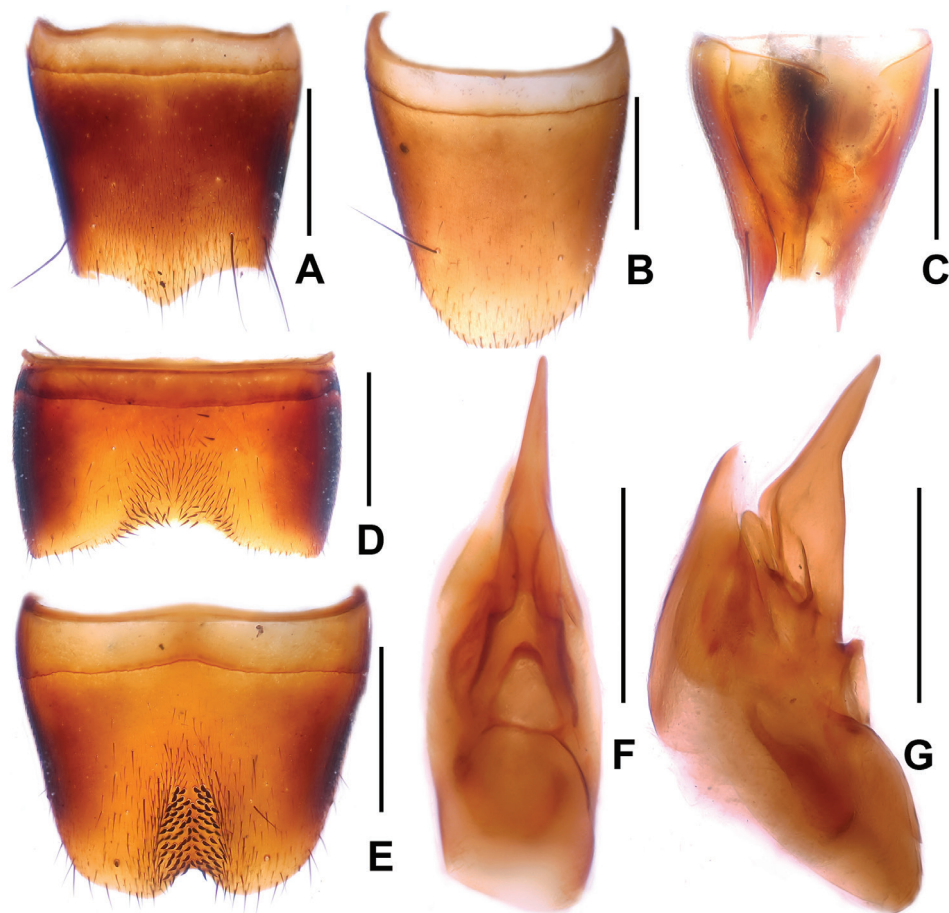
Figs 4A, 5

**Type material.** HOLOTYPE: ♂, labelled 'China: Guangxi Prov., Jinxiu Hsien, Qigongli, 24°09'07"N, 110°12'29"E, 1300 m, 16.VII.2014, Peng, Song, Yan & Yu leg.' (SNUC). Paratypes: 1 ♀, same label data as holotype (SNUC).

**Description.** Measurements (in mm) and ratios: BL 6.85–6.91, FL 2.96–3.06, HL 0.78–0.85, HW 0.80–0.84, AnL 1.58–1.63, PL 1.05–1.09, PW 0.84–0.87, EL 0.59–0.63, AL 0.85, HL/HW 0.98–1.01, HW/PW 0.95–0.97, HL/PL 0.74–0.78, PL/PW 1.25, EL/PL 0.56–0.58.

Habitus as in Fig. 4A. Body brown, legs yellowish brown, antennae light brown.

Head approximately as long as broad; punctation moderately coarse and sparse, sparser in median dorsal portion; interstices with distinct microreticulation; eyes very small and composed of approximately 20 ommatidia.



**Figure 3.** *Domene chenaе* (from Shengtang Shan). **A** female tergite VIII **B** female sternite VIII **C** female tergites IX–X **D** male sternite VII **E** male sternite VIII **F** aedeagus in ventral view **G** aedeagus in lateral view. Scale bars: 0.5 mm.

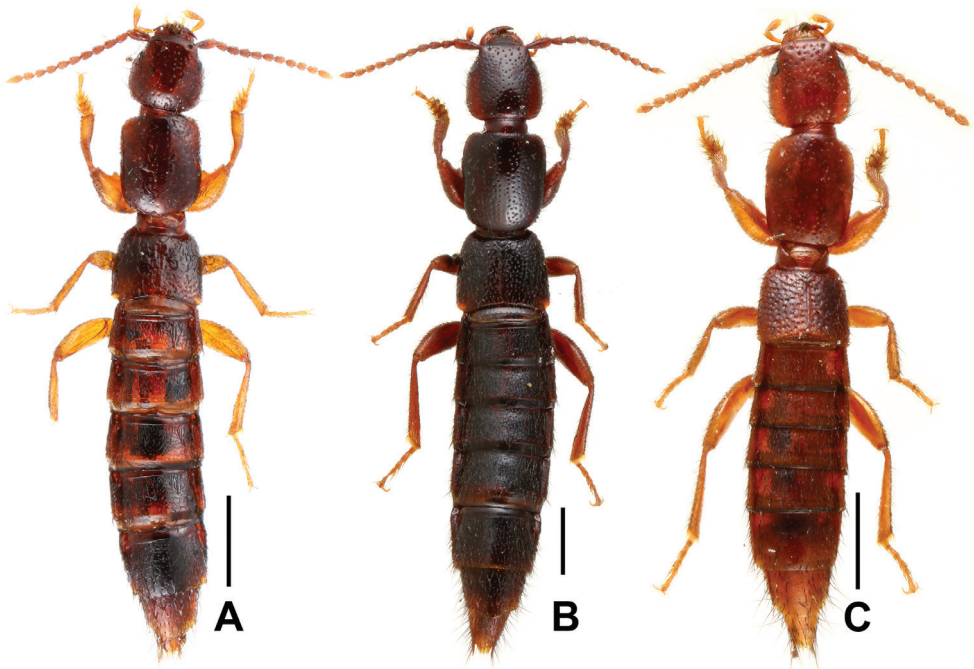
Pronotum nearly parallel-sided; punctation similar to that of head; impunctate midline broad; interstices without microsculpture.

Elytral punctation moderately dense, shallow and ill-defined. Hind wings completely reduced. Protarsi with weakly pronounced sexual dimorphism.

Abdomen with fine and moderately dense punctation, that of tergite VII somewhat sparser than that of anterior tergites; interstices with shallow microsculpture; posterior margin of tergite VII without palisade fringe; tergite VIII without sexual dimorphism, posterior margin (Fig. 5A) obtusely angled in the middle.

Male. Sternites III–VI unmodified; sternite VII (Fig. 5D) with very shallow postero-median impression, this impression without distinctly modified setae, posterior





**Figure 4.** Habitus of *Lathrobium* spp., **A** *L. jinxiuense* **B** *L. kuan* **C** *L. leii*. Scale bars: 1.0 mm.

margin weakly concave in the middle; sternite VIII (Fig. 5E) with very shallow median impression posteriorly, this impression without distinctly modified setae, posterior excision small and symmetrical; aedeagus as in Figs 5F, G, ventral process nearly symmetrical in ventral view and acute apically; dorsal plate with long and strongly sclerotized apical portion and with moderately sclerotized basal portion; internal sac with single long sclerotized spine.

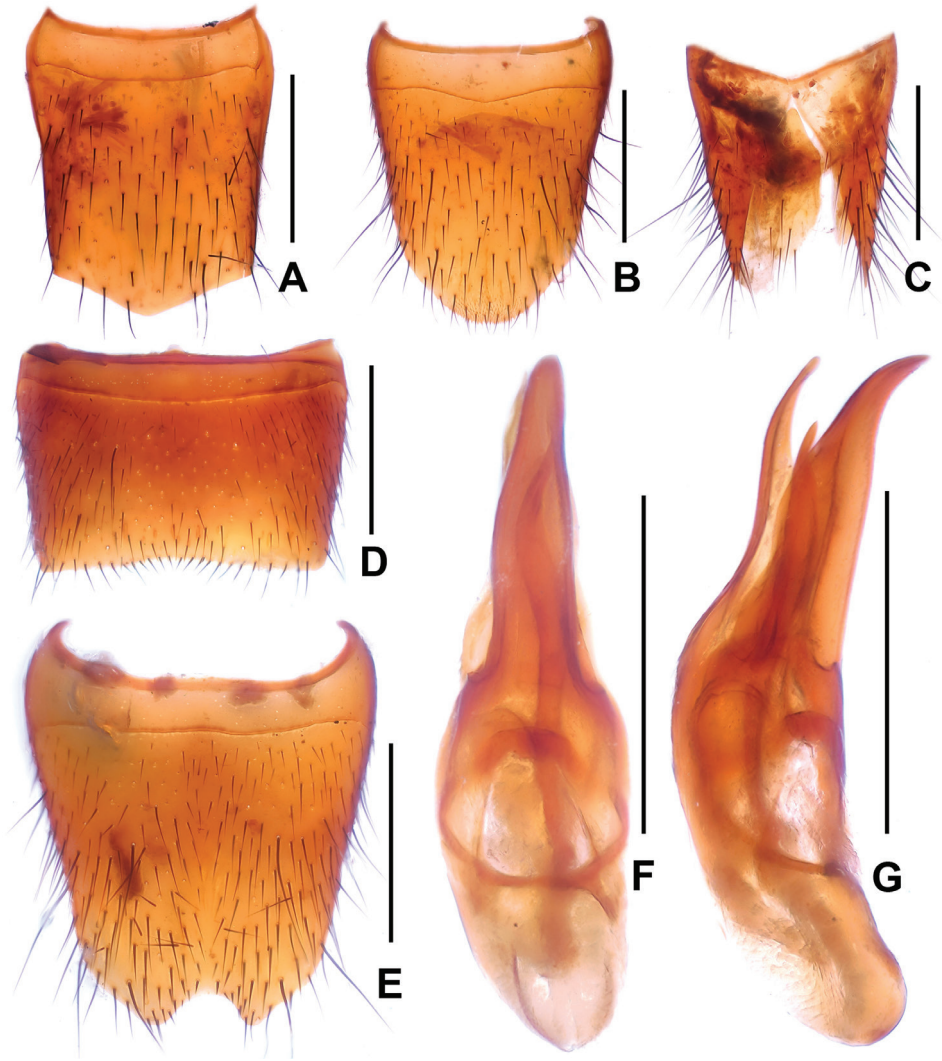
Female. Posterior margin of sternite VIII (Fig. 5B) convex and with moderately dense micropubescence; tergite IX (Fig. 5C) almost completely divided; tergite X (Fig. 5C) nearly reaching anterior margin of tergite IX.

**Comparative notes.** The new species resembles *L. maoershanense* Peng & Li, 2012 in habitus and the similarly derived morphology of the aedeagus, but differs from this species by the smaller posterior excision of the male sternite VIII, the more slender ventral process of the aedeagus and the shape of the female tergite VIII. For illustrations of *L. maoershanense* see Peng et al. (2012).

**Etymology.** The specific epithet is derived from Jinxiu, where the type locality is situated.

**Distribution and natural history.** The type locality is situated in Qigongli to the north of Jinxiu, central Guangxi. The specimens were sifted from leaf litter and humus in a beech forest at an altitude of 1,300 m, together with *L. dayaoshanense* Peng & Li, 2012.





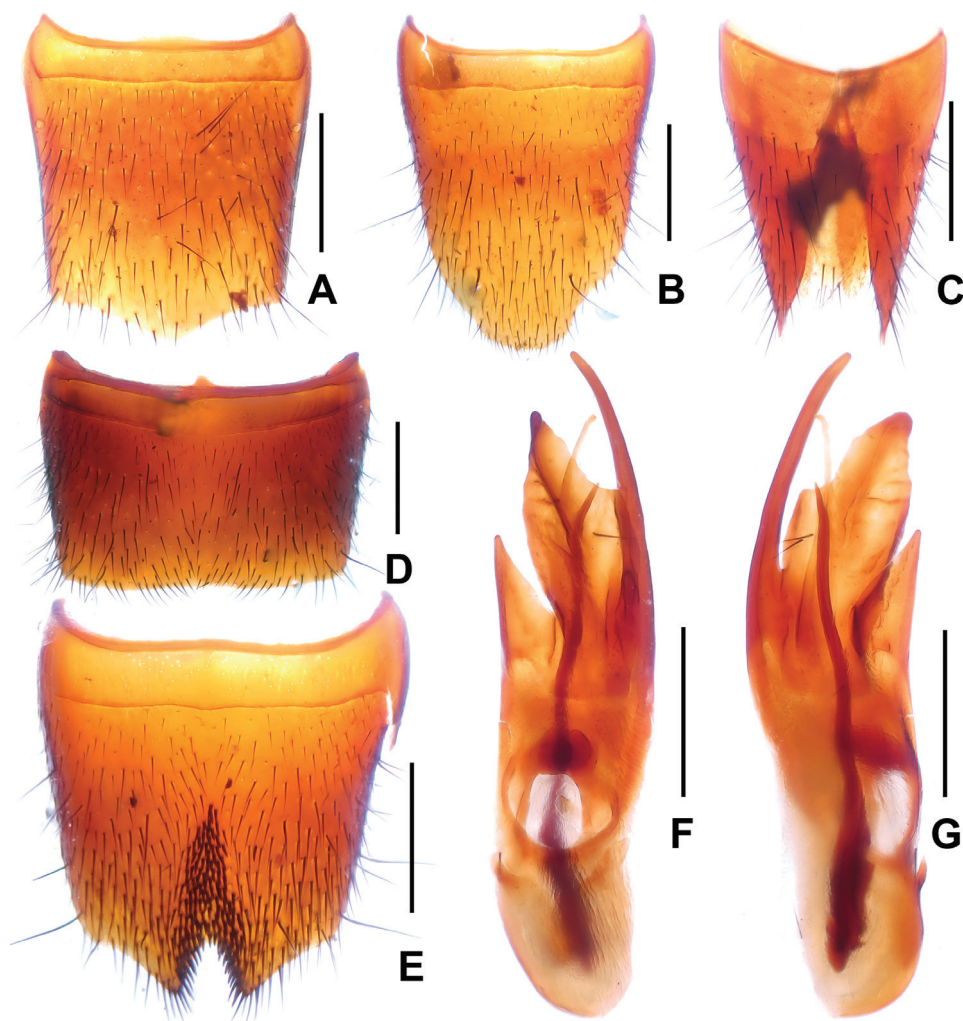
**Figure 5.** *Lathrobium jinxiuense*. **A** female tergite VIII **B** female sternite VIII **C** female tergites IX–X **D** male sternite VII **E** male sternite VIII **F** aedeagus in ventral view **G** aedeagus in lateral view. Scale bars: 0.5 mm.

***Lathrobium kuan* Peng & Li, sp. n.**

<http://zoobank.org/48016D68-2999-44B6-868C-A62DCFF3F851>

Figs 4B, 6

**Type material.** HOLOTYPE: ♂, labelled 'China: Guangxi Prov., Jinxiu Hsien, Shengtang Shan, 23°59'32"N, 110°06'26"E, 1160 m, 23.VII.2014, Peng, Song, Yan & Yu leg.' (SNUC). Paratypes: 1 ♂, 1 ♀, same label data as holotype (SNUC).



**Figure 6.** *Lathrobium kuan*. **A** female tergite VIII **B** female sternite VIII **C** female tergites IX-. **D** male sternite VII **E** male sternite VIII **F** aedeagus in ventral view **G** aedeagus in lateral view. Scale bars: 0.5 mm.

**Description.** Measurements (in mm) and ratios: BL 9.17–9.40, FL 4.00–4.11, HL 1.05–1.11, HW 1.05–1.15, AnL 2.00–2.15, PL 1.48–1.51, PW 1.18–1.26, EL 0.78–0.83, AL 1.90–1.93, HL/HW 0.97–1.00, HW/PW 0.89–0.91, HL/PL 0.71–0.74, PL/PW 1.20–1.25, EL/PL 0.53–0.55.

Habitus as in Fig. 4B. Body dark brown, legs and antennae brown.

Head approximately as long as broad; punctation coarse and moderately dense, somewhat sparser in median dorsal portion; interstices with shallow microreticulation; eyes small and composed of approximately 30 ommatidia.

Pronotum nearly parallel-sided; punctation similar to that of head; impunctate midline moderately broad; interstices glossy and without microsculpture.

Elytral punctation dense and well-defined. Hind wings completely reduced. Pro-tarsi without sexual dimorphism.

Abdomen with fine and dense punctation, that of tergite VII sparser than that of anterior tergites; interstices with distinct microsculpture; posterior margin of tergite VII without palisade fringe; tergite VIII without sexual dimorphism, posterior margin (Fig. 6A) obtusely angled in the middle.

Male. Sternites III–VI unmodified; sternite VII (Fig. 6D) with very shallow postero-median impression, this impression with weakly modified setae, posterior margin nearly truncate; sternite VIII (Fig. 6E) with very narrow median impression posteriorly, this impression with numerous short dark setae, posterior excision V-shaped and symmetrical; aedeagus as in Figs 6F, G; ventral process asymmetrical, broad and furcate; internal sac with a long strong sclerotized spine and a shorter weakly sclerotized spine.

Female. Posterior margin of sternite VIII (Fig. 6B) strongly convex; tergite IX (Fig. 6C) almost completely divided; tergite X (Fig. 6C) nearly reaching anterior margin of tergite IX.

**Comparative notes.** Based on the modifications of the male sternite VIII, the furcate ventral process of the aedeagus, the presence of long sclerotized spines in the internal sac of the aedeagus, and the divided female tergite IX, *Lathrobium kuan* belongs to the *L. fissispinosum* group, which was previously known from Shaanxi, Gansu, Hubei, Guizhou and Sichuan. The new species is distinguished from the other representatives of this group by the chaetotaxy and shapes of the male sternites VII and VIII, the strongly asymmetrical ventral process and the presence of two (rather than one) sclerotized spines in the internal sac of the aedeagus. For illustrations of the species of the *L. fissispinosum* group see Assing (2013b, in press b) and Peng et al. (2013b, 2014b).

**Etymology.** The specific name is the Chinese adjective “kuan” (broad). It refers to the broad ventral process of the aedeagus of this species, when compared with the other species known from Dayao Mountains.

**Distribution and natural history.** The type locality is situated in the Shengtang Shan to the southwest of Jinxiu, central Guangxi. The specimens were sifted from leaf litter in a beech forest at an altitude of 1,160 m.

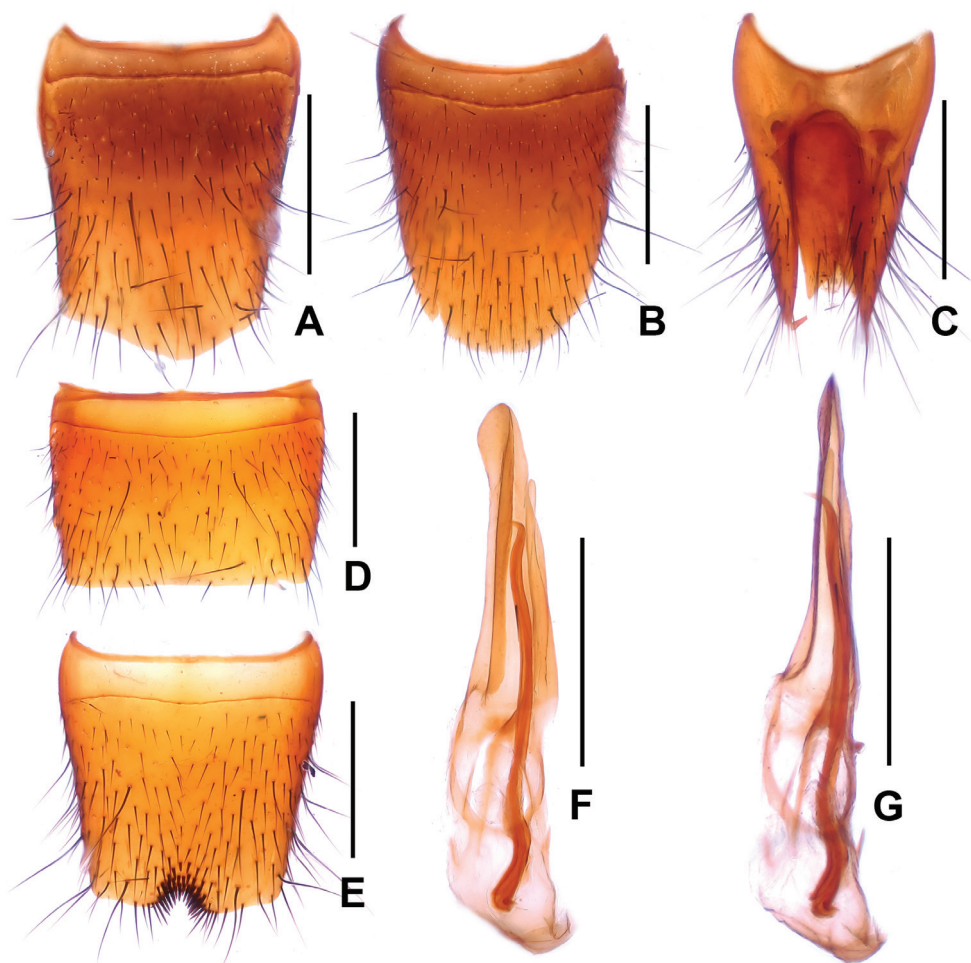
### *Lathrobium leii* Peng & Li, sp. n.

<http://zoobank.org/135F9705-E94F-4E4D-B93D-0F663999A135>

Figs 4C, 7

**Type material.** HOLOTYPE: ♂, labelled ‘China: Guangxi Prov., Jinxiu Hsien, Shengtang Shan, 23°57'37"N, 110°06'46"E, 1300 m, 21.VII.2014, Peng, Song, Yan & Yu leg.’ (SNUC). Paratypes: 1 ♀, same label data as holotype (SNUC).

**Description.** Measurements (in mm) and ratios: BL 6.61–6.67, FL 3.11–3.34, HL 0.83–0.87, HW 0.82–0.88, AnL 1.66–1.68, PL 1.12–1.14, PW 0.85–0.90, EL 0.65–0.68, AL 1.26, HL/HW 0.99–1.01, HW/PW 0.96–0.98, HL/PL 0.74–0.76, PL/PW 1.27–1.32, EL/PL 0.58–0.60.



**Figure 7.** *Lathrobium leii*. **A** female tergite VIII **B** female sternite VIII **C** female tergites IX–X **D** male sternite VII **E** male sternite VIII **F** aedeagus in ventral view **G** aedeagus in lateral view. Scale bars: 0.5 mm.

Habitus as in Fig. 4C. Body light brown, legs yellowish brown, antennae light brown.

Head approximately as long as broad; punctation coarse and moderately sparse, distinctly sparser in median dorsal portion; interstices with shallow microreticulation; eyes small and composed of approximately 35 ommatidia.

Pronotum with weakly convex lateral margins in dorsal view; punctation similar to that of head; impunctate midline broad; interstices without microsculpture.

Elytral punctation moderately dense and defined. Hind wings completely reduced. Protarsi without pronounced sexual dimorphism.

Abdomen with fine and moderately dense punctation, that of tergite VII somewhat sparser than that of anterior tergites; interstices with shallow microsculpture; posterior



margin of tergite VII without palisade fringe; tergite VIII without sexual dimorphism, posterior margin (Fig. 7A) asymmetrical obtusely angled.

Male. Sternites III–VI unmodified; sternite VII (Fig. 7D) with truncate posterior margin and without distinctly modified setae; sternite VIII (Fig. 7E) with very shallow postero-median impression, this impression with short dark setae posteriorly, posterior excision small and weakly asymmetrical; aedeagus as in Figs 7F, G; ventral process long and slender; dorsal plate with very long moderately sclerotized apical portion and short weakly sclerotized basal portion; internal sac with a long sclerotized spine.

Female. Posterior margin of sternite VIII (Fig. 7B) convex and with moderately dense micropubescence; tergite IX (Fig. 7C) without median suture; tergite X (Fig. 7C) 4.7 times as long as antero-median portion of tergite IX.

**Comparative notes.** Based on the different general morphology of the aedeagus, *L. leii* represents a different lineage than the other species recorded from Dayao Mountains. It is additionally distinguished from them by smaller body size, yellowish brown legs, the chaetotaxy of the male sternite VIII, and a slender aedeagus with a long sclerotized spine.

**Etymology.** The species is dedicated to Yu-Yang Lei, who supported us on our field trips.

**Distribution and natural history.** The type locality is situated in the Shengtang Shan to the southwest of Jinxiu, central Guangxi. The specimens were sifted from leaf litter and humus in a rhododendron forest at an altitude of 1,300 m, together with *L. shengtangshanense*.

### *Lathrobium dayaoshanense* Peng & Li, 2012

**Material studied.** China: Guangxi: 2 ♂♂, 3 ♀♀, Jinxiu Hsien, Houzi Shan, Yinshan Station, 24°10'01"N, 110°14'38"E, 1200 m, 10.VII.2014, Peng, Song, Yan & Yu leg. (SNUC); 1 ♀, Jinxiu Hsien, Changtanghe, 24°16'00"N, 110°13'29"E, 860 m, 15.VII.2014, Peng, Song, Yan & Yu leg. (SNUC); 1 ♀, Jinxiu Hsien, Qigongli, 24°09'07"N, 110°12'29"E, 1300 m, 16.VII.2014, Peng, Song, Yan & Yu leg. (SNUC); 1 ♂, Jinxiu Hsien, Laoshan, 24°07'02"N, 110°11'51"E, 950 m, 26.VII.2014, Peng, Song, Yan & Yu leg. (SNUC).

**Comment.** The above material was collected in several localities in the region to the north, east, and northwest of Jinxiu, central Guangxi. The specimens were sifted from deep leaf litter layers in mixed forests at altitudes of 860–1,300 m.

### *Lathrobium shengtangshanense* Peng & Li, 2012

**Material studied.** China: Guangxi: 5 ♂♂, 4 ♀♀, Jinxiu Hsien, Shengtang Shan, 23°57'37"N, 110°06'46"E, 1300 m, 21.VII.2014, Peng, Song, Yan & Yu leg. (SNUC).

**Comment.** *Lathrobium shengtangshanense* has been recorded only from the Shengtang Shan in Guangxi.



***Lathrobium* sp.**

**Material studied.** China: Guangxi: 1 ♀, Jinxiu Hsien, Shiliugongli, 24°08'25"N, 110°15'38"E, 960 m, 13.VII.2014, Peng, Song, Yan & Yu leg. (SNUC).

**Comment.** The above micropterous female represents an undescribed species distinguished from the other species known from Dayao Mountains by somewhat smaller body size, the slender pronotum and the female secondary sexual characters.

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# A review of Norwegian *Gymnometriocnemus* (Diptera, Chironomidae) including the description of two new species and a new name for *Gymnometriocnemus volitans* (Goetghebuer) sensu Brundin

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## Abstract

Examination of the syntypes of *Metriocnemus volitans* Goetghebuer, 1940 revealed that these specimens belong to the genus *Chaetocladius* and are not con-specific with *Gymnometriocnemus volitans* (Goetghebuer, 1940) sensu Brundin (1956) and Sæther (1983). A literature search showed that *Gymnometriocnemus kamimegavirgus* Sasa & Hirabayashi, 1993 fits well with the species figured and diagnosed by Brundin (1956) as well as with specimens of this species from Norway. We present arguments for *Chaetocladius volitans* (Goetghebuer) comb. n. and for the use of *G. kamimegavirgus* for *G. volitans* sensu Brundin. In addition, we provide DNA barcode data that indicate the presence of at least seven *Gymnometriocnemus* species in Norway of which six are collected as male adults. Two of these, *Gymnometriocnemus* (*Gymnometriocnemus*) *pallidus* **sp. n.** and *Gymnometriocnemus* (*Raphidocladius*) *autumnalis* **sp. n.** are regarded as new to science and diagnosed based on adult male morphology and DNA barcodes. The species *Gymnometriocnemus* (*Gymnometriocnemus*) *marionensis* Sæther, 1969 is re-established and a key to all Holarctic species is provided.

## Keywords

Chironomidae, Orthoclaadiinae, DNA barcodes, new species, taxonomy, non-biting midges

## Introduction

The orthoclad genus *Gymnometriocnemus* was suggested by Goetghebuer (1932), but without designation of a type species. Edwards (1932) designated *Gymnometriocnemus subnudus* (Edwards, 1929) as type species and made the name available according to the International Code of Zoological Nomenclature. Edwards is thus credited authorship of the genus (Spies and Sæther 2004). The genus was revised by Sæther (1983) who distinguished two subgenera based on adult male and pupal morphology. There currently are 15 *Gymnometriocnemus* species recognized (Ashe and O'Connor 2012). Larvae of most species have previously been regarded as terrestrial (Andersen et al. 2013), but there is evidence for at least semi-aquatic lifestyle in larvae of subgenus *Raphidocladius* from Norway (own data).

*Metriocnemus volitans* was described by Goetghebuer (1940) based on material collected in Abisko, Sweden by Thienemann in 1939. The species was placed in genus *Gymnometriocnemus* by Brundin (1956), probably without consulting the type material. Since then, chironomid workers have used Brundin's interpretation of this species and even Sæther (1983) in his revision of Holarctic *Gymnometriocnemus* relied on the characters presented by Brundin (1956). He stated explicitly, however, that he had not examined Goetghebuer's types.

Sæther (1983) erected the subgenus *Raphidocladius* for *Gymnometriocnemus* species possessing an extremely long virga with needle-like sclerotization in the adult males. The species *G. brumalis* (Edwards, 1929) and *G. acigus* Sæther, 1983 were listed as members of the group, while *G. volitans* was considered a possible member since immatures were unknown and virga had not been examined (Sæther 1983). Two species in this subgenus were later described from Japan, but both *Gymnometriocnemus kamimegavirgus* Sasa & Okazawa, 1994 and *G. tairaprimus* Sasa & Okazawa, 1994 were diagnosed without considering *G. volitans* sensu Brundin (1956) and Sæther (1983) (Sasa and Hirabayashi 1993; Sasa and Okazawa 1994). Cranston and Oliver (1988) argued for a synonymy of the type species for *Raphidocladius*, *G. (R.) acigus*, with *G. (R.) brumalis* claiming that the characters used by Sæther (1983) to separate these species were not reliable and interspecifically variable even within the same population. This leaves four valid species in subgenus *Raphidocladius* in the World Catalogue of Chironomidae: *G. (R.) brumalis*, *G. (R.) kamimegavirgus*, *G. (R.) tairaprimus* and *G. (R.) volitans* (Ashe and O'Connor 2012).

Subgenus *Gymnometriocnemus* is characterised by a short virga and a weakly developed crista dorsalis in the adult male hypopygium (Sæther 1983). Cranston and Oliver (1988) synonymized *Gymnometriocnemus marionensis* Sæther, 1983 with *G. subnudus*, leaving 11 valid species in subgenus *Gymnometriocnemus* the World Catalogue of Chironomidae: *G. (G.) ancudensis* (Edwards, 1931), *G. (G.) benoiti* (Freeman, 1956), *G. (G.) brevitarsis* (Edwards, 1929), *G. (G.) johanasecundus* Sasa & Okazawa, 1994, *G. (G.) lobifer* (Freeman, 1956), *G. (G.) longicostalis* (Edwards, 1931), *G. (G.) subnudus*, *G. (G.) terrestris* Krüger, Thienemann & Goetghebuer, 1941, *G. (G.) mahensis* (Kieffer, 1911) *G. (G.) nitidulus* (Skuse, 1889) and *G. (G.) wilsoni* Freeman, 1961 (Ashe and O'Connor 2012).

DNA barcoding using partial cytochrome c oxidase subunit 1 sequences (COI) (Hebert et al. 2003) has been shown to perform well for species identification of many Chironomidae groups (Brodin et al. 2012; Ekrem et al. 2010; Ekrem et al. 2007; Stur and Ekrem 2011). We have therefore had a strong focus on developing a barcode library of chironomids for use in taxonomy, life stage association and future environmental monitoring in Norway. In connection with biosurveillance projects in central, eastern and northern Norway, we have barcoded adult specimens of *Gymnometriocnemus* species from various habitats.

The motivation for this study was to clarify the identity of *Gymnometriocnemus volitans* (Goetghebuer), describe hitherto unknown species of *Gymnometriocnemus* and to present the DNA barcodes of Norwegian *Gymnometriocnemus* as a resource for future studies of this genus.

## Material and methods

We examined eight syntypes of *Metriocnemus volitans* Goetghebuer from the Royal Belgian Institute of Natural Sciences (RBINS), five male and three female adults mounted between cellophane strips on two separate pins. Both pins bear the label “Env. d. Abisko, Aout 1939, Dr. Thienemann” and “*Metriocnemus volitans* n sp” (Figs 1A-B). Thienemann (1941) uses Goetghebuer’s name and state that only adults were collected in a groundwater spring area at the beginning of the Njúlja road 29.viii.1939.

We also examined the male holotype and a male paratype of *Gymnometriocnemus* (*Raphidocladus*) *acigus* Sæther, 1983 (University Museum of Bergen, Norway (ZMBN)) and two male syntypes and a female syntype of *Gymnometriocnemus* (*Gymnometriocnemus*) *terrestris* (RBINS).

Additional material of *Gymnometriocnemus* was collected using a variety of methods in different biosurveillance projects: Malaise traps, sweep netting and fogging of oak canopies (Supplementary file 1). This material is deposited in the NTNU University Museum insect collection (NTNU-VM). One to three legs were dissected off the specimens and submitted to the Canadian Centre for DNA Barcoding. Metadata, photos, sequences and trace-files are available in the Barcode of Life Data Systems (BOLD, [www.boldsystems.org](http://www.boldsystems.org)) through the dataset DS-GYMNO with doi: 10.5883/DS-GYMNO. GenBank accessions are given in Supplementary file 1.

DNA extracts and partial COI gene sequences were generated using standard primers and bi-directional Sanger sequencing with BigDye 3.1 termination at the Canadian Centre for DNA Barcoding in Guelph. Protocols and original trace-files are available through the dataset DS-GYMNO in BOLD. Alignments were done on amino acid sequences and was trivial as indels were absent; only sequences > 300bp were used in the final alignment. The taxon ID-tree was generated using neighbour joining analysis and 1000 bootstrap replicates on Kimura 2-parameter (K2P) genetic distances in MEGA 6 (Tamura et al. 2013).



**Table 1.** Antennal ratios (AR) and fore leg ratios (LR<sub>1</sub>) of male *Gymnometriocnemus* from Norway.

Species	AR	LR <sub>1</sub>
<i>G. (Raphidocladus) kamimegavirgus</i>	0.88–1.14, 1.04 [n=5]	0.51–0.52, 0.51 [n=3]
<i>G. (Raphidocladus) brumalis</i>	1.03–1.31, 1.19 [n=5]	0.50–0.53, 0.51 [n=5]
<i>G. (Raphidocladus) autumnalis</i>	0.87–1.00, 0.94 [n=4]	0.56–0.58, 0.57 [n=4]
<i>G. (Gymnometriocnemus) subnudus</i>	1.00–1.08, 1.06 [n=3]	0.58–0.63, 0.61 [n=3]
<i>G. (Gymnometriocnemus) pallidus</i>	1.05–1.10, 1.07 [n=5]	0.62–0.69, 0.65 [n=5]
<i>G. (Gymnometriocnemus) marionensis</i>	1.00–1.08, 1.05 [n=4]	0.60–0.68, 0.65 [n=4]

Morphological terminology and abbreviations follow Sæther (1980). Antennal and fore leg ratios of Norwegian *Gymnometriocnemus* are given in Table 1. Measurements are given as ranges followed by the mean. Anal point lengths were measured from posterior margin of anal tergite to tip of anal point.

## Results and discussion

### *Chaetocladus volitans* (Goetghebuer, 1940), comb. n.

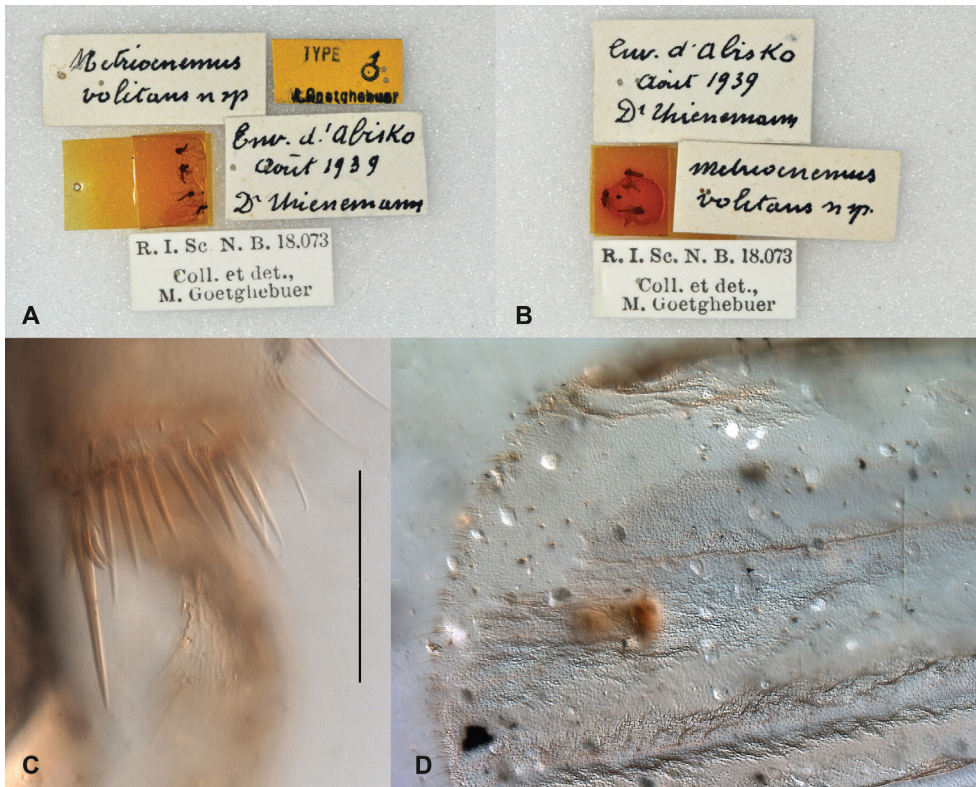
*Metriocnemus volitans* Goetghebuer, 1940: 59.

*Metriocnemus volitans* Goetghebuer in Thienemann (1941: 150, 172).

**Remarks.** Several of the type specimens are quite damaged and many characters are difficult or impossible to observe (Figs 1–2). However, it is obvious that they do not belong to *Gymnometriocnemus*, and the better preserved male specimens show characters that fits the diagnosis of *Chaetocladus*: wings with coarse punctuation and without macrotrichia on the membrane (Fig. 1D), divergent lateral spinules on the tibial spurs (Fig. 1C) and a fore leg ratio of 0.75. We are confident that this species should be placed in *Chaetocladus*, but due to the state of the specimens and the unrevised nature of the genus, we have not attempted to compare *C. volitans* with other species in *Chaetocladus*.

### *Gymnometriocnemus brevitarsis* Edwards, 1932

**Remarks.** We have only seen two females from eastern and central Norway. The two specimens fit Edwards' description for *G. brevitarsis* and represent the first records of this species in Norway. The wing and antenna are photographed (Fig. 3A, B) and leg ratios of fore- mid- and hind legs are measured to be LR<sub>1</sub> 0.41–0.42 [n=2], LR<sub>2</sub> 0.38–0.39 [n=2], LR<sub>3</sub> 0.50–0.53 [n=2] respectively. Only females are known and



**Figure 1.** Syntype specimens of *Metriocnemus volitans*. **A, B** specimens and labels on pins **C** hind tibial comb and spur (scale bar = 50  $\mu$ m) **D** wing tip.

partial COI gene sequences do not reveal close relationships to any other species in *Gymnometriocnemus*. Subgeneric placement of *G. brevitarsis* is therefore not possible.

### *Gymnometriocnemus* (*Gymnometriocnemus*) *marionensis* Sæther, 1969

**Remarks.** Only one specimen of this species from Norway has been available to us. It fits Sæther's description of *G. (G.) marionensis* in having very slightly larger megasetae on the gonostyli than specimens of *G. (G.) subnudus* (Figs 4A, C), but this character is not trustworthy as it is dependent on the orientation of the gonostyli in the slide-mount. The larger anal point will separate *G. (G.) marionensis* (c. 38  $\mu$ m long) from *G. subnudus* (c. 17  $\mu$ m long). Cranston and Oliver (1988) synonymized *G. (G.) marionensis* with *G. (G.) subnudus* doubting the diagnostic value of the size of the megaseta and position of the end of  $R_{2+3}$  (Fig. 5A). We have not examined the types of *G. (G.) marionensis*, but specimens from Michigan and North Carolina (ZMBN) identified by Ole Sæther as belonging to this species. This record of the species is the first from Norway and Europe.



**Figure 2.** Syntype of *Metriocnemus volitans*, hypopygium (scale bar = 100  $\mu$ m).

***Gymnometriocnemus (Gymnometriocnemus) pallidus* sp. n.**

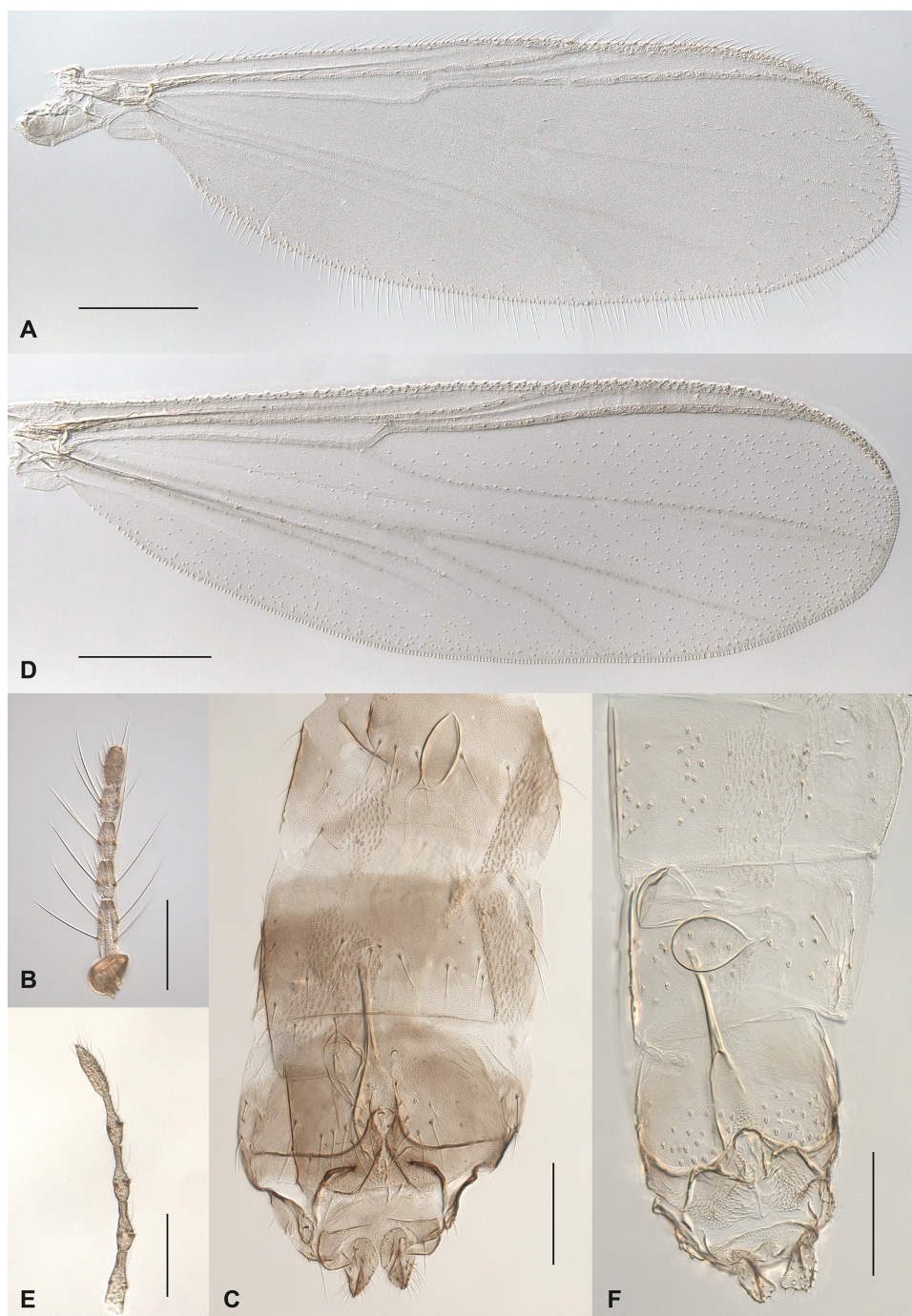
<http://zoobank.org/8C19C165-6923-4A17-9C9D-380DD9413E41>

**Type material.** Holotype: Male adult (NTNU-VM slide 143840), Norway, Hordaland, Kvam, Berge, oak canopy fogging, tree #3, 60.30921°N; 6.16453°E, 23.vi.2011, leg. Karl H. Thunes [BOLD ID: CH-eik131]. 5 Paratypes: 2 male adults as holotype except tree #1 60.314°N; 6.167°E, 21.vi.2011; 1 female adult as holotype except tree #18, 59.201°N; 9.920°E, 5.vii.2012; 2 male adults, Norway, Telemark, Porsgrunn, Brevik, Frierflaune, 59.0579°N; 9.66485°E, Malaise trap, 30.vi–27.vii.2010, leg. Geir Søli.

**Etymology.** The species is named “pallidus”, Latin adjective meaning pale, referring to the conspicuous pale body colour compared to other Holarctic *Gymnometriocnemus*.

**Diagnosis.** *Gymnometriocnemus (G.) pallidus* can be separated from other *Gymnometriocnemus* species by the following combination of characters in the adults: body pale yellow-green; male with short inconspicuous virga, gonostylus with convex outer margin and weakly developed crista dorsalis. Female with setae on most of wing surface, including numerous in cell m; antenna with apical flagellomere pointed and longer than flagellomere 4; genitalia with long rami, about the same length as notum.





**Figure 3.** Female *Gymnometriocnemus*. **A–C** *Gymnometriocnemus brevitarsis* (CH-OSF33): **A** Wing **B** Antenna **C** genitalia **D–F** *Gymnometriocnemus* (*G.*) *pallidus* sp. n. (CH-eik47): **D** Wing **E** Antenna **F** genitalia. Specimen codes in parenthesis correspond to codes in BOLD and in figure 6. Scale bar: 200  $\mu$ m (**A**, **D**); 100  $\mu$ m (**B**, **C**, **E**, **F**).

**Description.** Male adult ( $n = 5$  unless otherwise stated). Wing length 1.21–1.30, 1.28 mm. Colouration pale yellow-green body, legs and antennae; slightly darker bands on scutum; postnotum, dorsal side of head, ventral part of preepisternum pale brown; eyes dark brown.

Head. Antennal ratio in Table 1. Temporal setae 9–10; palp lengths (in  $\mu\text{m}$ ): 25/30/90–100/80–90 (4)/110–115 (4).

Thorax. Anteprepronotum with 2–6, 3 setae. Dorsocentrals 10–17, 13; acrostichals 7–12, 10, minute and difficult to discern; prealars 3–4; scutellars 6–7.

Wing (Fig. 5B). Costa moderately extended, not reaching half way to apex of  $M_{1+2}$ ;  $R_{2+3}$  approaching costa at 1/3 distance between  $R_1$  and  $R_{4+5}$ . Macrotrichia present on membrane in apical half of wing, 0–2 setae in cell m. Veins Sc,  $R_{2+3}$ , M and pseudovein without setae.

Legs. Fore tibia with one spur, 35  $\mu\text{m}$  long; mid tibia with two spurs ca. 20–25  $\mu\text{m}$  long; hind tibia with well-developed comb and 2 spurs, ca. 20 and 35  $\mu\text{m}$  long. Fore leg ratios in Table 1.

Hypopygium (Fig. 4B). Ninth tergite with 10–15, 13 setae, median setae stronger and situated on an obvious anal point. Virga 15–20  $\mu\text{m}$  long consisting of two spines. Inferior volsella well-developed lobe; gonostylus with slightly convex outer margin.

Female adult ( $n = 1$ ). Wing length 1.23 mm. Colouration as male.

Head. Antenna (Fig. 3E) with five flagellomeres, lengths (in  $\mu\text{m}$ ): 75/60/70/60/70. Temporal setae 9; palp lengths (in  $\mu\text{m}$ ): 25/30/95/-/-.

Thorax. Anteprepronotum with 6 setae. Dorsocentrals 18; acrostichals 11; prealars 3; scutellars 6.

Wing (Fig. 3D). Costa well extended, reaching slightly past half way to apex of  $M_{1+2}$ ;  $R_{2+3}$  approaching costa at 1/3 distance between  $R_1$  and  $R_{4+5}$ . Macrotrichia present on membrane in whole wing. Veins M, Sc and  $R_{2+3}$  without setae.

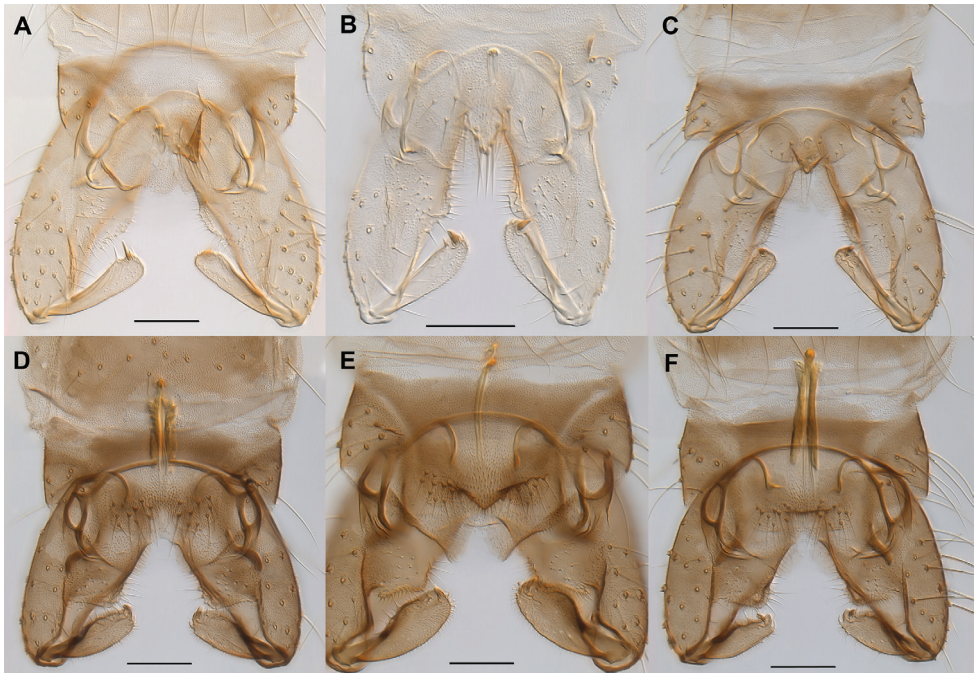
Legs. Fore tibia with one spur, 20  $\mu\text{m}$  long; mid tibia lost; hind tibia with well-developed comb and 2 spines, ca. 35–40  $\mu\text{m}$  long. Tarsus of fore leg lost ( $LR_1$  not measurable).

Genitalia (Fig. 3F). Gonocoxite IX with 7 setae. Ninth tergite undivided, semi-circular with 12 setae; cercus 50  $\mu\text{m}$  long; seminal capsules about 70  $\mu\text{m}$  long and 45  $\mu\text{m}$  wide, seminal tubules about 325  $\mu\text{m}$  long. Notum as long as rami, 87  $\mu\text{m}$ . Inner lobe of gonapophysis VIII broadly rounded with numerous long medially directed microtrichiae.

Immature stages unknown

**Remarks.** The species is morphologically similar to *G. (G.) subnudus* and *G. (G.) johanasecundus*, but paler (see whole specimen figures in BOLD dataset DS-GYMNO). Males and females are almost completely yellow-green with pale brown posterior side of head and postnotum; pale brown scutal bands and ventral side of preepisternum. *Gymnometriocnemus (G.) pallidus* is also similar to these species in having a short, triangular anal point and a small virga, but the hypopygium of *G. (G.) pallidus* has a more prominent inferior volsella than *G. (G.) johanasecundus* and considerably stronger anal tergite setae than *G. (G.) subnudus* (Figs 4B, C). Comparison with DNA barcode data in BOLD indicates that the species also has records from Germany and France.





**Figure 4.** Hypopygia of Norwegian *Gymnometriocnemus*. **A** *Gymnometriocnemus* (G.) *marionensis* (Finnmark06) **B** *Gymnometriocnemus* (G.) *pallidus* sp. n. (CH-eik131) **C** *Gymnometriocnemus* (G.) *subnudus* (ATNA398) **D** *Gymnometriocnemus* (R.) *autumnalis* sp. n. (Finnmark201) **E** *Gymnometriocnemus* (R.) *brumalis* (Finnmark75) **F** *Gymnometriocnemus* (R.) *kamimegavirgus* (Finnmark76). Scale bar = 50  $\mu$ m. Specimen codes in parenthesis correspond to codes in BOLD and in figure 6.

***Gymnometriocnemus* (*Gymnometriocnemus*) *subnudus* (Edwards, 1929)**

**Remarks.** We have seen specimens from eastern and central Norway that fit well with the original and later descriptions of the species, except for having a lower AR (1.0–1.1) compared to what Edwards (1929) reported. The specimens group in a well-defined barcode cluster (Fig. 6) separated from *G. (G.) marionensis* and *G. (G.) pallidus* sp. n. and is fairly widely distributed throughout Europe.

***Gymnometriocnemus* (*Raphidocladius*) *autumnalis* sp. n.**

<http://zoobank.org/CAB0F99B-1A0A-4078-A68D-D8DB31D0DEB1>

Figs 4D, 5D

**Type material.** Holotype: Male adult (NTNU-VM slide no. 136299), Norway, Finnmark, Porsanger, small pond near Gaggavann, 69.8306°N; 25.1856°E, 107 m a.s.l., 03.ix.2010, leg. Alyssa Anderson [BOLD ID: Finnmark201]. 3 Paratypes, male adults: 1 Norway, Finnmark, Vardø, Nedre Domen, lake and pond at road E75, 70.3215°N;

31.0341°E, 120 m a.s.l., 05.ix.2010, leg. Alyssa Anderson; 1 Norway, Finnmark, Nordkapp, Nordkapp-Plateau, 71.1446°N; 25.7641°E, 220 m a.s.l., 01-ix-2010, leg. Trond Andersen; 1 Norway, Oppland, Dovre, Rondane National Park, Vidjedalsbekken (upper), Malaise trap, 61.9717°N; 9.83606°E, 1280 m a.s.l., 15.ix.2008, leg. Terje Hoffstad.

**Etymology.** The species is named “autumnalis”, Latin adjective meaning belonging to autumn, referring to the time of the year when the type material was collected.

**Diagnosis.** *Gymnometriocnemus* (*R.*) *autumnalis* can be separated from other *Gymnometriocnemus* species by the following combination of characters in the adult male: body brown, dark brown; virga long and conspicuous with strong lateral sclerotization, anal tergite without dorsal anal point or ridge, gonostylus with convex outer margin and well-defined median crista dorsalis.

**Description.** Male adult (n = 4 unless otherwise stated). Wing length 1.30–1.52, 1.43 mm. Colouration completely brown, dark brown except for pale transverse bands posteriorly on abdominal tergites V–VIII, narrower on tergite V.

Head. Antennal ratio in Table 1. Temporal setae 8-10, 9; palp lengths (in  $\mu\text{m}$ ): 25/35/80-90/75-90/105-125.

Thorax. Antepnotum with 2 setae. Dorsocentrals 10-11; acrostichals 8-9, minute and difficult to discern; prealars 4-6; scutellars 2-5.

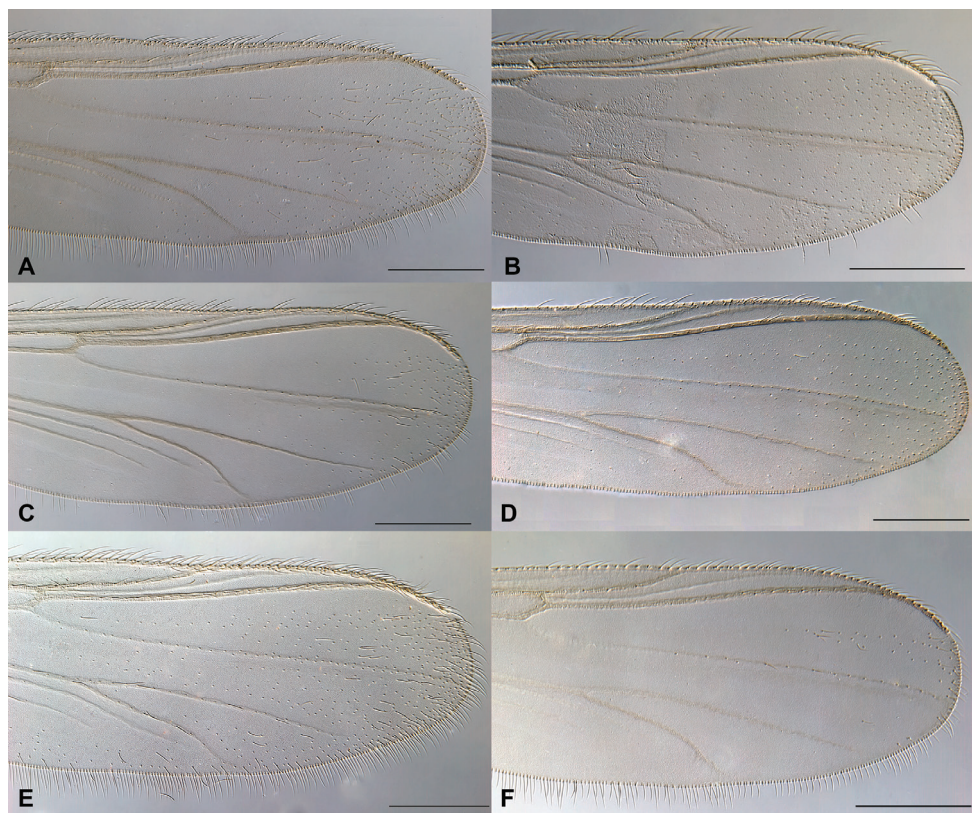
Wing (Fig. 5D). Costa moderately extended, not reaching half way to apex of  $M_{1+2}$ ;  $R_{2+3}$  approaching costa at  $\frac{1}{2}$  distance between  $R_1$  and  $R_{4+5}$ . Macrotrichia frequent on membrane in apical  $\frac{1}{3}$  of wing, absent from cell m, few (0-6) in cells cu+an. Veins Sc,  $R_{2+3}$ , M, Cu, PCu and pseudovein without setae.

Legs. Fore tibia with one spur, 40  $\mu\text{m}$  long; mid tibia with two spurs ca. 20  $\mu\text{m}$  long; hind tibia with well-developed comb and 2 spines, ca. 50  $\mu\text{m}$  long. Fore leg ratios in Table 1.

Hypopygium (Fig. 4D). Ninth tergite with 16-19, 18 setae, without anal point or ridge. Virga 110-120, 115  $\mu\text{m}$  long consisting of two spines, with strong lateral sclerotization. Inferior volsella well-developed lobe; gonostylus with slightly convex outer margin and well-defined median crista dorsalis.

Female and immature stages unknown.

**Remarks.** The species is morphologically similar to *G. (R.) brumalis* and *G. (R.) kamimegavirgus*, but different in lacking an anal point or ridge on the anal tergite. This character is similar to characters reported for *G. terrestris* and *G. tairaprimus*, but these two species can according to original descriptions be separated by having a higher AR (1.4 in *G. terrestris*) and a different shape of the superior volsella (Krüger and Thienemann 1941; Sasa and Okasawa 1994). The virga of *G. terrestris* has not been described, but the species is listed as a member of subgenus *Gymnometriocnemus* in the World Catalogue of Chironomidae (Ashe and O'Connor 2012). We have examined two males and one female between cellophane strips on two separate pins from the Goetghebuer collection; labels reading “Allemagne, Dr Thienemann, Bonn 1939”. Only one pin with a female bears a type label, but all specimens are likely part of the type series and regarded as syntypes. A small virga is visible in a rather well mounted and cleared



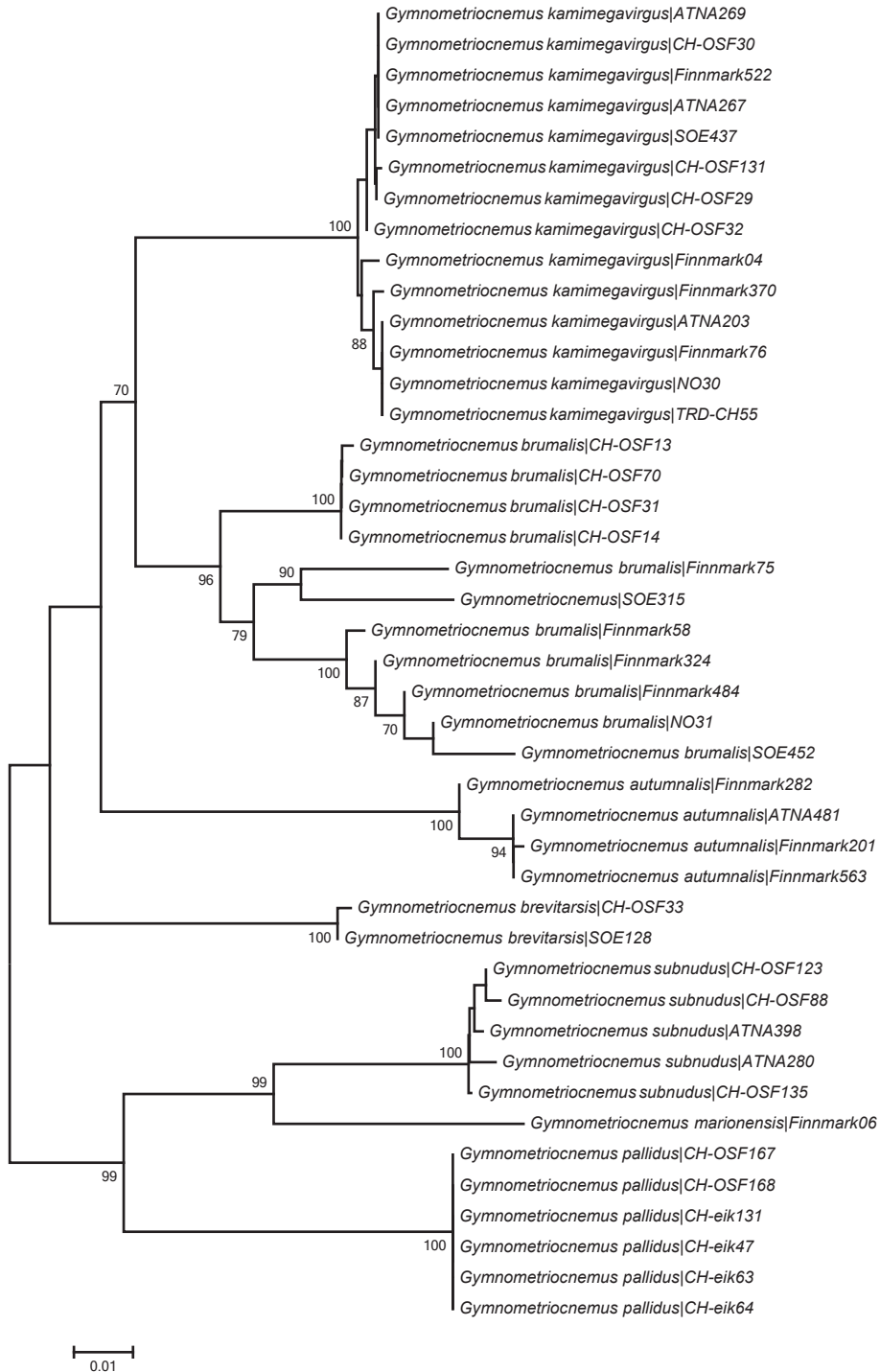
**Figure 5.** Distal part of wing for Norwegian *Gymnometriocnemus*. **A** *Gymnometriocnemus* (*G.*) *marionensis* (Finnmark06) **B** *Gymnometriocnemus* (*G.*) *pallidus* sp. n. (CH-eik131) **C** *Gymnometriocnemus* (*G.*) *subnudus* (ATNA98) **D** *Gymnometriocnemus* (*R.*) *autumnalis* sp. n. (Finnmark201) **E** *Gymnometriocnemus* (*R.*) *brumalis* (CH-OSF70) **F** *Gymnometriocnemus* (*R.*) *kamimegavirgus* (ATNA269). Scale bar = 200  $\mu$ m. Specimen codes in parenthesis correspond to codes in BOLD and in figure 6.

hypopygium on one of the specimens. We can confirm placement in subgenus *Gymnometriocnemus* and that none of the specimens we have collected in Norway belong to this species. *G. (R.) autumnalis* has so far only been recorded from the very north of mainland Norway and the Rondane mountains (1280 m a.s.l.) in Central Norway.

### *Gymnometriocnemus (Raphidocladus) brumalis* (Edwards, 1929)

**Remarks.** We have barcoded specimens from eastern, central and northern Norway that fall within the same genetic cluster although with quite large intraspecific divergence (0–6.8%, mean 3.42% K2P-distance) (Fig. 6). We are at present not able to find morphological differences that fully correspond to the internal groupings inside the *G. (R.) brumalis* cluster and all our specimens fit the description by Sæther (1983) with





**Figure 6.** Taxon ID-tree from Neighbour Joining analysis on K2P-distances using 1000 bootstrap replicates. Bootstrap values >70 are given on branches.

the additions by Cranston and Oliver (1988). There is some variation observed in the length of the virga in relationship with the length of the gonocoxite, one of the characters used to separate *G. (R.) acigus* from *G. (R.) brumalis* (Cranston and Oliver 1988; Sæther 1983), but we have not examined or barcoded a sufficient number of specimens to confidently conclude if the divergent genetic clusters in our *G. (R.) brumalis* can be regarded as separate species. We have examined the male holotype and a male paratype of *G. (R.) acigus* and can confirm that the species fits our and Cranston and Oliver's (1988) interpretation of *G. (R.) brumalis*. The species has a Holarctic distribution.

### ***Gymnometriocnemus (Raphidocladus) kamimegavirgus* Sasa & Hirabayashi, 1993**

*Gymnometriocnemus kamimegavirgus* Sasa & Hirabayashi (Sasa and Hirabayashi 1993; Sasa and Okasawa 1994).

*Gymnometriocnemus volitans* (Goetghebuer), misidentifications (e.g. Brundin 1956).

*Gymnometriocnemus (Raphidocladus?) volitans* (Goetghebuer) sensu Brundin (1956), misidentification (Sæther 1983).

*Gymnometriocnemus (Raphidocladus) volitans* (Goetghebuer) sensu Brundin (1956) (Ashe and O'Connor 2012; Sæther and Spies 2013).

**Diagnosis.** *Gymnometriocnemus (R.) kamimegavirgus* can be separated from other species of the genus *Gymnometriocnemus* by having well-developed, long virga (about the length of the gonocoxite); AR 0.9-1.1 (n=5); LR<sub>1</sub> about 0.53-0.56 (n=3); wing membrane with setae at the apex only, occasionally with 1-2 setae proximally in cell an; R<sub>2+3</sub> situated in the middle between R<sub>1</sub> and R<sub>4+5</sub>; dark brown almost blackish thorax and head, slightly paler abdomen and legs.

**Remarks.** Our examined material is from eastern, central and northern Norway, frequently collected near streams, rivers and moors. Male adults fit well with Brundin's description of *G. volitans*, and Sasa & Hirabayashi's description of *G. kamimegavirgus* except for slightly fewer setae on the abdominal tergites (Brundin 1956; Sasa and Hirabayashi 1993; Sasa and Okasawa 1994). The species is Holarctic in distribution.

### **Key to Holarctic male adults of the genus *Gymnometriocnemus***

The species *Gymnometriocnemus brevitarsis* is only known as female and therefore not included in the key.

- |   |   |   |
|---|---|---|
| 1 | Large, needle-like virga well-developed, sometimes with strong lateral sclerotization (Fig. 4D–F) ..... | 2 |
| – | Virga small and inconspicuous, without lateral sclerotization (Fig. 4A–C) ....                          | 5 |
| 2 | Anal tergite without ridge or anal point (Fig. 4D) .....  | 3 |
| – | Anal tergite with at least a median triangular ridge (Fig. 4E, F) .....                                 | 4 |



- 3 Inferior volsella with obvious dorsal and ventral lobe (Japan)..... *G. (R.) tairaprimus*
- Inferior volsella with single lobe (Norway) (Fig. 4D) ..... *G. (R.) autumnalis*
- 4 Wing membrane with setae on wing tip only; often strong sclerotization laterally of virga (Fig. 5F) ..... *G. (R.) kamimegavirgus*
- Wing membrane with setae on at least half of wing; no strong sclerotization laterally of virga (Fig. 5E) ..... *G. (R.) brumalis*
- 6 Body pale yellow-green with pale brown scutal markings.... *G. (G.) pallidus*
- Body completely brown, or when yellowish ground colour with dark brown scutal markings.....7
- 7 Edge of anal tergite broadly rounded (possibly an anal point, but difficult to discern in syntypes); gonostylus strongly curved inwards ... *G. (G.) terrestris*
- Anal point present, triangular; gonostylus at most with a weakly convex outer margin (Fig. 4A, C) .....8
- 8 Ground colour of thorax yellowish (Japan) ..... *G. (G.) johanasecundus*
- Ground colour of thorax brown.....9
- 9 Anal point moderately well developed, c. 38 µm long (Fig. 4A) ..... *G. (G.) marionensis*
- Anal point weakly developed, c. 17 µm long (Fig. 4C) ..... *G. (G.) subnudus*

## Final remarks

As a result of this study, there are now 17 species of *Gymnometriocnemus* registered worldwide and the genus is present in all major biogeographical regions except Antarctica. Our findings through moderate sampling in Norway indicate that the number of species could be considerably higher also on a global scale and show that molecular data can be a great advantage in diversity assessments of targeted groups. Moreover, our study also highlights the importance of consulting type material for correct identification of Chironomidae if we are to avoid long term misconceptions of species.

## Acknowledgements

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Bergen for loan of specimens of *G. (G.) marionensis* and types of *G. (R.) acigus*. We are grateful for the help provided by Martin Spies in obtaining copies of relevant literature from the library at the Zoologische Staatssammlung München. Thanks to Rick Jacobsen and two anonymous reviewers for comments on the submitted manuscript.

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## Supplementary material I

### Examined and DNA barcoded *Gymnometriocnemus* species from Norway

Authors: Elisabeth Stur, Torbjørn Ekrem

Data type: Excel spreadsheet with specimen data and GenBank accessions

Explanation note: The spreadsheet contains the following information for all examined species: sample id, catalogue number, voucher status, locality, date of collection, collector and GenBank accession.

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