ZooKeys 589: 1–21 (2016)
doi: 10.3897/zookeys.589.8361
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Species of *Elasmogorgia* and *Euplexaura* (Cnidaria, Octocorallia) from Japan with a discussion about the genus *Filigella*

Asako K. Matsumoto[1], Leen P. van Ofwegen[2]

1 *Planetary Exploration Research Center (PERC), Chiba Institute of Technology (Chitech), Tsudanuma 2-17-1, Narashino, Chiba 275-0016, Japan* **2** *Naturalis Biodiversity Center, Darwinweg 2, P.O. Box 9517, 2300 RA Leiden, The Netherlands*

Corresponding author: *Asako K. Matsumoto* (amatsu@gorgonian.jp)

Academic editor: *B.W. Hoeksema* | Received 3 March 2016 | Accepted 8 April 2016 | Published 16 May 2016

http://zoobank.org/3B006BA1-16A8-408B-9FCC-5E812D081608

Citation: Matsumoto AK, van Ofwegen LP (2016) Species of *Elasmogorgia* and *Euplexaura* (Cnidaria, Octocorallia) from Japan with a discussion about the genus *Filigella*. ZooKeys 589: 1–21. doi: 10.3897/zookeys.589.8361

Abstract

Octocorals with thread-like colony shape have been re-examined, mainly from Japanese waters. The holotypes of *Elasmogorgia filiformis* and *Filigella boninensis* and a syntype of *F. mitsukurii* have been studied. *Euplexaura arbuscula* is identified and *Euplexaura yayoii* **sp. n.** described.

Keywords

Astrogorgia, *Thesea*, Plexauridae, Alcyonacea, deep-water octocorals, Indo-Pacific, new species, Challenger Expedition

Introduction

The octocoral genera *Elasmogorgia*, *Filigella* and *Thesea* have been underexplored and their taxonomic position remains confusing. One of the Japanese species of these genera, *Filigella mitsukurii*, is classified with three different genera in WoRMS, as *Elasmogorgia mitsukurii* (Ofwegen 2016a), *Filigella mitsukurii* (Ofwegen 2016b), and *Thesea mitsukurii* (Ofwegen 2016c). In this manuscript, a revision is presented of the

genera *Elasmogorgia* and *Filigella* and their species in Japan, as well as some Japanese species of *Euplexaura*.

The genus *Filigella* Gray, 1868 was established to accommodate *F. gracilis* from Brazil. Later on Wright and Studer (1899) established the Pacific genus *Elasmogorgia* with the remark that their new species *E. filiformis* could be identical to *Filigella gracilis*. Next, Hickson (1905) described *Elasmogorgia flexilis* from the Maldives, Kinoshita (1909) described *Filigella mitsukurii* from Japan, Nutting (1912) described *Elasmogorgia ramosa*, also from Japan, and finally Aurivillius (1931) described *Filigella boninensis* from the Ogasawara Islands (Bonin Islands), and Thomson and Dean (1931) described *Elasmogorgia filigella* from Kalimantan (Indonesia). Both Kinoshita and Aurivillius considered *Elasmogorgia* and *Filigella* synonymous and Aurivillius doubted whether *Elasmogorgia ramosa* of Nutting (1912) belonged to one of these two genera. Kükenthal (1919) first treated them as two separate genera but he synonymized them five years later (Kükenthal 1924).

Bayer (1959: 17) was the first to include *F. gracilis* in the genus *Thesea* Duchassaing & Michelotti, 1860, although he did not directly synonymize the genus *Filigella* with *Thesea*, but much later in his key to the octocoral genera, Bayer (1981: 945). However, he did not re-examine six Pacific species referred to *Filigella* or *Elasmogorgia*, and therefore the status of these species has remained doubtful.

In the present study, the type material of *Elasmogorgia filiformis*, *E. filigella*, *Filigella mitsukurii*, and *F. boninensis*, is examined and their previous identifications are discussed. In addition, two specimens identified as *E. filiformis* by Nutting (1910) and by Thomson and Dean (1931) were examined. *Elasmogorgia filigella* Thomson and Dean (1931) from Kalimantan clearly does not belong to *Elasmogorgia* because it has a red colony and also red sclerites. The type specimen of *E. filigella* (ZMA 2536) appears to consist of a few branch fragments with disintegrated sclerites. It is considered to represent a species of *Astrogorgia* in the present study.

Finally, a new thread-like *Euplexaura* species is decribed from the Pacific side of northern Japan, *Euplexaura yayoii* sp. n., in addition to *E. arbuscula* Broch, 1935 from off Chishima Is. (Kuril Is.), which previously was reported from the west coast of Kamchatka, Sea of Okhotsk. These two species are both from northern Japan and northeastern Russia (Figure 1).

Material and methods

Abbreviations

BMNH	British Museum of Natural History, London, UK
NBC (RMNH)	Naturalis Biodiversity Center, formerly Rijksmuseum van Natuurlijke Historie, Leiden, The Netherlands
UMUTZ	University Museum of University of Tokyo, Tokyo, Japan
UUZM (UPSZTY)	Museum of Evolution, Uppsala, Sweden

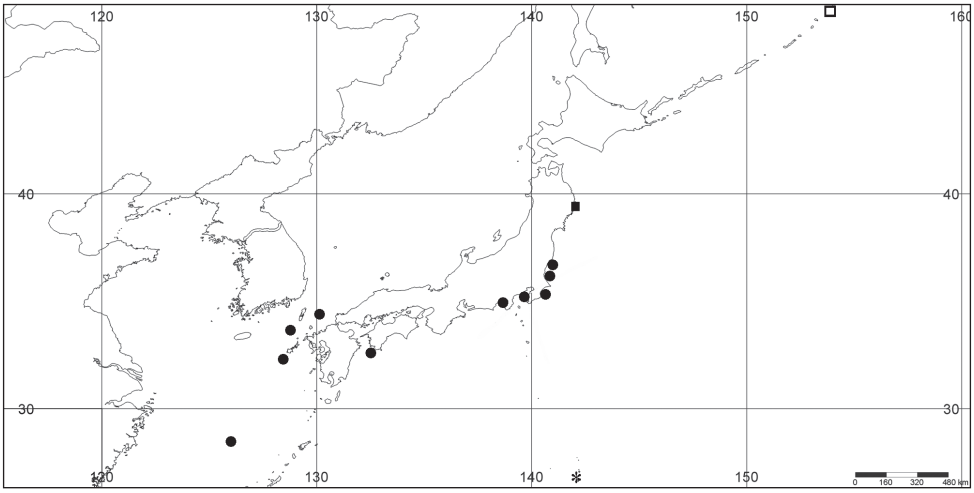


Figure 1. Distribution map of *Euplexaura boninensis* (*), *E. mitsukurii* (●), *E. arbuscula* (□), and *E. yayoii* sp. n. (■).

ZMUC	Zoological Museum University of Copenhagen, Copenhagen, Denmark
ZIN	Museum of the Zoological Institute of the Russian Academy of Sciences St. Petersburg, Russia
ZMA	Zoological Museum Amsterdam (ZMA), now part of NBC.

Material

Material was collected from depths between 38 and 366 m by dredging, trawling or fishing net onboard *RV Tansei-maru*, University of Tokyo and Japan Agency for Marine-earth Science and Technology and *RV Yayoi*, the University of Tokyo, during the years 1975–2010. Type specimens and other historical museum material was examined in collections of the BMNH, NBC, UMUTZ, UUZM, ZIN, and ZMUC.

From each specimen a small piece of the distal part of a branch was dissolved in a solution of household bleach (4% hypochlorite) to isolate sclerites. The sclerites were washed with demineralised water, dried on a hot plate, mounted on SEM stubs, and coated with Pd/Au for SEM imaging. For this, either a JEOL JSM6490LV scanning electron microscope was operated at high vacuum at 10 kV, or a JEOL JSM6510LA scanning electron microscope with a Quick Carbon Coater SC-701C, SANYU ELECTRON was used. For terminology, see Bayer et al. (1983).

Descriptions of old Japanese material collected by Japanese used "hiro" (Japanese fathom) as the depth unit. One Japanese fathom (hiro) is usually 1.43 m, occasionally 1.51 m, whereas, it is 1.818 m for the length unit on land. The old depth unit fathom

is also converted to 1.8288 m. When it was not clear whether the collector used fathom or hiro, the converted depth has wider ranges.

Taxonomy

Genus *Elasmogorgia* Wright & Studer, 1889

Elasma (non *Elasma* Jaennicke 1866); Studer (and P. Wright) 1887: 58.
Elasmogorgia Wright & Studer, 1899: 132; Kükenthal 1919: 836; Soest 1979: 88.
?*Elasmogorgia*; Hickson 1905: 814; Thomson and Simpson 1909: 238; Thomson and Russell 1910: 159; Nutting 1912: 85.
NOT *Elasmogorgia*; Nutting 1909: 717; 1910: 45; 1912: 85; Thomson and Dean 1931: 199.
Partly *Elasmogorgia*; Kükenthal 1924: 148.

Diagnosis. Plexauridae with sparsely branched colonies lacking a holdfast. Calyces dome-shaped. Polyps with collaret and points. Sclerites are colourless spindles.

Elasmogorgia filiformis Wright & Studer, 1889

Figures 2a, 3–4

Elasmogorgia filiformis Wright & Studer, 1889: 133 (Indonesia, Arafura Sea); Kükenthal 1924: 148.
? *Elasmogorgia filiformis*; Nutting 1912: 85 (Tateisha zaki Light, Japan); Thomson and Russell 1910: 159 (Amirantes); Thomson and Simpson 1909: 238 (Birma, India); Tixier-Durivault 1966: 403 (Madagascar); all not re-examined.
NOT *Elasmogorgia filiformis*; Nutting 1909: 717 (California = *Thesea*); 1910: 45 (Timor = *Euplexaura*); Thomson and Dean 1931: 199 (Sulawesi = *Astrogorgia*).

Material examined. Holotype BMNH 1889.5.27.77, Arafura Sea, South of Papua, 28 fms, Challenger st. 188, 10 September 1874; ZMA Coel. 2537, Siboga st. 213, Saleyer anchorage, Sulawesi, Indonesia, 38 m, 26 September 1899 (= *Astrogorgia*); ZMA Coel. 2538, Timor, 112 m, Siboga st. 289, 09°00.3'S, 126°24.5'E (= *Euplexaura*).

Diagnosis. Colony thread-like (Figure 2a). Calyces dome-shaped, arranged all around the branches (Figure 2a). Coenenchyme with spindles up to 0.45 mm long, with simple tubercles (Figures 3–4). Colony white with colourless spindles.

Remarks. One somewhat flattened spindle was found, 0.35 mm long, maybe referable to a collaret (Figure 4b), and one capstan (Figure 3b). As the microscope slide that was made only shows heavily oxidized black sclerites it could not be really ascertained where different types of sclerites came from. The little fragment available was not sufficient for more extensive examination. Wright and Studer (1889) mentioned

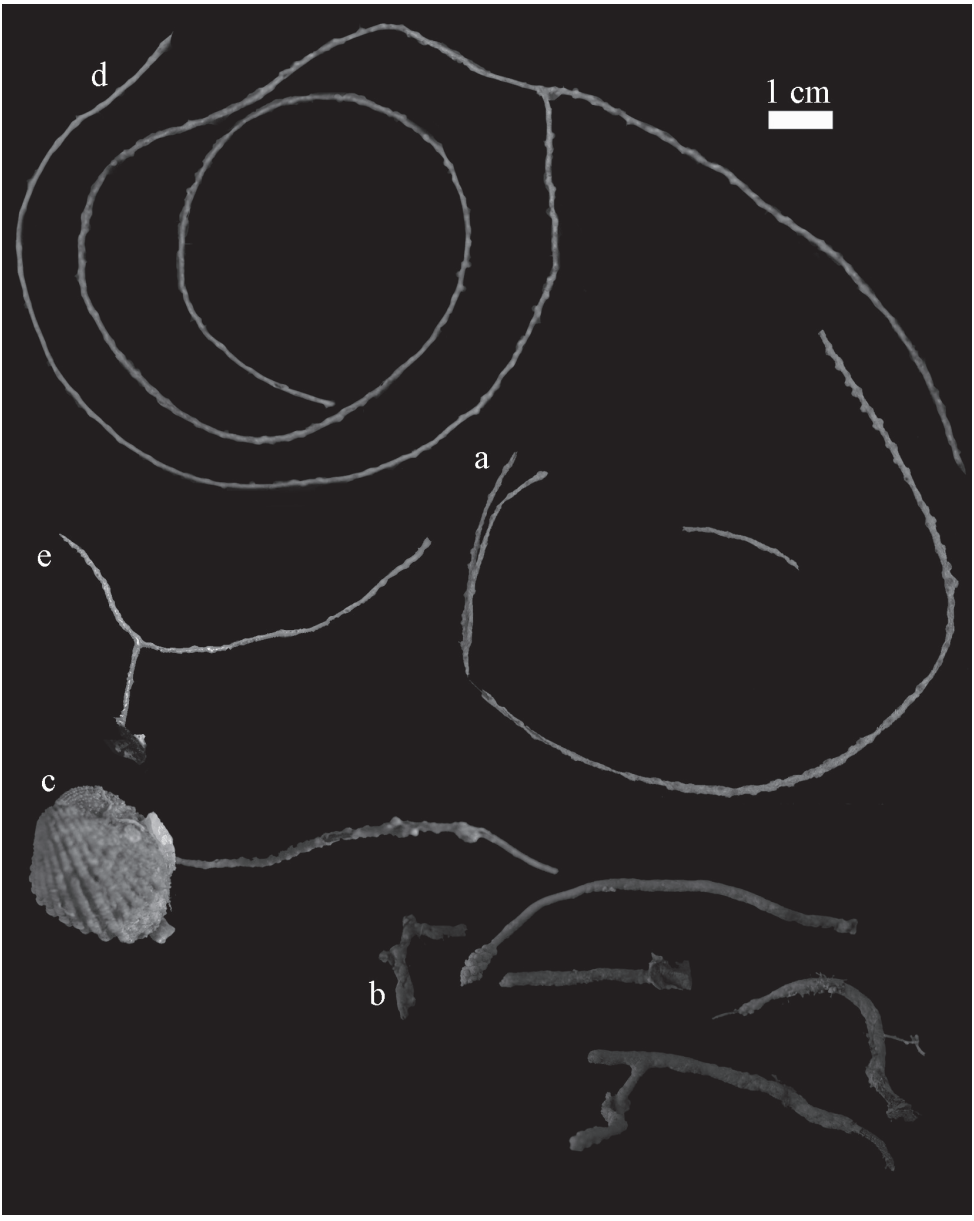


Figure 2. a *Elasmogorgia filiformis* Wright & Studer, 1889, holotype BMNH 1889.5.27.77 **b** *Euplexaura arbuscula* Broch, 1935, ZIN 11667 **c** *E. boninensis* (Aurivillius, 1931), holotype UPSZTY2165 (UUZM 68) **d** *E. mitsukurii* (Kinoshita, 1909), syntype UMUTZ-CnidG-222 **e** *E. yayoii* sp. n., holotype RMNH 42104.

spindles up to 0.62 mm long. They also mentioned the basal portion of the tentacles has spindle-shaped sclerites of up to 0.18 mm long. *Elasmogorgia filiformis* mostly resembles a species of *Astrogorgia* but differs in not having polyp body sclerites and

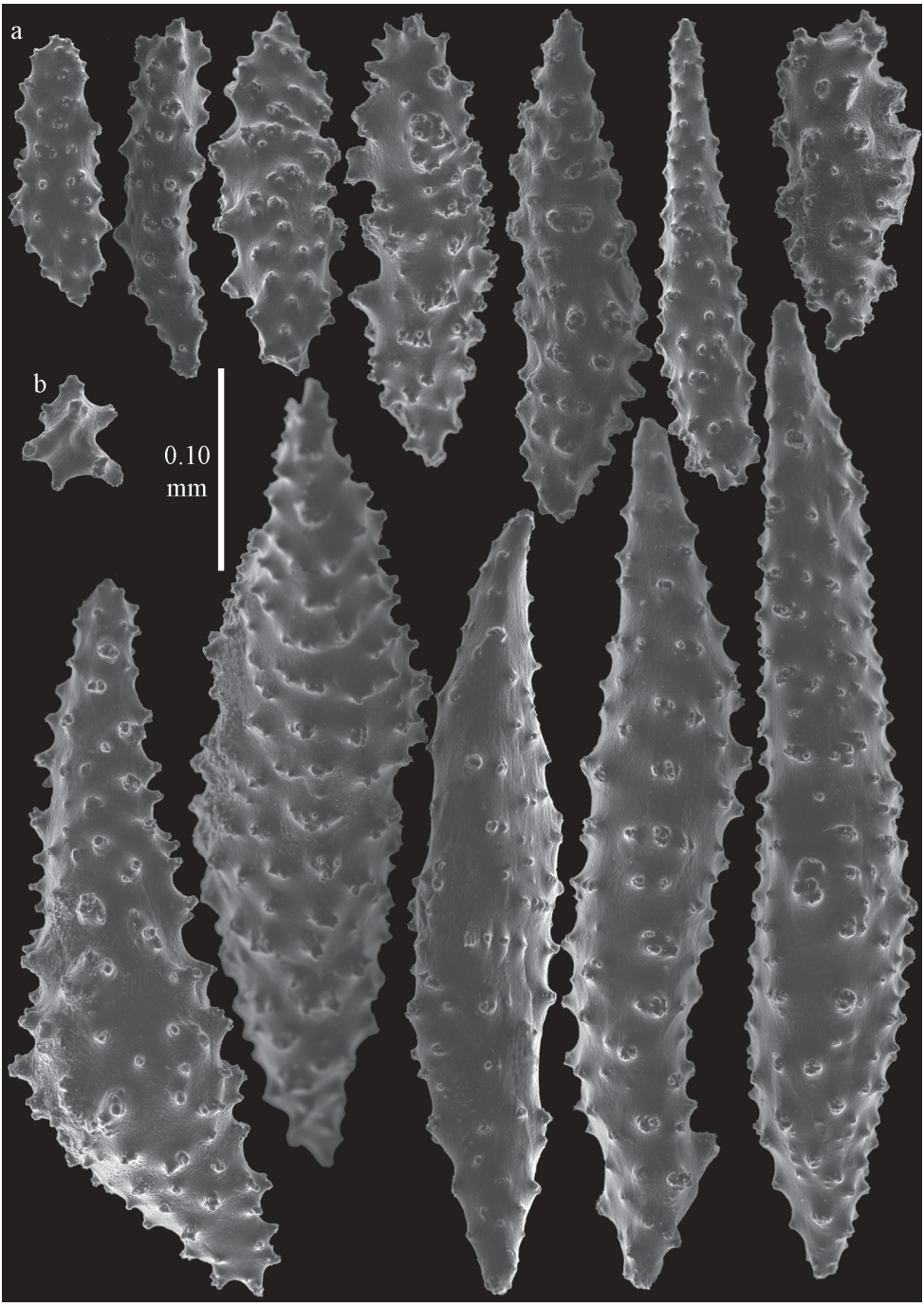


Figure 3. *Elasmogorgia filiformis* Wright & Studer, 1889, holotype BMNH 1889.5.27.77, **a** spindles from surface layer of coenenchyme **b** capstan.

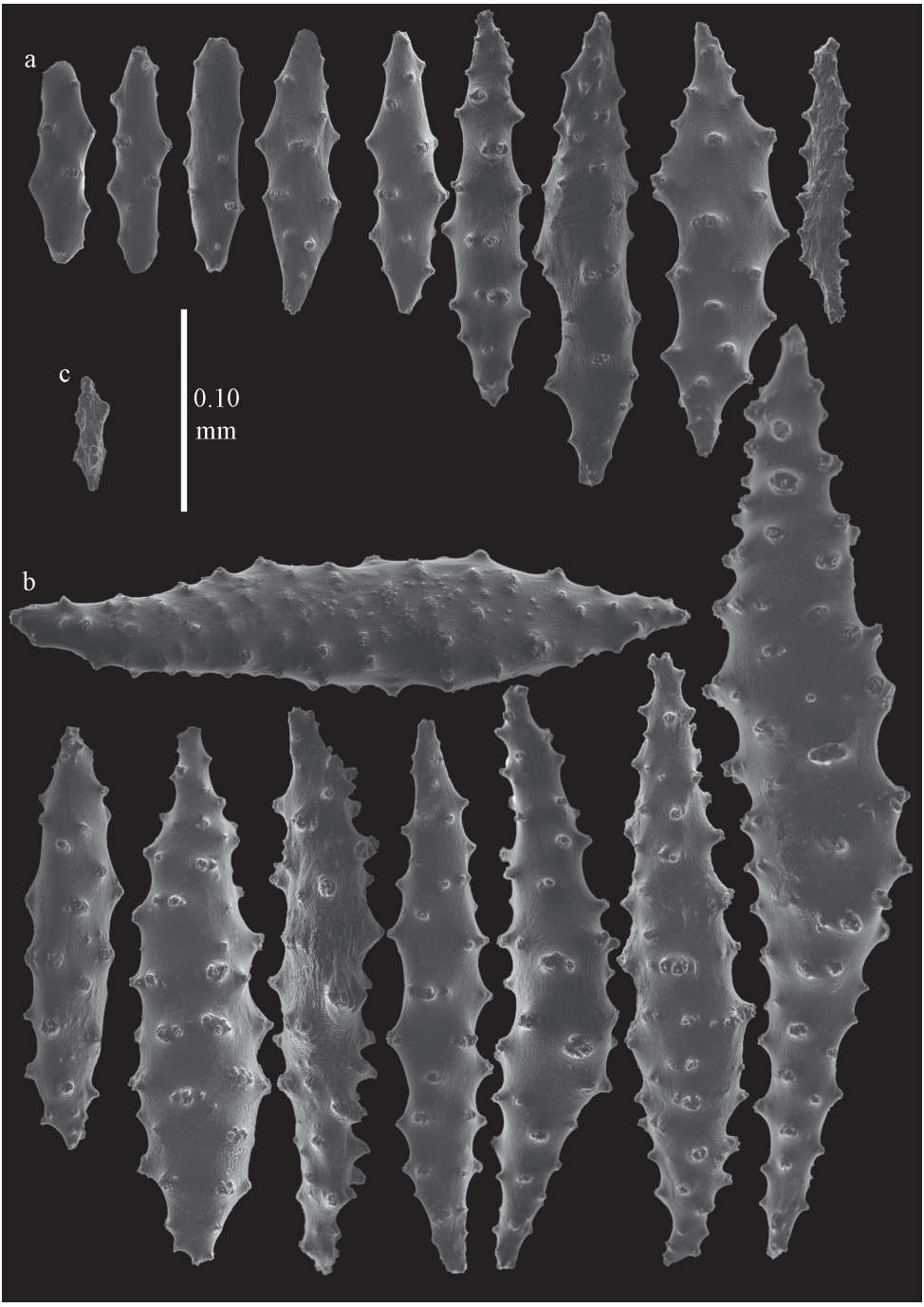


Figure 4. *Elasmogorgia filiformis* Wright & Studer, 1889, holotype BMNH 1889.5.27.77, **a** spindles from interior of coenenchyme **b** possible collaret spindle **c** rod.

extremely weak ornamentation of spindles. Until new material becomes available for a more thorough examination the genus *Elasmogorgia* is retained.

ZMA 2537 of Thomson and Dean (1931) is a thread-like colony fragment containing colourless disintegrated sclerites, which were sufficiently recognizable to identify it as a species of *Astrogorgia*. In a comparison with *Astrogorgia bayeri* Ofwegen and Hoeksema, 2001, from Sulawesi, the latter species appears to have shorter spindles, up to 0.5 mm long, whereas Thomson and Dean's (1931) specimen has spindles of over 1 mm long. Because of the disintegrated state of its sclerites, no more differences could be ascertained.

ZMA 2538 of Nutting (1910) was also re-examined; it has characters of the genus *Euplexaura*. *Elasmogorgia filiformis* of Nutting (1912) is also unlikely an *Elasmogorgia*.

Genus *Euplexaura* Verrill, 1869

Euplexaura Verrill, 1869: 75; Kükenthal 1924: 90 (synonymy of the genus).

Diagnosis. Plexauridae with colonies branched in one plane. Calyces may be present but are mostly absent. Polyps with collaret and points, only point sclerites, or no sclerites at all. The surface of the coenenchyme with robust ovals or spindles with complex tubercles; sometimes with one side that is less tuberculate. The interior with rods or small spindles with simple tubercles. All sclerites colourless.

Euplexaura arbuscula Broch, 1935

Figures 1, 2b, 5

Euplexaura arbuscula Broch, 1935: 20, fig. 12.

Material. ZIN 11667(ZIN110824-018-040), Skaly Lovushki I., off Chishima Is. (= Kuril Is.), 154°44'5E, 48°15'5N, depth 140 m, Bottom: gravel with stones, Ship *Odissey*, Grab "Ocean" 50 cm^2 (bottom sampler), coll. Boris Sirenko and Mikhail Kolesnikov, 3 August 1984.

Diagnosis. Branches thread-like. Calyces dome-shaped, arranged all around the branches (Figure 2b). Polyps without sclerites. The surface layer of the coenenchyme has spindles and blunt ellipsoids (Figure 5a), up to 0.15 mm long, with complex tubercles. The interior has small spindles, capstans, and a few crosses, up to 0.15 mm long (Figures 5b-d), all with simple tubercles.

Remarks. The material examined was fragmentary (Figure 2b) and therefore it resembles a species of *Elasmogorgia*.

Euplexaura abietina Kukenthal, 1909 resembles *E. arbuscula* regarding its sclerites, but it differs in having polyp spindles.

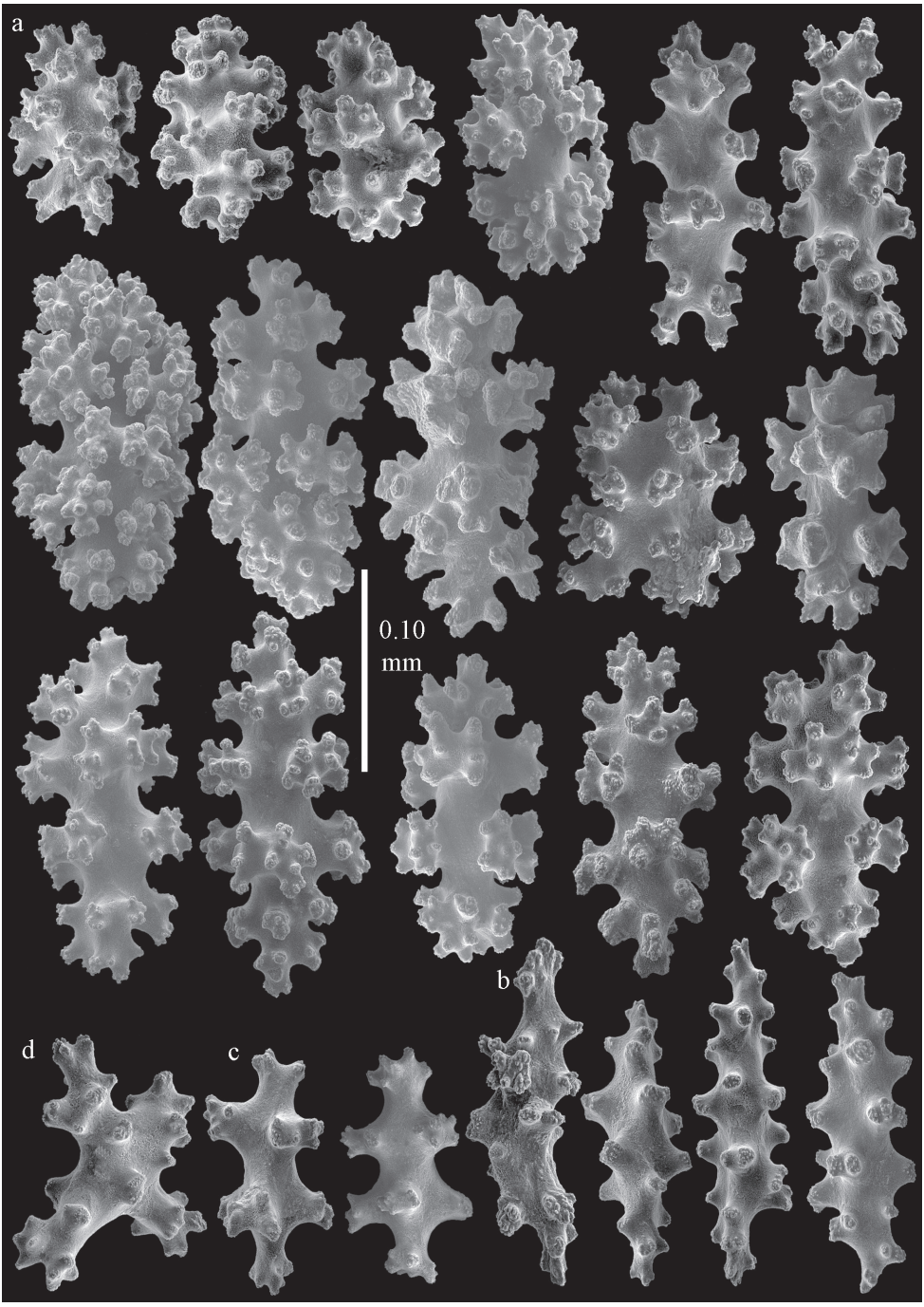


Figure 5. *Euplexaura arbuscula* Broch, 1935, ZIN 11667 **a** spindles and blunt ellipsoids from surface layer of coenenchyme **b–d** sclerites from interior of coenenchyme **b** spindles **c** capstans **d** cross.

Since its original description, the species was not found again and its type material could not be retraced, hence some doubts remain about the identification of this species. Broch (1935) described the species only from one specimen. It is not present in the Natural History Museum, University of Oslo, and ZIN.

Distribution. Kamchatka, Sea of Okhotsk, off Chishima Is. (= Kuril Is.).

Euplexaura boninensis (Aurivillius, 1931)

Figures 1, 2c, 6–7

Filigella boninensis Aurivillius, 1931: 139 (Bonin Islands).
Thesea boninensis; Matsumoto 2014: 158 (Table 1, listed only).

Material examined. Holotype UPSZTY2165 (UUZM 68), East of Chichijima I., Ogasawara Is. (= Bonin Is.), Japan, depth 100 m, in formalin, Dr. Sixten Bock's, Japan Expedition, coll. Dr. Sixten Bock, 1 August 1914.

Diagnosis. Branches thread-like, 6 cm in length. Calyces dome-shaped, arranged all around the branches (Figure 2c). The polyps have points with flattened spindles, up to 0.15 mm long (Figure 6b), with simple tubercles and spiny distal end. Collaret present, with slightly bent, flattened spindles, up to 0.25 mm long, with simple tubercles (Figure 6c). Tentacles with small scales, up to 0.10 mm long (Figure 6a).

The surface layer of the coenenchyme has spindles (Figure 6d) and blunt ellipsoids (Figure 6e), up to 0.65 mm long, with complex tubercles. Several of them with one side less tuberculate. The interior has small spindles and rods, up to 0.25 mm long (Figure 7), with simple tubercles.

Remarks. Because the sclerites of this species are spindles and ellipsoids with complex tubercles it actually represents an *Euplexaura* species. It is the only species of *Euplexaura* with thread-like colony shape which has many sclerites with one side that is less tuberculate.

Euplexaura mitsukurii (Kinoshita, 1909)

Figures 1, 2d, 8–9

Filigella mitsukurii Kinoshita, 1909: 1(Sagami Bay); Aurivillius 1931: 129 (Kiu-Shiu = Kyushu); Utinomi 1961: 213; Bayer 1956: F206, fig. 148,3.
Elasmogorgia mitsukurii; Kükenthal 1924: 149.
Thesea mitsukurii; Matsumoto et al. 2007: 240 (Table 2, listed); Matsumoto 2014: 158, 159, 160 (Table 1, listed).
Thesea sp. Matsumoto 2014: 161 (Table 1, listed).

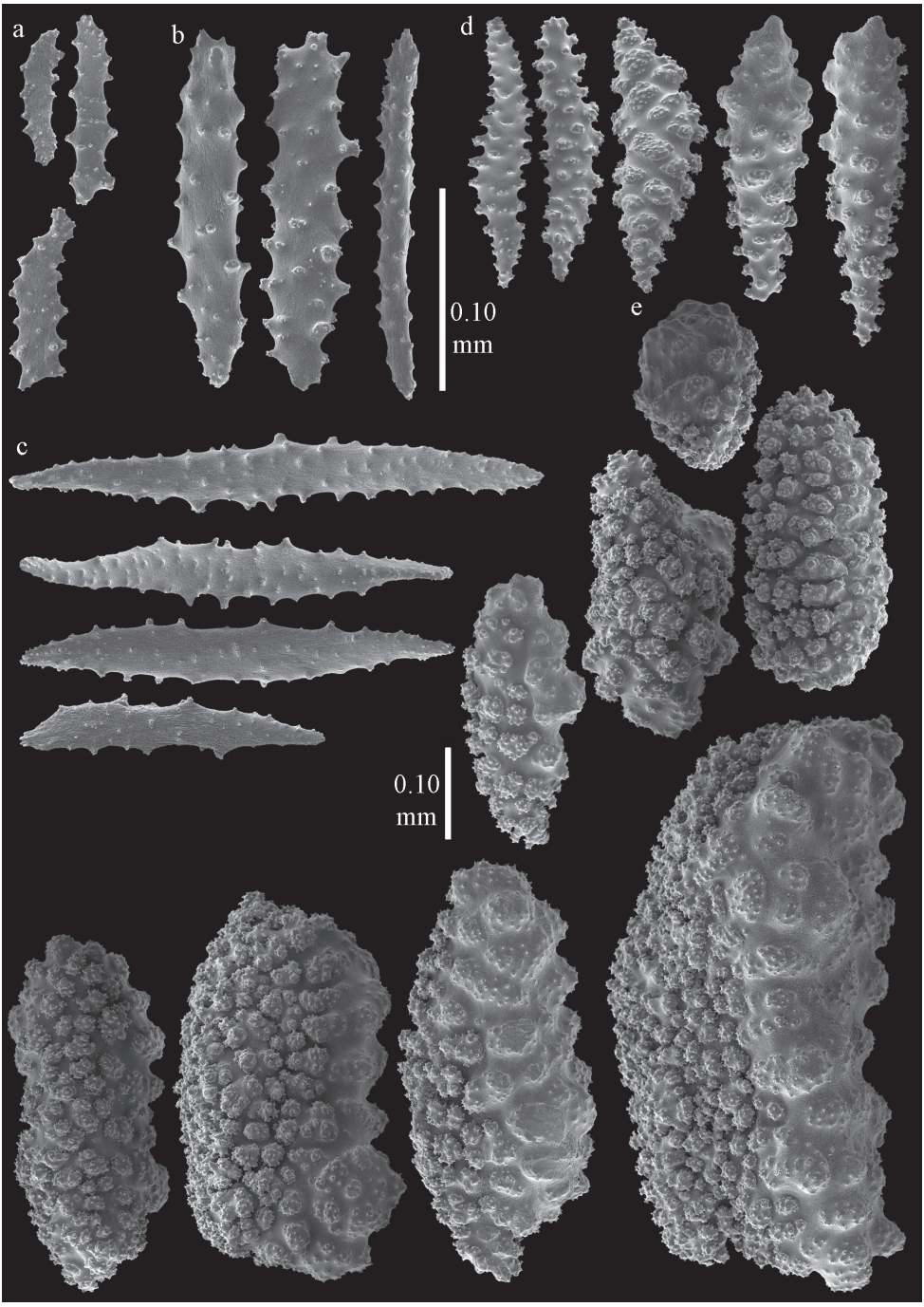


Figure 6. *Euplexaura boninensis* (Aurivillius, 1931), holotype UPSZTY2165 (UUZM 68), **a** tentacle scales **b** point spindles **c** collaret spindles **d** spindles from surface layer of coenenchyme **e** blunt ellipsoids from surface layer of coenenchyme.

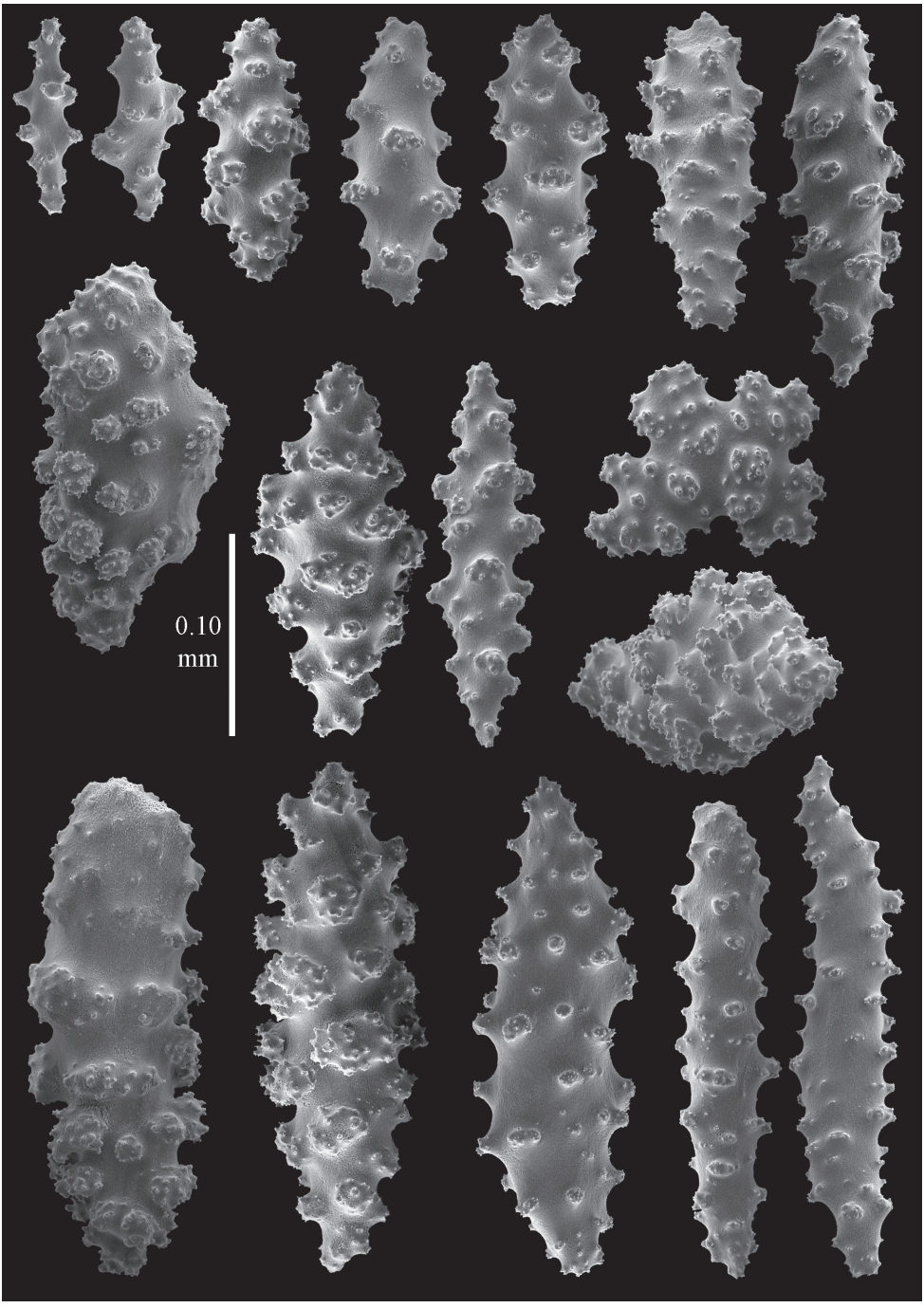


Figure 7. *Euplexaura boninensis* (Aurivillius, 1931), holotype UPSZTY2165 (UUZM 68), sclerites of interior of coenenchyme.

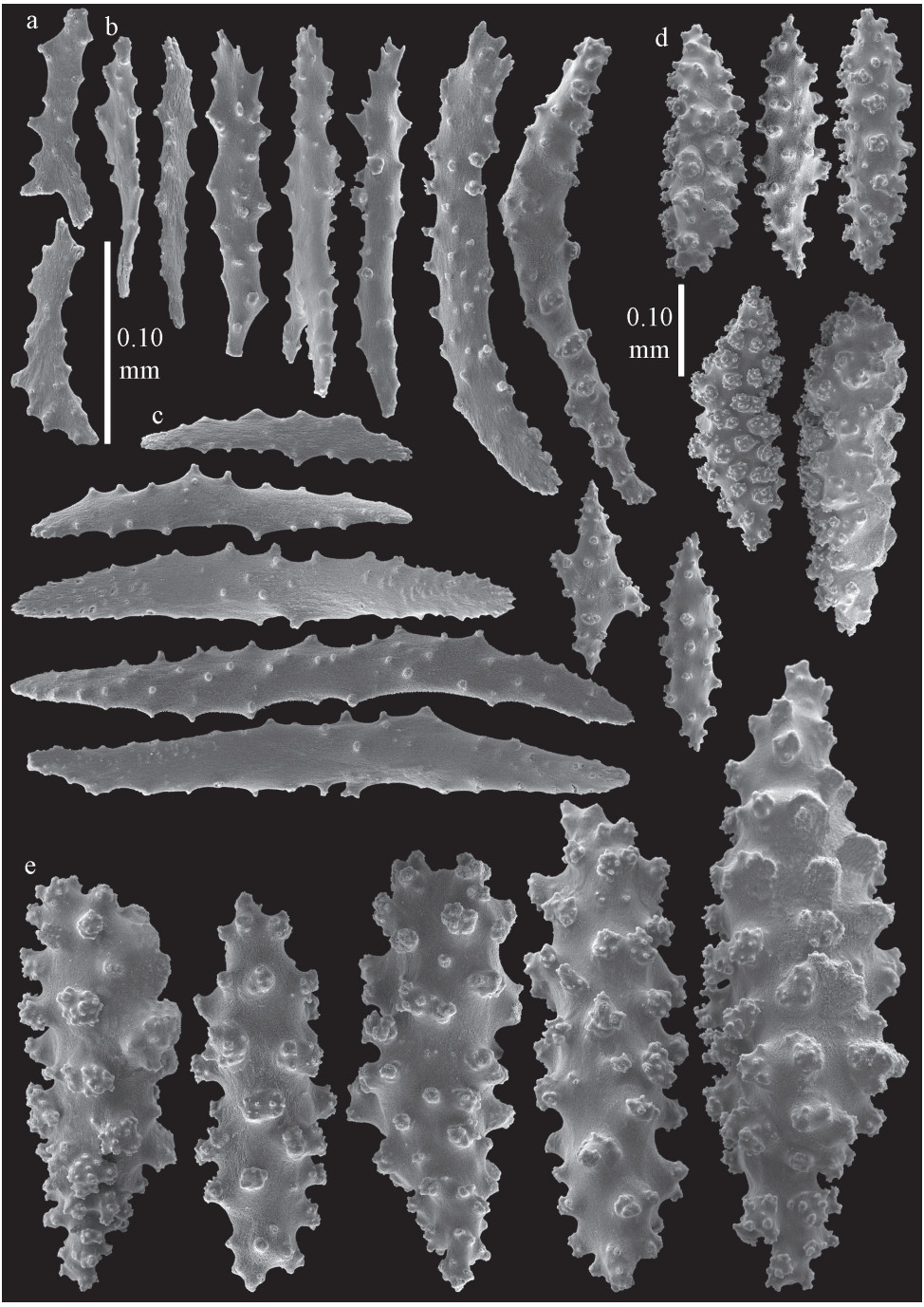


Figure 8. *Euplexaura mitsukurii* (Kinoshita, 1909), syntype UMUTZ-CnidG-222 **a** tentacle scales **b** point spindles **c** collaret spindles **d–e** spindles from surface layer of coenenchyme. Scale at d only applies to **d**.

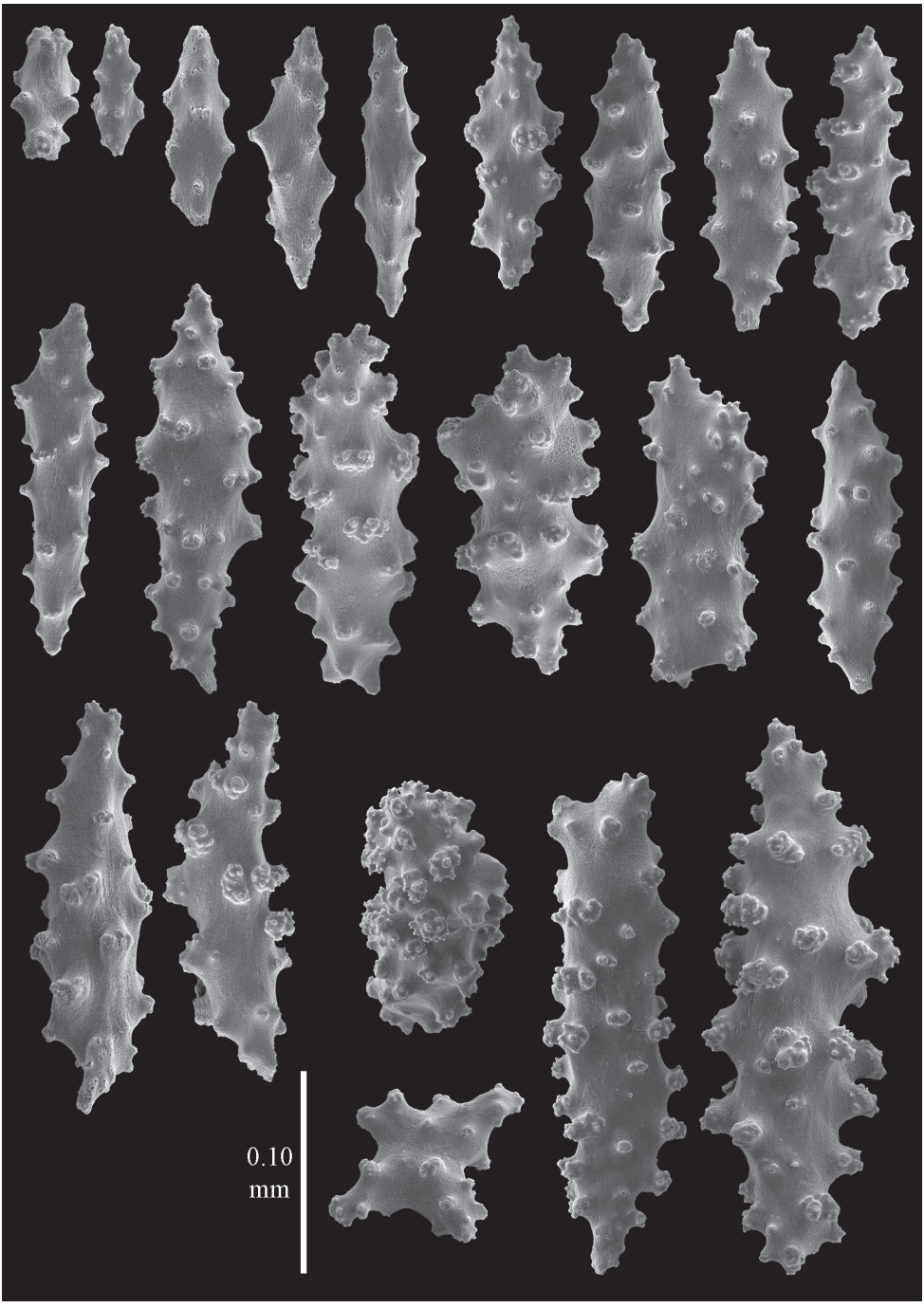


Figure 9. *Euplexaura mitsukurii* (Kinoshita, 1909), syntype UMUTZ-CnidG-222 sclerites of interior of coenenchyme.

Material examined. Syntypes UMUTZ-CnidG-222, off Jogashima I., Sagami Bay, Japan, depth 70 Japanese fathoms (100-106 m), secured with Hydra dredge, 26 August 1901; UMUTZ-CnidG-223, Japanese 2 nautical miles (5 km in Kinoshita, 1909) of West South off Jogashima I., Sagami Bay, Japan, saba-nawa line, 31 July 1892. Identified museum material UMUTZ-CnidG-122, off Torishima I., Japan, East China Sea, 28°10'N, 126°2'E - 28°20'N, 126°11'E, depth 64 fms (117 m), trawl, coll. N. Yanaghi, 22 June 1913, det. F.M. Bayer, ca.1950, as *Filigella mitsukurii*; UMUTZ-CnidG-126 same data as UMUTZ-CnidG-122, as *Filigella mitsukurii*. Unidentified museum material. ZMUC ANT-000611 (ZMUC120604-09), East China Sea, 33°41'N, 128°50'E, depth 75 fms (137 m), sand, *Hyateri maru*, trawl, coll. Dr. Th. Mortensen, 17 May 1914; ZMUC ANT-000616 (ZMUC120604-16), East China Sea, 32°15'N, 128°17'E, depth 90 fms (165 m), hard bottom, *Hyateri maru*, coll. Dr. Th. Mortensen, 15 May 1914; ZMUC ANT-000664 (ZMUC120604-59), 34°20'N, 130°10'E, depth 60 fms (110 m), sand, coll. Dr. Th. Mortensen, 18 May 1914; ZMUC ANT-000655 (ZMUC120604-67), off Misaki Biological Station, Sagami Bay, Japan, depth 200 fms (366 m), sand, coll. Dr. Th. Mortensen, 30 June 1914; AKM1630, Sukumo Bay, Bungo Channel, Japan, ca.32°38'N, ca.132°29'-30'E, depth 144-150 m, *RV Tansei-maru*, KT86-16, st.A-8, 1 m ORI biological dredge, coll. S. Ohta, 1 November 1986; AKM1631, off Kashima, Kashima Sea, Japan, 36°07'N, 140°49.0'E, depth 63-71 m, *RV Tansei-maru*, KT79-13, st. KB2, 2 m Beam trawl, coll. S. Ohta, 7 August 1979; AKM1632, off Toi, Suruga Bay, Japan, depth 192-207 m, 34°55.83'N, 138°44.85'E - 34°56.62'N, 138°45.0'E, *RV Tansei-maru*, KT75-15, st. 02, 2 m Beam Trawl, coll. S.Ohta, 24 November 1975; AKM1566, South East off Taito-saki Cape, Boso Peninsula, Japan, 35°21.259'N, 140°45.27'E - 35°21.359'N, 140°45.613'E, depth 104-105 m, *RV Tansei-maru*, KT01-08, st. TZ-7, 1 m ORI biological dredge, coll. S. Ohta, 22 June 2001; AKM 1644, off Hitachi, Kashima sea, Japan, 36°36.4'N, 140°50.1'E - 36°35.2'N, 140°50.5'E, depth 79–82 m, *RV Tansei-maru*, KT79-13, st. KB14, 2 m Beam trawl, coll. S. Ohta, 9 August 1979.

Diagnosis. Branches thread-like. The examined syntype has two branches arising from the main stem with a length of 19 cm and 28.5 cm, respectively; the main stem is 9 cm long. Calyces dome-shaped, arranged all around the branches (Figure 2d). The polyps have points with flattened spindles, up to 0.20 mm long (Figure 8b), with simple tubercles. Collaret present, with slightly bent, flattened spindles, up to 0.30 mm long, with sparse, simple tubercles (Figure 8c). Tentacles with small scales, up to 0.10 mm long (Figure 8a).

The surface layer of the coenenchyme has spindles (Figure 8d–e), up to 0.35 mm long, with complex tubercles. Some of them with one side that is less tuberculate. The interior has small spindles and rods, up to 0.25 mm long (Figure 9), with simple tubercles.

Remarks. Because the sclerites of this species are spindles with complex tubercles this is actually a species of *Euplexaura*.

Kinoshita (1909) mentioned 13 specimens of *Filigella mitsukurii* and three of them were complete. He used two specimens for his original description. Nowadays

two specimens are present in UMUT and the data fit Kinoshita's, two specimens in his description.

The locality name "Jogaschima, Pagamibai" of this species in Kükenthal (1924) is a mistyping of "Jogashima, Sagamibai (Sagami Bay)".

Distribution. Sagami Bay, off Boso Peninsula, Kashima Sea, Suruga Bay, Bungo Channel, East China Sea, Japan.

***Euplexaura yayoii* sp. n.**
http://zoobank.org/65B660AC-70D9-4697-8411-A1F3116FFD47
Figures 1, 2e, 10–11

Material examined. Holotype RMNH 42104 (AKM1551), Off Ohako-zaki cape, Otsuchi Bay, Iwate Prefecture, Japan. 142°00.640'E 39°31.400'N, depth 77.0 m, *RV Yayoi*, st. 4-1, coll. A.K. Matsumoto, 27 April 2010; paratypes RMNH 42105 (AKM592), entrance of Otsuchi Bay, Iwate Prefecture, Japan, 39°21.858'N, 141°59.972'E, depth 65.6 m, *RV Yayoi*, st. 1, coll. A.K. Matsumoto, 12 September 2005; RMNH 42106 (AKM597), same data as AKM 592; RMNH 42107 (AKM623), off Ohako-zaki cape, Otsuchi Bay, Iwate Prefecture, Japan, 142°00.556'E 39°21.452'N, depth 63 m, *RV Yayoi*, st. 2, coll. A.K. Matsumoto, 12 September 2005.

Description. The holotype is 2.5 cm high and 5.5 cm wide (Figure 2e). The colony is branched only once, 1 cm above the base. The two branches are very slender, only 1 mm thick; the calyces are low, dome-shaped, arranged spirally around the branches.

The polyps have points with slightly bent, flattened spindles, up to 0.30 mm long, with a few tubercles and a slightly spiny distal end (Figure 10b). The collaret has bent, flattened spindles, up to 0.30 mm long, with simple tubercles, the largest tubercles present in the middle (Figure 10c). The tentacles have flattened rods, up to 0.15 mm long, with hardly any tubercles (Figure 10a). The surface layer of the branches has spindles and blunt ellipsoids, up to 0.15 mm long, with complex tubercles (Figure 11). The deeper layer has short spindles, up to 0.10 mm long, and a few crosses (Figure 10d-e); all with simple tubercles.

Etymology. Named after the research vessel that was used to collect the specimens.

Remarks. The live colony has blue-coloured polyps. *E. yayoii* differs from the two other Japanese *Euplexaura* species with thread-like branches, *E. boninensis* and E. *mitsukurii*, by its very small sclerites.

Distribution. Otsuchi Bay, Iwate Prefecture, Japan.

Discussion

Originally, there were four species of *Elasmogorgia*: *E. filiformis* Wright & Studer, 1889, *E. filigella* Thomson and Dean 1931, *E. flexilis* Hickson, 1905, and *E. ramosa* Nutting, 1912. Based on the present re-examination, it is obvious that *Elasmogorgia filiformis*,

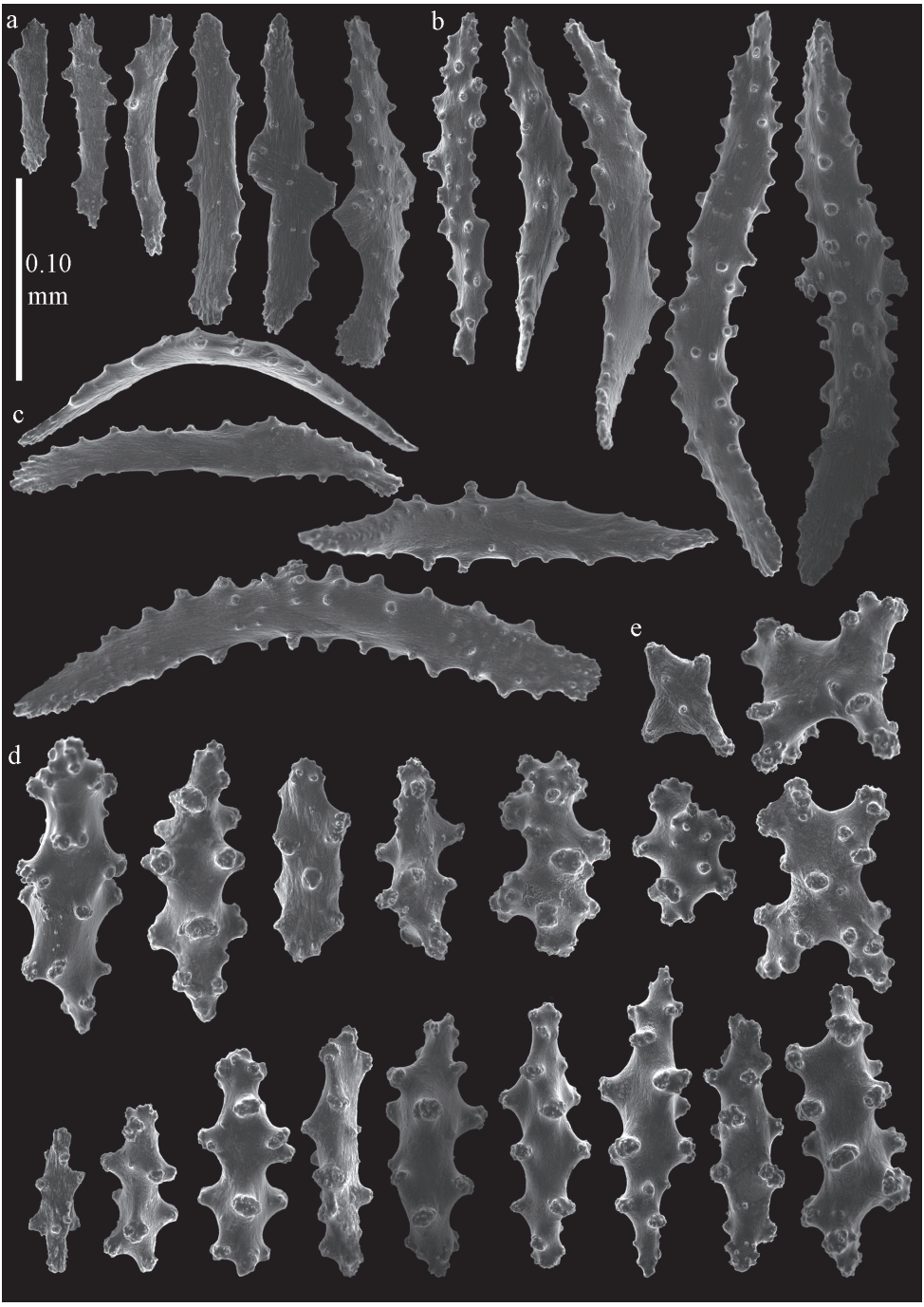


Figure 10. *Euplexaura yayoii* sp. n., holotype RMNH 42104 **a** tentacle scales **b** point spindles **c** collaret spindles **d–e** sclerites of interior of coenenchyme **d** spindles **e** crosses.

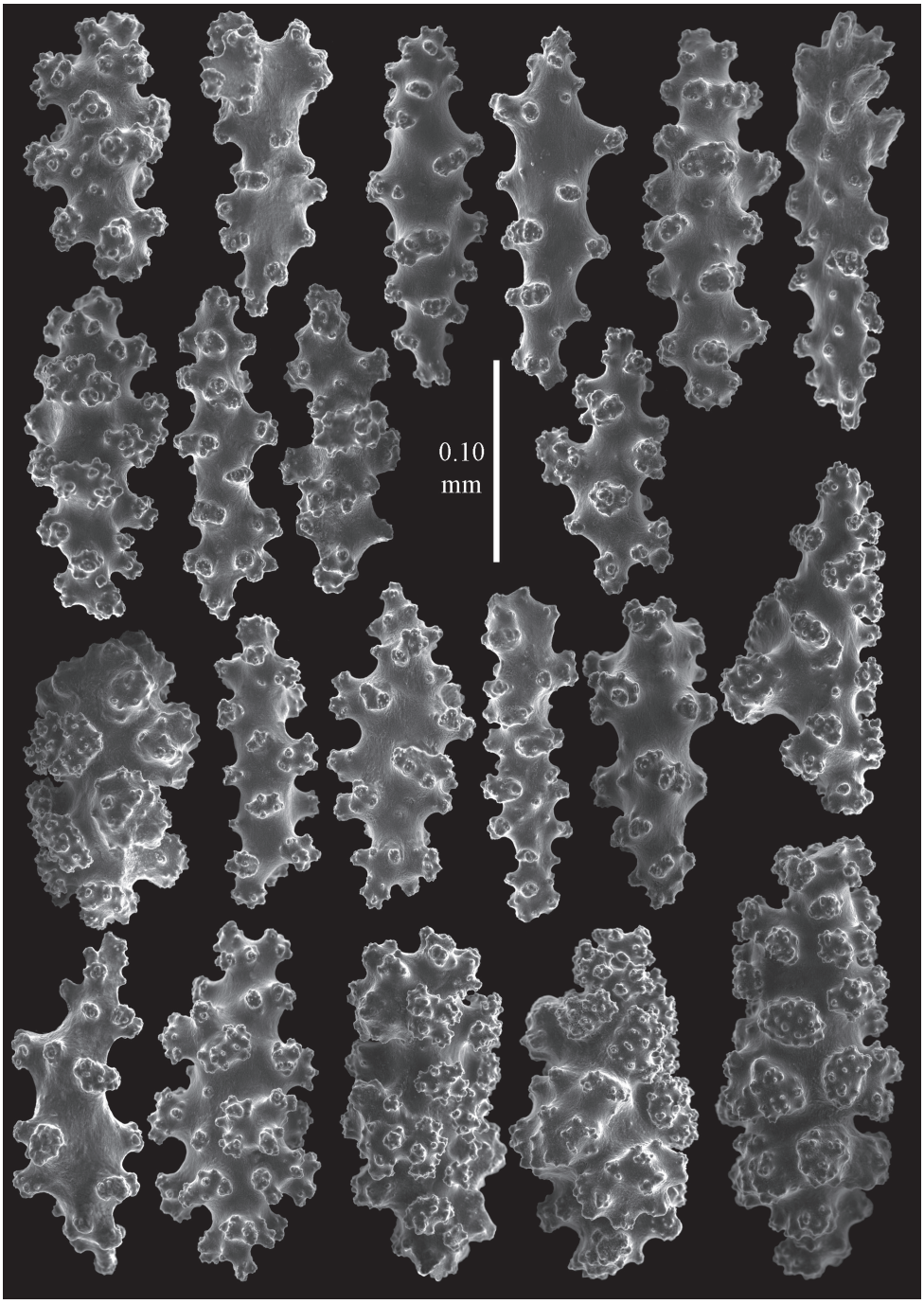


Figure 11. *Euplexaura yayoii* sp. n., holotype RMNH 42104 sclerites from surface layer of coenenchyme.

with spindles covered by simple tubercles, is not a species of *Thesea*. Corals of this genus have coarse rugose plates, sometimes tuberculate spindles and double heads (Bayer 1981). Therefore the genus *Elasmogorgia* is reinstated here. The only two species from Japan previously recognized as *Filigella*, *i.e.*, *F. mitsukurii* and *F. boninensis*, were re-examined and both proved to belong to the genus *Euplexaura*. *Elasmogorgia filigella* from Kalimantan is a species of *Astrogorgia*. This leaves *E. ramosa* and *E. flexilis* unexamined. *E. ramosa* was collected by the Steamer Albatross at Satamisaki Light, south of Kyushu I., Kagoshima prefecture, Japan, 103 fms (188 m), and *E. flexilis* from the Maldives. From the descriptions of these two species it is obvious that *E. ramosa*, with a heavily branched colony, is not a *Thesea* or *Elasmogorgia*. *Elasmogorgia flexilis*, with spindles with complex tubercles probably is a species of *Euplexaura*, and therefore the genus *Elasmogorgia* is considered here monotypic with *E. filiformis* as its only member. *E. filiformis* mostly resembles a species of *Astrogorgia*. Following Bayer (1981) we also consider *Filigella* a synonym of *Thesea.* All Japanese species previously included in *Filigella* are assigned to *Euplexaura* in this study.

All Japanese thread-like plexaurid material South of Kashima Sea was previously identified as *F. mitsukurii* and it clearly is the most common thread-like plexaurid species of Japan.

Acknowledgements

We thank the following persons for allowing us to examine material and for hosting: Ms. Miranda Lowe and Mr. Andrew Cabrinovic, BMNH, London, UK; Dr. Rei Ueshima, UMUTZ, Tokyo, Japan; Ms. Erica Sjölin and Mr. Hans Mejlon, ME, Uppsala, Sweden; Dr. Ole Tendal, Ms. Majken Toettrup, Ms. Laura Pavesi, and Dr. Martin V. Sørensen, ZMUC, Copenhagen, Denmark; Dr. Sergey D. Grebelnyi, ZIN, St. Petersburg, Russia. Dr. Suguru Ohta, University of Tokyo, the captain and crew of *RV Tansei-maru* and *RV Yayoi* are thanked for collecting. Dr. Tina Molodtsova, Institute of the Russian Academy of Sciences (IORAS), Moscow, Russia, is acknowledged for arranging for visiting ZIN and translating Russian to English. The first author would like to thank Dr. Manfred Grasshoff, Senkenberg Nature Museum, Germany, for his suggestion to study specimens in ME, Uppsala. We are grateful to the International Coastal Research Center in Otsuchi for the use of its facilities. The SEM facility and financial support of the Planetary Exploration Research Center (PERC), Chiba Institute of Technology is thanked. The research was partially financially supported by Cooperation Research from the Ocean Research Institute, University of Tokyo (H10-109, H13-110, H14-132, H16-131, H17-131, H18-110, H20-001, H21-001, H22-007, H23-129) and by the Higashi Nippon International University. We thank Dr. Catherine McFadden and Dr. Bert W. Hoeksema for comments on the manuscript.

References

Aurivillius M (1931) The Gorgonarians from Dr. Sixten Bock's, expedition to Japan and Bonin Islands 1914 (Vol. 3). Kungliga Svenska Vetenskapsakademien handlingar 9(4): 1–337.

Bayer FM (1959) Octocorals from Suriname and the adjacent coasts of South America. Studies on the Fauna of Suriname and other Guyanas 6: 1–43.

Bayer FM (1981) Key to the genera of Octocoralia exclusive of Pennatulacea (Coelenterata: Anthozoa), with diagnosis of new taxa. Proceedings of the Biological Society of Washington 94(3): 902–947.

Bayer FM, Grasshoff M, Verseveldt J (1983) Illustrated Trilingual Glossary of Morphological and Anatomical Terms Applied to Octocorallia. E. J. Brill, Leiden, 75 pp.

Broch H (1935) Oktokorallen des Nördlichsten Pazifischen Ozeans. Avhancllinger utgitt av det Norske Videnskaps-Akademi i Oslo. I. Mat-Naturv. 1935(1): 1–53.

Duchassaing P, Michelotti J (1864) Supplément au mémoire sur les coralliaires des Antilles. Memoria Reale accademia delle scienze di Torino (2) 23: 97–206. doi: 10.5962/bhl.title.105196

Gray JE (1868) Descriptions of some new Genera and Species of Alcyonoid Corals in the British Museum. Annals and magazine of natural history 2(4): 441–445. doi: 10.1080/00222936808695849

Hickson SJ (1905) The Alcyonaria of the Maldives part III. The families Muriceidae, Gorgonellidae, Melitodidae, and the Genera *Pennatula*, *Eunephthya*. In: Gardiner JS (Ed.) The Fauna and Geography of the Maldive and Laccadive Archipelagoes 2(4). Cambridge, 807–826.

Kinoshita K (1909) On some Muriceid Corals belonging to the Genera *Filigella* and *Acis*. Journal of the College of Science, Imperial University (Tokyo) 27(7): 1–16.

Kükenthal W (1909) Japanische Gorgoniden. 2 Teil: Die Familien der Plexauriden, Chrysogorgiiden und Melitodiden. In: Doflein F (Ed.) Beitrage zur Naturgeschichte Ostasiens. Abhandlungen der Mathematisch-Physikalische Klasse der Königlich-Bayerische Akademie der Wissenschaften, Supplment Band 1(5): 1–78.

Kükenthal W (1919) Gorgonaria. Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899 13(2): 1–946.

Kükenthal W (1924) Gorgonaria – Das Tierreich 47. Berlin and Leipzig, 478 pp.

Matsumoto AK (2014) The Relation between Telegraph Cables of Great Nordic Ltd. (Store Nordiske) and Japanese Octocoral Collection in Copenhagen, Denmark and UUZM, Uppsala, Sweden. Journal for the Comparative Study of Civilizations (JCSC) 19: 153–186. [In Japanese, with English Tables]

Matsumoto AK, Iwase F, Imahara Y, Namikawa H (2007) Bathymetric distribution and biodiversity of deep-water octocorals (Coelenterata: Octocorallia) in Sagami Bay and adjacent waters of Japan. Bulletin of Marine Science 81(Suppl. 1): 231–252.

Nutting CC (1909) Alcyonaria of the Californian coast. Proceedings of the United States National Museum 35: 681–727. doi: 10.5479/si.00963801.35-1658.681

Nutting CC (1910) The Gorgonacea of the Siboga Expedition III. The Muriceidae. Siboga-Expedition Monograph 13b: 1–108.

Nutting CC (1912) Descriptions of the Alcyonaria collected by the U.S. Fisheries steamer "Albatros", mainly in Japanese waters, during 1906. Proceedings of the United States National Museum 43: 1–104. doi: 10.5479/si.00963801.43-1923.1

Ofwegen LP van (2016a) *Elasmogorgia*. Accessed through: World Register of Marine Species (WoRMS) at http://www.marinespecies.org/aphia.php?p=taxdetails&id=267406 [on 24 February 2016]

Ofwegen LP van (2016b) *Filigella*. Accessed through: World Register of Marine Species (WoRMS) at http://www.marinespecies.org/aphia.php?p=taxdetails&id=291506 [on 24 February 2016]

Ofwegen LP van (2016c) *Thesea*. Accessed through: World Register of Marine Species (WoRMS) at http://www.marinespecies.org/aphia.php?p=taxdetails&id=125315 [on 24 February 2016]

Ofwegen LP van, Hoeksema BW (2001) *Astrogorgia bayeri*, a new gorgonian octocoral species from South Sulawesi (Coelenterata: Octocorallia: Plexauridae). Bulletin of the Biological Society of Washington 10: 66–70.

Soest RWM van (1979) A catalogue of the coelenterate type specimens of the Zoological Museum of Amsterdam. IV. Gorgonacea, Actiniaria, Scleractinia. Beaufortia 29(353): 81–126.

Studer T (1887) Versuch eines Systemes der Alcyonaria. Archiv für Naturgeschichte 53(1): 1–74.

Thomson JA, Dean LM (1931) The Alcyonacea of the Siboga Expedition with an addendum to the Gorgonacea. Siboga Expedition Monograph 13d: 1–227.

Thomson JA, Russell ES (1910) Alcyonarians collected on the Percy Sladen Trust Expedition by Mr. J. Stanley Gardiner. Part 1, the Axifera. Transactions of the Linnean Society of London (2)13(2): 139–164.

Thomson JA, Simpson JJ (1909) An account of the Alcyonarians collected by the RIM SS Investigator in the Indian Ocean. II. The Alcyonarians of the littoral area: XII, 319 pp.

Tixier-Durivault A (1966) Octocoralliaires de Madagascar et des îles avoisinantes. Faune Madagascar 21: 1–456.

Utinomi H (1961) Noteworthy octocorals collected off the southwest coast of Kii Penin., middle Japan. Pt. 2, Telestacea, Gorgonacea and Pennatulacea. Publications of the Seto Marine Biological Laboratory 9(1): 197–228.

Verrill AE (1869) Synopsis of the polyps and corals of the North Pacific Exploring Expedition, under Commodore C. Ringgold And Capt. John Rodgers, U.S.N., from 1853 to 1856. Collected by Dr. Wm. Stimpson Naturalist to the Expedition. Proceedings of the Essex Institute 6: 51–104.

Wright EP, Studer T (1889) Report on the Alcyonaria collected by H.M.S. Challenger during the years 1873-1876. Report on the scientific results of the voyages of H.M.S. Challenger during the years 1873–1876, Zoology 31: 1–314.

ZooKeys 589: 23–53 (2016)
doi: 10.3897/zookeys.589.7933
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

The first revision of the carnivorous land snail family Streptaxidae in Laos, with description of three new species (Pulmonata, Stylommatophora, Streptaxidae)

Khamla Inkhavilay[1], Thanit Siriboon[1], Chirasak Sutcharit[1], Ben Rowson[2], Somsak Panha[1]

1 *Animal Systematics Research Unit, Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand* **2** *Department of Natural Sciences, National Museum of Wales, Cathays Park, Cardiff CF10 3NP, United Kingdom*

Corresponding authors: *Chirasak Sutcharit* (jirasak4@yahoo.com); *Somsak Panha* (somsak.pan@chula.ac.th)

Academic editor: *E. Gittenberger* | Received 26 January 2016 | Accepted 12 April 2016 | Published 16 May 2016

http://zoobank.org/EECEC70C-A983-43C4-86A2-08CD536293EB

Citation: Inkhavilay K, Siriboon T, Sutcharit C, Rowson B, Panha S (2016) The first revision of the carnivorous land snail family Streptaxidae in Laos, with description of three new species (Pulmonata, Stylommatophora, Streptaxidae). ZooKeys 589: 23–53. doi: 10.3897/zookeys.589.7933

Abstract

The family Streptaxidae in Laos is revised. Twelve species are known, mainly from limestone areas, in the genera *Discartemon* Pfeiffer, 1856, *Perrottetia* Kobelt, 1905, *Haploptychius* Möllendorff, 1906, and *Indoartemon* Forcart, 1946. Three new species, *P. unidentata* **sp. n.** and *P. megadentata* **sp. n.** from northern and central Laos, and *I. diodonta* **sp. n.** from central Laos, are described. All eight species of these three genera previously recorded from Laos are revised and discussed based on examined material from Laos, Cambodia, Vietnam and Thailand. Type material was examined and lectotypes are designated. Details of genital anatomy and radulae are provided, including the first detailed genitalia and radula descriptions from *Haploptychius*. Two novelties in Streptaxidae, a vaginal caecum, and the occurrence of aphallic individuals, are reported from *H. pellucens* (Pfeiffer, 1863).

Keywords

Limestone, tropical forest, systematics, type specimen, Southeast Asia, predator, taxonomy, aphally

Introduction

The Streptaxoidea currently comprises two families, the worldwide Streptaxidae Gray, 1860 and the Southeast Asian endemic Diapheridae Panha and Naggs, 2010 (Richardson 1988, Schileyko 2000, Rowson et al. 2010a, Sutcharit et al. 2010). The Streptaxidae Gray, 1860 are active predators with an eccentric to cylindrical shell, usually with apertural dentition, and a yellowish to orange soft body (Zilch 1960, Schileyko 2000, Rowson et al. 2010b, Siriboon et al 2013, 2014a, b).

Early classifications of the family such as W. Kobelt (1905–6), used mainly shell shape and the arrangement of apertural dentition. However, many shell characters are highly conserved or occur recurrently, making some species and genera difficult to separate. Fortunately, the reproductive organs of streptaxids can also be taxonomically significant (e.g. Schileyko 2000, Rowson and Tattersfield 2013, Siriboon et al. 2013, 2014a, b). Few reports have contributed data on the genitalia of Southeast Asian taxa (e.g. Stoliczka 1871, Berry 1963, 1965) until recently (Siriboon et al. 2013, 2014a, b, Páll-Gergely et al. 2015).

In Indochina, streptaxid diversity was throught to comprise only 10 genera and about 40 species (Bruggen 1967). However, in the last decade 21 new species (more than half the previous total) and one new genus have been described from Indochina (Siriboon et al. 2013, 2014a, b, Do and Do 2015). Thirty-seven species are recorded from Thailand (Panha 1996, Hemmen and Hemmen 2001, Siriboon et al. 2013, 2014a, b), 10 from Myanmar (Blanford and Godwin-Austen 1908), and 45 from Vietnam (Schileyko 2011). In contrast, only three species were reported from Laos in the past two centuries (Pfeiffer 1863, Möllendorff 1898), with three others added in recent years (Schileyko 2011, Do and Do 2015).

Almost all groups of the land snail fauna in Laos have been less-well studied than those of neighbouring areas. The Lao People's Democratic Republic, until recently encompassed some of the most significant forest areas remaining in Southeast Asia such as mountainous areas in the north and limestone karsts in central area, and some of the most intact biota left in Asia (Kemp 2011). Those habitat characteristics also harbor diverse of terrestrial molluscan fauna. The present paper focuses on the four genera, *Discartemon* Pfeiffer, 1856, *Perrottetia* Kobelt, 1905, *Haploptychius* Möllendorff, 1906, and *Indoartemon* Forcart, 1946 that were formerly recorded from Laos. Genital anatomy and shell micro-structures of are carefully investigated. The type specimens of all known species were examined, and the penial hooks and radula morphology of *Haploptychius* are defined for the first time. This adds significantly to knowledge of the Streptaxidae in Indochina and especially in Laos.

Materials and methods

Animals were collected from evergreen forest in the north, and limestone karsts and dipterocarp forest in the south of Laos. Live specimens were photographed

and then stored at -20 °C and then preserved in 70% ethanol (v/v) for anatomical studies. The identifications were based on Pfeiffer (1863), Möllendorff (1898), Kobelt (1905–1906), Blanford and Godwin-Austen (1908), Bavay and Dautzenberg (1903, 1908), and Siriboon et al. (2013, 2014b). Shell height (H), shell width (W), whorl count and H/W ratio were measured and interpreted following Siriboon et al. (2013). Shells and genitalia were investigated and digital images taken using Cell'D Imaging Software. All live adult specimens of each species were dissected and the genitalia examined under a stereo-microscope and representatives selected for illustrations under a camera lucida. The buccal masses were removed, and the radulae were soaked in 10% NaOH then cleaned in distilled water. Radula, penial hooks and vaginal hooks were examined and photographed under SEM (JEOL, JSM-5410 LV). In the descriptions, 'proximal' relates to the genital orifice, and 'distal' refers to the region furthest away from the genital orifice. The term 'vaginal caecum' is defined herein.

Anatomical abbreviations. ag, albumen gland; at, atrium; fo, free oviduct; gd, gametolytic duct; gs, gametolytic sac; hd, hermaphroditic duct; ov, oviduct; p, penis; pr, penial retractor muscle; ps, penial sheath; psr, penial sheath retractor muscle; sv, seminal vesicle; ta, talon; v, vagina; vc, vaginal caecum; vd, vas deferens (Sutcharit et al. 2010, Siriboon et al. 2013, 2014a, b).

Institutional abbreviations. Materials examined in this study were deposited in the following institutions:

CUMZ Chulalongkorn University Museum of Zoology, Bangkok.
MNHN Muséum National d'Histoire Naturelle, Paris.
NHMUK The Natural History Museum, London.
NUOL National University of Laos, Vientiane.
SMF Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main.

Systematics

Family Streptaxidae Gray, 1860

Genus *Discartemon* Pfeiffer, 1856

Discartemon Pfeiffer, 1856: 173. Siriboon et al. 2014a: 48, 49.
Odontartemon (*Discartemon*)—Kobelt 1905: 91, 96.

Type species. *Streptaxis discus* Pfeiffer, 1851, by subsequent designation by Ancey (1884: 399).

Remark. The genus was recently revised. For complete illustrations, species descriptions and dichotomous key see Siriboon et al. (2014a).

Discartemon discus (Pfeiffer, 1853)

Streptaxis discus Pfeiffer, 1853: 252. Type locality: Unknown.
Streptaxis (*Discartemon*) *paradiscus* Möllendorff, 1900: 117. Type locality: Phucson bei Touranne, Annam [Da Nang Province, Vietnam].
Discartemon discus—Siriboon et al. 2014a: 53–55, figs 4a–c, 11a–c, 22a.

Material examined. Lectotype of *Streptaxis discus* Pfeiffer, 1853 NHMUK 20130684. Lectotype of *Streptaxis paradiscus* Möllendorff, 1900 SMF 108534 and paralectotypes SMF 108535 (5 shells).

Remarks. *Discartemon discus* has been recently re-described from the shell, genitalia and radula, and type specimens were re-investigated and illustrated (see Siriboon et al. 2014a).

All previous records of this species were all from "Annam" (Siriboon et al. 2014a). This term is a historical political division during the colonial period, with an uncertain boundary. The distribution of *D. discus* (=*D. paradiscus*) in Laos was reported by Schileyko (2000: 784, 2011: 23). However, no specimens were found by the present study and the records from Laos remain to be confirmed.

Genus *Haploptychius* Möllendorff, 1906

Haploptychius Möllendorff in Kobelt 1906: 127. Zilch 1960: 562. Richardson 1988: 211. Schileyko 2000: 796, 797.
Odontartemon (*Haploptychius*)—Thiele 1931: 730. Forcart 1946: 215.
Oophana (*Haploptychius*)—Benthem Jutting 1954: 76, 95.

Type species. *Streptaxis sinensis* Gould, 1859, by original designation.

Description. Shell depressed to very distorted, mostly white-hyaline or transparent. Shell surface smooth and glossy or with fine radial ridges. Embryonic shell smooth; following whorls increasing regularly; penultimate whorls slightly to strongly extended beyond body whorl. Last whorl rounded and more or less deviated from the vertical axis. Umbilicus narrowly open and deep. Aperture sub-circular to semi-ovate. Peristome expanded and reflected. Apertural dentition always consisting of a single parietal lamella. Schileyko (2000) includes species with a "smooth" parietal wall, i.e. without a lamella in *Haploptychius*, but whether such taxa belong in this genus requires further investigation.

Live specimens exhibit a semi-transparent bright yellow body, sometimes with brownish spots; skin reticulated. Upper tentacles yellow to orange, long, with black eye-spot on tip; lower tentacles short. Brownish digestive gland and black kidney may be visible through transparent shell. Foot narrow, undivided and with short tail.

Genitalia with long and slender penis; penial sheath long, about a half to whole length of penis. Internal wall of penis with numerous long and slender penial hooks in

longitudinal arrangement. Vas deferens passes under penial sheath before connecting apically to penis. Vagina and free oviduct short. Seminal vesicle present, convoluted and short. Vaginal hooks not found.

Remarks. Currently, the genus *Haploptychius* consists of about 40 nominal species distributed from India to Indochina, south of China and Greater Sunda Islands (Kobelt 1906, Zilch 1961, Richardson 1988, Schileyko 2000). Fifteen species were reported from Indochina, of which only three species: *H. pellucens* (Pfeiffer, 1863), *H. porrectus* (Pfeiffer, 1863) and *H. fischeri* (Morlet, 1887) were recorded from Laos (see Gude 1903, Kobelt 1906, Schileyko 2011).

General shell morphology of *Haploptychius* is quite similar to *Oophana* Ancey, 1884 and *Indoartemon* Forcast, 1946. However, it differs in having only a parietal lamella; while *Oophana* usually has parietal, palatal, columellar and basal lamellae, and *Indoartemon* always has parietal and basal lamellae. In addition, the genitalia of *Haploptychius* have a penial sheath extends about a half to entire the penis length, vas deferens passes through penial sheath, and long slender penial hooks. In *Oophana*, the vas deferens enter the penial sheath apically with very short vagina (Berry 1963, Schileyko 2000); and *Indoartemon*, the vas deferens attached (not pass through) the penial sheath, with small and short penial hooks (Siriboon et al. 2013).

Carinartemis Siriboon & Panha, 2013 resembles *Haploptychius* in having only a parietal lamella. However, it differs from *Haploptychius* in its very sharp peripheral keel and having the last whorl more deviated from the vertical axis. In addition, the genitalia has thick or thin penial sheath, penial hook short and stout, and vaginal hooks present (Siriboon et al. 2014b).

The relatively large, distorted heliciform shell and dentition restricted to a parietal lamella clearly differentiate *Haploptychius* from *Discartemon* Pfeiffer, 1856 and *Perrottetia* Kobelt, 1905 (Schileyko 2000, Siriboon et al. 2013, 2014a, b).

***Haploptychius pellucens* (Pfeiffer, 1863)**

Figs 1, 2A, 3A–C, 7A, B, 8A–D, 9A–F, 10G; Table 1

Streptaxis pellucens Pfeiffer 1863 [1862]: 273, pl. 36, fig. 6. Type locality: Lao Mountain, Camboja [Cambodia]. Martens 1867: 85. Pfeiffer 1868: 441. Pfeiffer 1871: 29, pl. 115, figs 11, 12. Morlet 1883: 105, pl. 4, fig. 2, 2a. Tryon 1885: 71, 72, pl. 14, figs 98–100. Gude 1903: 212.

Haploptychius pellucens—Kobelt 1906: 132, 133, pl. 61, figs 17–20. Richardson 1988: 217, 218. Schileyko 2011: 24, 25.

Material examined. This species was described from the H. Cuming collection. An illustration of the shell and one set of measurements were given in the original description. Three specimens from the Cuming collection at NHM have Pfeiffer's handwritten label stating the species name and locality. In order to stabilise the name, an identical specimen matching with the illustration and measurements given in the original de-

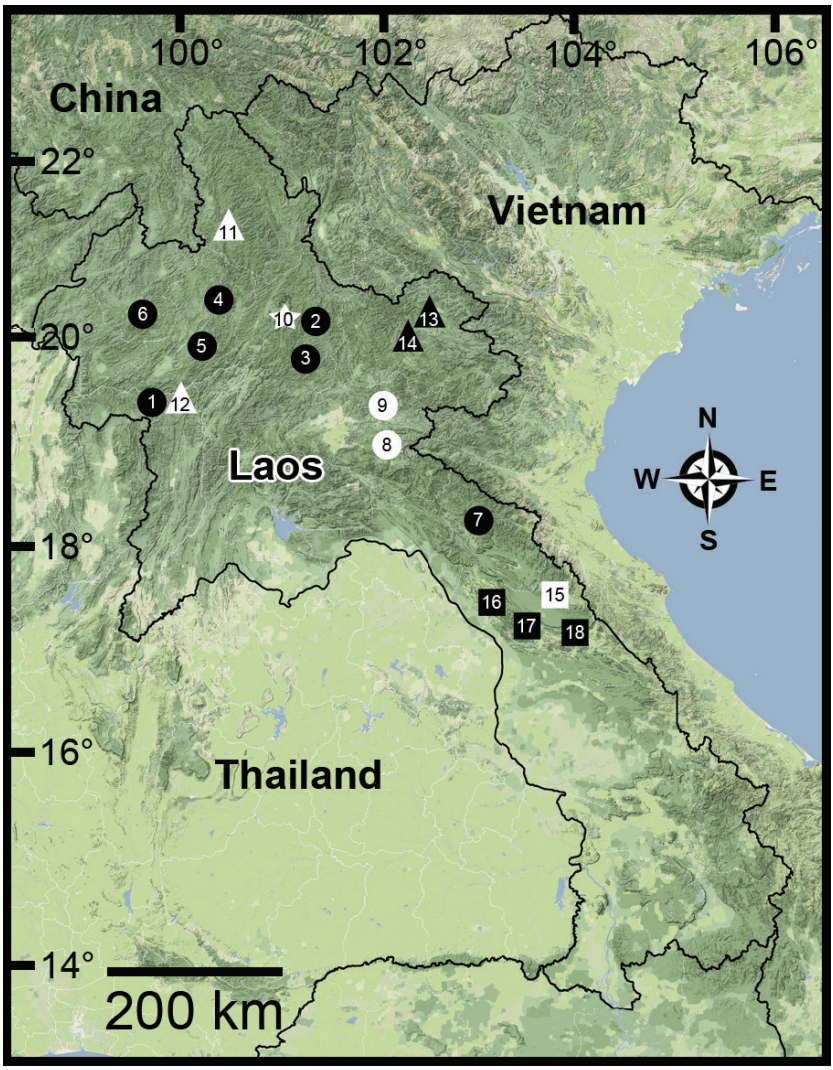


Figure 1. Approximate locations of the type localities of *Haploptychius* spp., *Perrottetia* spp., and *Indoartemon* spp. in Laos. Described species (●) *Haploptychius pellucens*, (○) *Haploptychius porrectus*, (★) *Haploptychius blaisei*, (△) *Perrotettia aquilonaria*, (▲) *Perrottetia unidentata* sp. n., (□) *Perrottetia megadentata* sp. n. and (■) *Indoartemon diodonta* sp. n. The numbered localities are detailed in Table 1, except locality no. 6 is from Tam Kao Rao, Vieng Phoukha, Luang Namtha, Laos, and no. 11 from Ban Bo Khoun, Boun Neua, Phongsaly, Laos.

scription is designated here as lectotype NHMUK 20160249.1 (Fig. 3A; H = 11.7, W = 11.2). The other two remaining shells from the same lot then became paralectotypes NHMUK 20160249.2 (2 shells; Fig. 3B; H = 11.1, W = 10.6 and H = 13.1, W = 13.4).

Cambodia: NHMUK MacAndrew coll. (4 shells). Ban Namone, Xayabouly, Laos (about 40 Km. from Ngeun District, Lao-Thai border to Xayabouly District): CUMZ

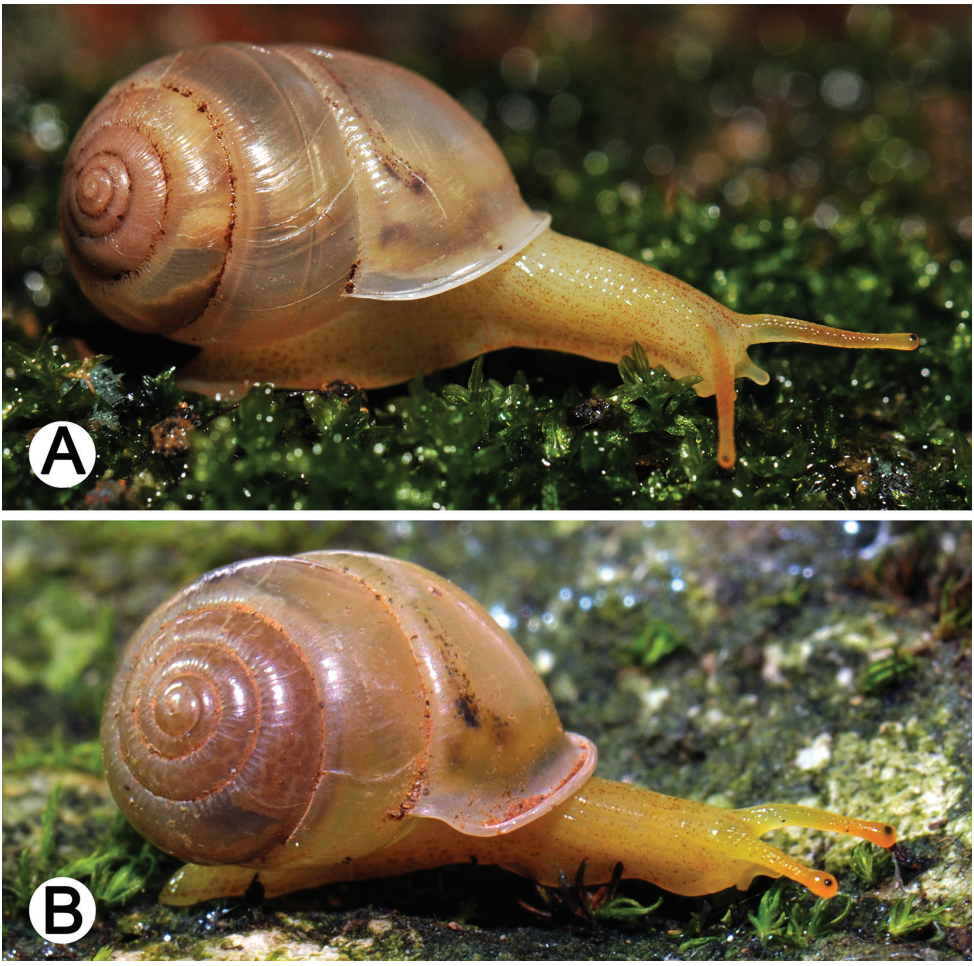


Figure 2. Living snails of **A** *Haploptychius pellucens* CUMZ 6265, from Xayabouly (shell width about 11 mm) and **B** *Haploptychius porrectus* CUMZ 6273, from Xieng Khuang (shell width about 7 mm).

6264 (Fig. 3C; 8 shells), 6265 (4 specimens in ethanol). Tam Phatok, Ngoi, Luang Phrabang, Laos: CUMZ 6267 (7 shells). Ngoi, Luang Phrabang, Laos: CUMZ 6268 (7 shells). Nam Ork Roo, Nathong, Namor, Oudomxay, Laos: CUMZ 6269 (29 shells), 2670 (6 specimens in ethanol; Figs 3A, B, 8A, 9A–F, 10G). Ban Oudom, Pak Beng, Oudomxay, Laos: CUMZ 6271 (15 shells). Tam Kao Rao, Vieng Phoukha, Luang Namtha, Laos: CUMZ 2672 (2 shells). Tam Mung Korn, Khamkeurt, Bolikhamxay, Laos: CUMZ 6266 (4 shells).

Description. Shell. Shell oblique-ovate, white and translucent. Whorls 6½, spire conical with distinct suture. Shell surface glossy with thin transverse ridges which diminish below periphery. Embryonic shell about 2½ whorls, with smooth surface; following whorls regularly coiled. Penultimate whorl and last whorl rounded, axially deflected.

Table 1. Shell measurements for populations of the three *Haploptychius*, four *Perrottetia*, and one *Indoartemon* species collected.

Specie and locality and CUMZ nos	No. of specimens	Rangs, mean ± S.D. in mm of:				Number of whorls
		Shell height (H)	Shell width (W)	H/W ration	Shell angle	
Haploptychius pellucens (Pfeiffer, 1863)						
Lectotype and paralectotypes	3	11.1–13.0 11.9±1.02	10.6–13.3 11.7±1.45	0.9–1.0 1.0±0.01	31.7–37.1 34.9±2.77	6–6½
1. Ban Namone, Xayabouly (about 40 Km. from Ngeun, Lao-Thai border to Xayabouly district): (6264, 6265)	12	10.1–12.10 11.0±0.55	9.6–12.0 10.6±0.63	0.9–1.18 1.0±0.09	33.6–53.02 44.2±5.26	6–6½
2. Tam Phatok, Luang Phrabang: (6267)	7	9.5–10.7 9.8±0.43	9.0–10.7 9.8±0.75	0.9–1.1 1.0±0.11	45.8–56.9 50.9±3.75	6–6½
3. Ngoi, Luang Phrabang: (6268)	7	9.7–12.4 11.1±0.90	10.4–11.8 10.9±0.48	0.9–1.1 1.0±0.01	48.8–54.1 51.2±1.91	6½
4. Nam Ork Roo, Nathong, Namor, Oudomxay: (6269, 6270)	35	9.5–11.5 10.2±0.53	9.3–12.0 10.8±0.61	0.8–1.1 0.9±0.08	42.5–54.1 48.9±3.19	6½
5. Ban Oudom, Pak Beng, Oudomxay: (6271)	15	10.6–13.1 12.0±0.77	9.5–12.8 11.2±0.76	0.8–1.3 1.0±0.12	37.6–58.3 47.9±5.21	6½–7
7. Tam Mungkorn, Khamkeurt, Bolikhamxay: (6266)	4	8.8–9.6 9.3±0.30	8.0–9.1 8.7±0.50	1.0–1.1 1.0±0.03	46.7–50.3 48.2±1.56	6½–7
Haploptychius porectus (Pfeiffer, 1863)						
8. Ban Nong Tang, Phoukood, Xieng Khuang: (6273, 6274)	19	6.2–8.1 7.4±0.50	6.3–8.4 7.4±0.52	0.8–1.2 0.9±0.11	41.1–59.6 49.6±4.58	6½
9. Tam Pew, Kham, Xieng Khuang: (6275)	4	6.5–7.2 7.0±0.34	7.3–8.5 7.7±0.59	0.8–0.9 0.9±0.06	44.1–47.1 45.0±1.52	6½
Haploptychius blaisei (Dautzenberg and Fischer, 1905)						
10. Tam Phatok, Ngoi, Luang Phrabang: (6276, 6277)	16	5.4–6.7 6.2±0.35	9.1–10.5 9.8±0.36	0.5–0.7 0.6±0.05	53.7–75.3 67.1±5.9	6½
Perrottetia aquilonaria Siriboon and Panha, 2013						
12. Ban Namone, Xayabouly (about 40 Km. from Ngeun, Lao-Thai border to Xayabouly District): (6278, 6279)	3	4.1–4.5 4.4±0.19	5.4–6.4 6.0±0.55	0.7–0.9 0.7±0.09	48.4–59.1 54.2±5.43	5½–6

Specie and locality and CUMZ nos	No. of specimens	Rangs, mean ± S.D. in mm of:				Number of whorls
		Shell height (H)	Shell width (W)	H/W ration	Shell angle	
Perrottetia unidentata sp. n.						
13. Ban Nawit, Viengxay: (6281, 6282, 6283)	8	4.0–5.8 5.0±0.05	8.9–9.7 9.3±0.25	0.4–0.6 0.5±0.06	67.0–88.9 76.8±6.74	6–6½
14. Tam Than Kaisone Phomvihan, Viengxay: (6284, 6285)	5	5.0–6.5 5.9±0.77	7.4–8.2 7.7±0.36	0.6–0.8 0.7±0.11	54.5–60.7 57.1±2.51	6
Perrottetia magnadenta sp. n.						
15. Km 70, Tha Khek, Yommalat: (6286, 6287)	36	6.0–7.6 6.7±0.36	7.2–8.8 7.8±0.42	0.7–0.9 0.8±0.06	47.4–59.9 54.5±3.34	6
Indoartemon diodonta sp. n.						
16. Tam Xang, Tha Khek, Khammouan: (6289, 6290)	49	6.8–8.0 7.4±0.33	6.9–8.6 7.7±0.37	0.8–1.0 0.9±0.06	42.1–58.1 51.8±3.03	6½–7
17. Tam Nang Ann, Tha Khek, Khammouan: (6291)	7	7.9–8.9 8.6±0.33	7.2–8.3 7.8±0.34	1.0–0.1 1.1±0.03	46.4–52.4 49.7±2.07	6½–7
18. Tam Xieng Lieb, Tha Khek, Khammouan: (6292)	15	6.8–7.8 7.3±0.27	6.4–7.8 7.3±0.46	0.8–1.1 1.0±0.09	41.3–61.0 51.8±5.04	7

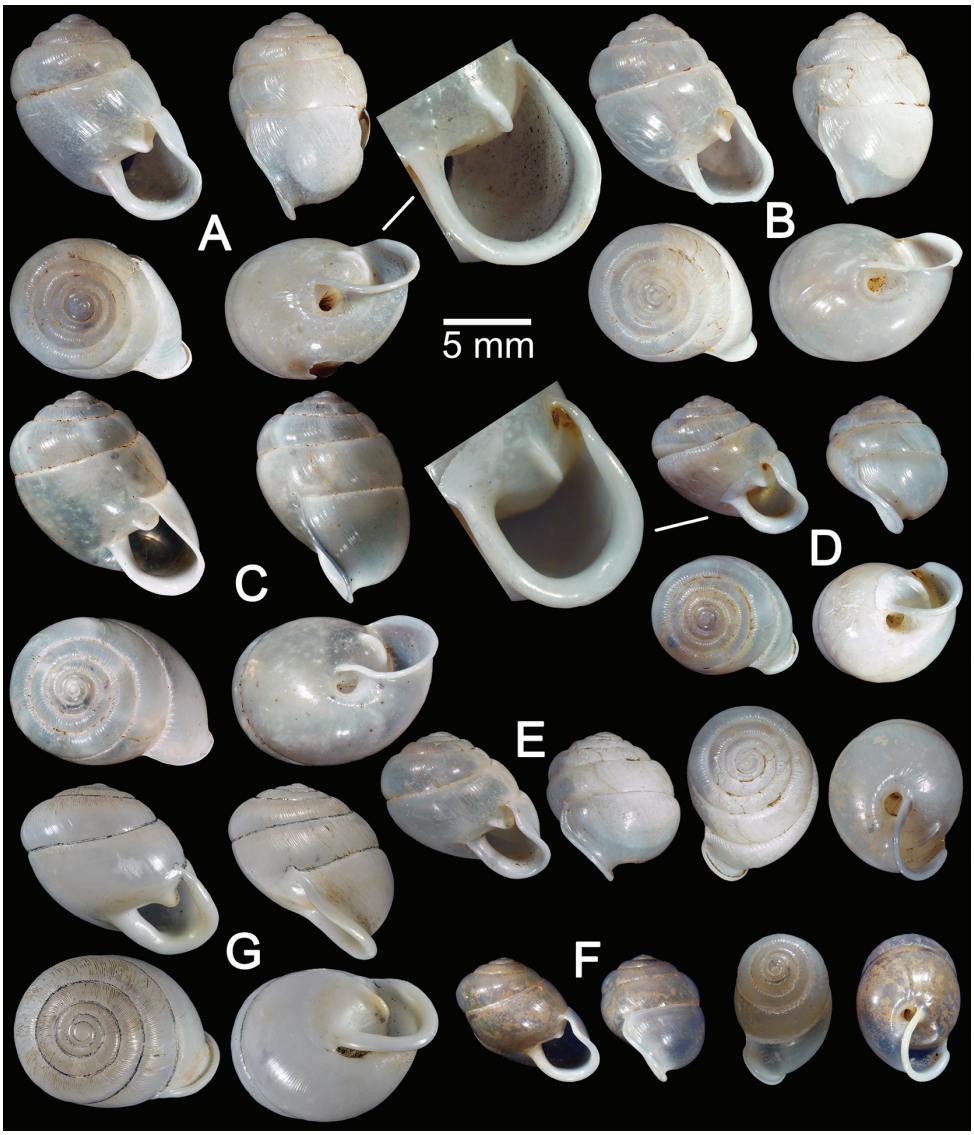


Figure 3. Shells of *Haploptychius* spp. **A–C** *Haploptychius pellucens* **A** lectotype NHMUK 20160249.1 with apertural dentition **B** paralectotype NHMUK 20160249.2, and **C** specimen CUMZ 6264, from Xayabouly **D–F** *Haploptychius porrectus* **D** lectotype NHMUK 20140750.1 **E** paralectotype NHMUK 20140750.2, and **F** specimen CUMZ 6273, from Xieng Khuang. **G** *Haploptychius fischeri*, lectotype MNHN-IM 200030873.

Aperture subcircular; peristome thin, little expanded and reflected. Apertural dentition with one more or less strong parietal lamella. Umbilicus open and deep (Fig. 3A–C).

Radula. Each row consists of 77–85 teeth with formula (38-42)-1-(38-42). Central tooth very small, triangular, with a pointed cusp. Lateral and marginal teeth undif-

ferentiated, lanceolate, unicuspid. Latero-marginal teeth gradually reduce in size, with outermost teeth much smaller and shorter than inner teeth (Fig. 10G).

Genital organs. Atrium (at) short. Proximal penis (p) stout about one-thirds of penis length; distal penis slender. Penial sheath (ps) thin, extending about half of penis length; penial sheath retractor muscle (psr) very thin, originating at atrium and inserted apically at penial sheath (Fig. 6A). Vas deferens (vd) passes through about one-third of penial sheath length before entering into penis apically. Penial retractor muscle (pr) very thick and connected at penis apically (Fig. 7A, B).

Internal wall of atrium generally corrugated with numerous atrial pores (Fig. 9A). Penial wall densely covered in light brown penial hooks, about 6 hooks/200 μm^2. Hooks located on low conical penial papillae, separated by thin reticulated folds. Penial hooks small (< 0.1 mm in length), long, slender, slightly expanded at base, tips pointed and curved towards genital orifice (Fig. 9B–E).

Vagina (v) short, about one-third of penis length. Gametolytic duct (gd) long tube extending to albumin gland; gametolytic sac (gs) ovate. Free oviduct (fo) proximally large with almost equal diameter to vagina, becoming narrower distally. Oviduct (ov) enlarged and folded; prostate gland inconspicuous and bound to oviduct. Talon (ta) small, short and club-shaped. Hermaphroditic duct (hd) bearing long and thin seminal vesicle. Seminal vesicle (sv) about three times longer than the length from talon to branching point of seminal vesicle (Fig. 7A).

Vaginal wall with series of transverse and undulated parallel vaginal folds; vaginal hooks absent (Fig. 9F).

Distribution. This species is known from several limestone areas from central to northern part of Laos. The animals can be found at altitudes from 150-300 meters above mean sea level.

Remarks. This species can be distinguished from *H. porrectus* by having a larger shell, more elevated spire elevated and less oblique aperture. The vas deferens passes through a shorter part of the penial sheath length, and the vagina wall has undulated transverse ridges rather than papillae. *Haploptychius pellucens* can be distinguished from *H. costulatus* (Möllendorff, 1881) from China by having a larger and thinner shell, narrower umbilicus andhaving the left periphery of the penultimate whorl extending beyond the diameter of the last whorl. *Haploptychius fischeri* differs from this species by having a larger, more depressed and thicker shell, with a more obtuse spire, and subquadrangular aperture (Fig. 3G).

All live adult specimens were dissected and the genitalia have been examined, and three different types of genitalia are observed. There are six fully adult specimens collected from Nam Ork Roo, Oudomxay with 'normal' genitalia (Fig. 8A). Two specimens from Ban Namone, Xayabouly have no male genital organs (penis, retractor muscle, vas deferens and prostate gland), while female genital organs are well developed and fully function (Fig. 8D). This is apparently the first report of aphallic animals in Streptaxidae. The other two specimens from Ban Namone, have a 'normal' penis, but have an enlarged and curved "vaginal caecum (vc)" near the penis and atrium junction (Fig. 8B, C). This too is an unusual or unique structure in Streptaxidae. Nevertheless, all these animals appear conspecific based on their shells and the causes of this variation are unknown.

***Haploptychius porrectus* (Pfeiffer, 1863)**
Figs 1, 2B, 3D–F, 7C, D, 9G–M, 10H; Table 1

Streptaxis porrecta Pfeiffer 1863 [1862]: 273. Type locality: Lao Mountains, Camboja [Cambodia]. Martens 1867: 85. Pfeiffer 1868: 442. Tryon 1885: 74. Fischer 1891: 18. Gude 1903: 217. Gude 1903: 275, 322, 325, pl. 12, figs 20–22.
Haploptychius porrectus—Kobelt 1906: 133, pl. 61, figs 24-26. Richardson 1988: 219.

Material examined. This species was described from the H. Cuming collection. The number of specimens was not indicated, but only one set of measurements was given in the original description. The NHM collection contains two specimens from the Cuming collection that has Pfeiffer's handwritten label stating the species name and collection locality. In order to stabilize the name, a specimen matching with the measurements given in the original description is designated here as lectotype NHMUK 20140750.1 (Fig. 3D; H = 8.0, W = 9.0). The other specimen from the same lot becomes a paralectotype NHMUK 20140750.2 (1 shell; Fig. 3E; H = 8.0, W = 8.2).

Laos: NHMUK 1906.1.1.770 (4 shells), NHMUK MacAndrew coll. (2 shells). Ban Nong Tang, Phoukood, Xieng Khuang, Laos: CUMZ 6273 (18 shells; Fig. 3F), 6274 (1 specimen in ethanol; Figs 2B, 7C, D). Tam Pew, Kham, Xieng Khuang, Laos: CUMZ 6275 (4 specimens in ethanol; Figs 9G–M, 10H).

Description. Shell. Shell oblique-heliciform, white and translucent. Whorls 6½, spire conical, suture distinct. Shell surface glossy, with transverse ridges that diminish below the periphery. Embryonic shell smooth with 2½ whorls; following whorls regularly coiled. Penultimate whorl rounded; last whorls rounded and axially deflected. Aperture subcircular; peristome thickened and reflected. Aperture dentition with one parietal lamella. Umbilicus open and deep (Fig. 3D–F).

Radula. Each row consists of 46–58 teeth with formula (23-29)-1-(23-29). Central tooth very small and triangular, with a pointed cusp. Lateral and marginal teeth undifferentiated, lanceolate, unicuspid. Latero-marginal teeth gradually reduce in size, with outermost teeth much smaller and shorter than inner teeth (Fig. 10H).

Genital organs. Atrium (at) short. Proximal penis (p) stout about one-fifth of penis length; distal penis slender. Penial sheath (ps) thin, extending about two thirds of penis length; penial sheath retractor muscle (psr) very thin, originating at atrium and inserting distally on penial sheath (Fig. 7C). Vas deferens (vd) passes through about a quarter of the penial sheath length before entering into penis apically (Fig. 7D). Penial retractor muscle (pr) thick, short and connected with penis apically.

Internal wall of atrium generally smooth (Fig. 9G). Proximal penial wall densely covered with brownish penial hooks, about 10 hooks/200 μm^2. Hooks located on low conical penial papillae, separated by very thin reticulated folds. Proximal penial hooks small and short (< 0.04 mm in length), slightly expanded at base, tip sharp and directed towards genital orifice (Fig. 9H, I). Distal penial wall less densely scattered with brownish penial hooks, about 4 hooks/200 μm^2; penial papillae absent. Distal hooks

very large, long and slender (< 0.5 mm in length), expanded at base, tip obtuse and directed towards genital orifice (Fig. 9J, K).

Vagina (v) short, about half of penis length. Gametolytic duct (gd) long tube extending as far as albumin gland; gametolytic sac (gs) small. Free oviduct (fo) short with almost the same diameter as vagina. Oviduct (ov) enlarged and folded; prostate gland inconspicuous and bound to oviduct. Talon (ta) small and club shape. Hermaphroditic duct (hd) bearing very long and enlarged seminal vesicle (sv) about ten times longer than the length from talon to branching point of seminal vesicle (Fig. 7C).

Vaginal wall generally corrugated with irregular vaginal papillae (Fig. 9L, M). Vaginal hooks absent.

Distribution. This species is known from the limestone outcrops in northeastern and central parts of Laos. The animals can be found at altitudes from 150-300 meters above mean sea level.

Remarks. This species can be distinguished from *H. dorri* (Dautzenberg, 1894) and *H. blaisei* (Dautzenberg & Fischer, 1905) in having a less depressed shell and less deviated last whorl. In addition, *H. blaisei* possesses a solid shell with an angular penultimate whorl, and *H. dorri* has a smaller and smooth shell with an angular penultimate whorl. *Haploptychius anceyi* (Mabille, 1887) is similar to *H. porrectus*, however it differs in its smaller shell, circular aperture, and nearly smooth shell surface.

Haploptychius fischeri **(Morlet, 1887)**

Fig. 3G

Streptaxis fischeri Morlet 1887 [1886]: 259, 274, pl. 12, fig. 1, 1a. Type locality: Baie d'Halong et Montagne de l'Éléphant [Elephant Mountain of Halong Bay, Quang Ninh Province, Vietnam]. Gude 1903: 212.

Haploptychius fischeri—Kobelt 1906: 136, pl. 61, fig. 21, Richardson 1988: 215. Schileyko 2011: 25.

Material examined. The species was described based on material from Jourdy's collection and an illustration was included in the original description (Morlet 1887: 259, pl. 12, fig. 1, 1a). There is a single specimen from L. Morlet in the MNHN collections with an original label stating "Type". In order to stabilize the name, a shell that matched well with the illustration and measurements given in the original description is designated here as lectotype MNHN-IM 200030873 (Fig. 3G).

Remark. *Haploptychius fischeri* is currently known only from the north of Vietnam (Schileyko 2011, Do and Do 2015). The type specimen was examined. Shell thickened, oblique-heliciform with depressed spire. Shell surface with strong radial ridges; penultimate whorl rounded; last whorl axially deflected. Aperture subquadrangular, parietal lamella strong and parietal callus thickened. Peristome wide; lip thickened and reflected. Umbilicus narrowly open.

Compared with *H. pellucens* and *H. porrectus*, this species differs in its larger and thicker shell, depressed spire, prominent transverse ridges, subquadrangular aperture, thicker parietal lamella, and narrower umbilicus.

Haploptychius blaisei (Dautzenberg & Fischer, 1905)

Figs 1, 4D–F; Table 1

Streptaxis blaisei Dautzenberg and Fischer 1905: 86, 87, pl. 3, figs 1–4. Type locality: Ile Krieu, Tonkin [Krieu Island, Ha Long, Quang Ninh Province, Vietnam].
Haploptychius blaisei—Kobelt 1906: 173, pl. 66, figs 4–7. Richardson 1988: 212.

Material examined. The original description was based on single specimen since stated "un seul examplaire" (a single example). The specimen of M. Blaise in the MNHN collections is considered as holotype MNHN-IM 200030866 (Fig. 4D).

Phu Ly, Dongson, Ha Nam, Vietnam: NHMUK Vermeulen coll. (4 shells). Tam Phatok, Ngoi, Luang Phrabang, Laos: CUMZ 6276 (1 shell; Fig. 4E), 6257 (15 shells; Fig. 4F).

Remarks. Shell oblique-heliciform, white and translucent. Whorls 6½; spire depressed to slightly convex, with distinct suture. Shell surface glossy, with thin transverse ridges that diminish below periphery and around umbilicus. Embryonic shell large, about 2½ whorls, with a smooth surface; following whorls regularly expanded. Penultimate whorl rounded; last whorl axially deflected. Aperture semi-ovate; peristome discontinuous, parietal callus thin; lip thickened and slightly expanded. Apertural dentition with one strong parietal lamella. Umbilicus widely open and shallow.

Haploptychius blaisei is superficially similar to *H. diespiter* (Mabille, 1887) and *H. dorri* from north Vietnam, but it has a larger shell, more depressed spire, rounded penultimate whorl, a wide and deep umbilicus, and thin transverse ridges on the upper periphery. For comparison, *H. diespiter* (Fig. 4B) has the last whorl less deviated from the vertical axis, and *H. dorri* (Fig. 4C) has a more depressed suture.

Genus *Perrottetia* Kobelt, 1905

Odontartemon (*Perrottetia*) Kobelt 1905: 91. Kobelt 1906: 108. Thiele 1931: 730. Forcart 1946: 215.
Oophana (*Perrottetia*)—Benthem Jutting 1954: 95.
Perrottetia—Zilch 1960: 562, 563, Richardson 1988: 237. Schileyko 2000: 777, 778. Siriboon et al. 2013: 44, 45.

Type species. *Helix peroteti* Petit, 1841, by subsequent designation of Forcart (1946: 215).

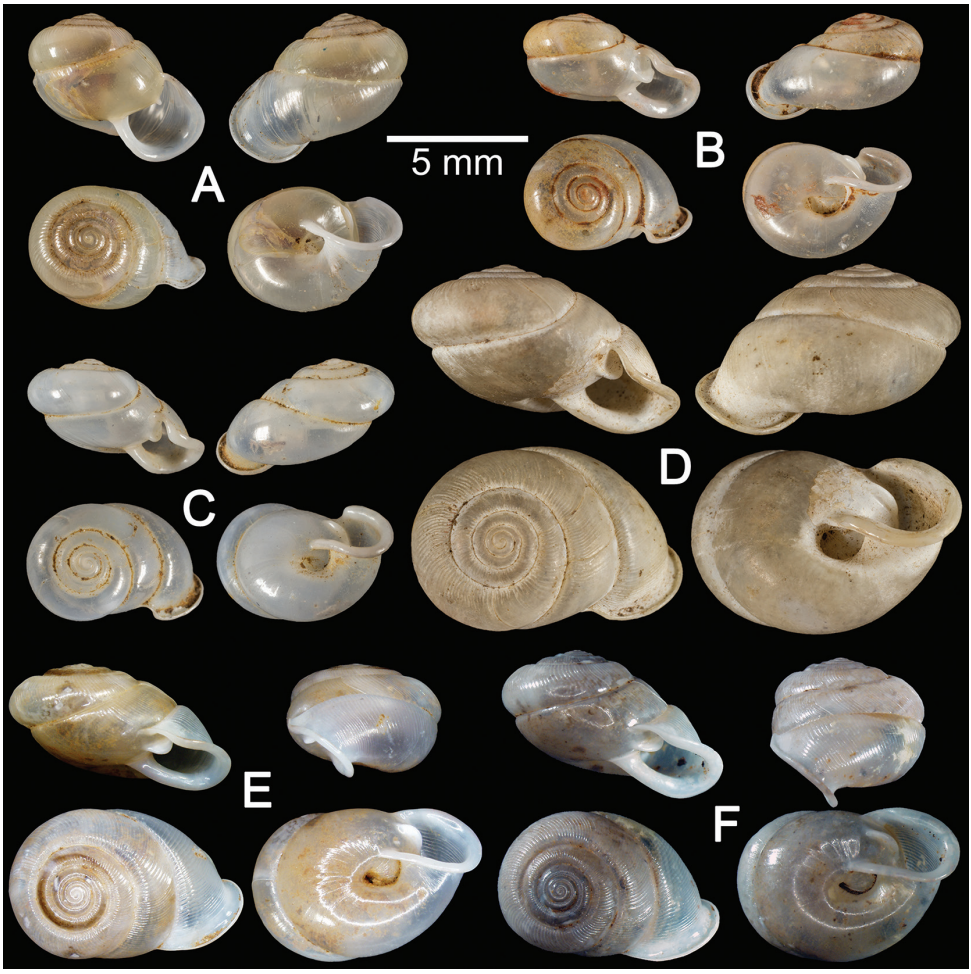


Figure 4. Shells of *Haploptychius* spp. **A** *Haploptychius anceyi* lectotype MNHN-IM 200030868 **B** *Haploptychius diespiter* syntype MNHN-IM 200030870 **C** *Haploptychius dorri* syntype MNHN-IM 200030869 **D–F** *Haploptychius blaisei* **D** holotype MNHN-IM 200030866 and **E, F** specimens from Luang Phrabang CUMZ 6257.

Remarks. The genus *Perrottetia* differs from all other Southeast Asian streptaxid genera in having two longitudinal furrows outside the aperture. Apertural dentition usually comprises one or two parietal lamellae, plus, palatal, basal and columellar lamellae. Genitalia with long penis, penial hooks present, and vaginal hooks sometimes present (Zilch 1960, Schileyko 2000, Siriboon et al. 2013).

Currently, 29 *Perrottetia* species are recognized, from India and Sri Lanka to Indochina and southern China (Kobelt 1906, Richardson 1988, Schileyko 2011, Siriboon et al. 2013). Two species have been reported from Laos, *P. dugasti* (Morlet, 1892) and *P. daedaleus* (Bavay & Dautzenberg, 1908) (see Schileyko 2011).

***Perrottetia dugasti* (Morlet, 1892)**
Fig. 5A

Streptaxis dugasti Morlet 1892: 82. Morlet 1893[1892]: 315, 316, pl. 7, fig. 5, 5a, 5b. Type locality: Laï-Chau, sur les bords de la Riviére Noire, Tonkin [on the banks of the Black River, Lai Chau Province, Vietnam]. Gude 1903: 255.
Perrottetia dugasti—Kobelt 1906: 123, 124, pl. 61, fig. 13. Richardson 1988: 239. Schileyko 2011: 23.

Material examined. The species was described based on material from L. Dugast collection but no illustration was given. Morlet (1893: 315, 316, pl. 7, fig. 5, 5a, 5b) subsequently published the description and illustrated a single specimen. There is a specimen of L. Morlet in the MNHN collections with an original label stating "Type". In order to stabilise the name, the shell that closely matched with the measurements given in the original description and illustration in Morlet (1893: pl. 7, fig. 5, 5a, 5b) is here designated as lectotype MNHN-IM 200030867 (Fig. 4A).

Remarks. Shell sub-oblique heliciform with depressed spire and 6 whorls. Shell surface smooth, glossy and with a distinct suture. Embryonic shell smooth, following whorl regularly expanded. Last whorl rounded, axially deflected, with longitudinal furrows present. Aperture narrow; peristome discontinuous, thick and expanded, and short sinulus present. Aperture dentition consisting of two parietal lamellae (lower one large; upper one small and close to sinulus), one palatal lamella, one basal lamella and one bifid columellar lamella.

Compared with *P. messageri* (Bavay & Dautzenberg, 1908), this species differs in having a strong lower parietal lamella, a bifid columellar lamella, and the left periphery of penultimate whorl not extended beyond the diameter of the last whorl. In contrast, *P. messageri* has a strong columellar lamella, a supracolumellar lamella is present, and the left periphery of the penultimate whorl extended beyond the diameter of the last whorl (Fig. 5D).

***Perrottetia daedaleus* (Bavay & Dautzenberg, 1908)**
Fig. 5C

Streptaxis daedaleus Bavay and Dautzenberg 1908: 230. Type locality: Pac-Kha [Pa Kha, Son La Province, Vietnam]. Bavay and Dautzenberg 1909: 164, 165, pl. 4, figs 1–4.
Streptaxis daedaleus var. *major* Bavay and Dautzenberg 1908: 231. Type locality: Pac-Kha [Pa Kha, Son La Province, Vietnam]. Bavay and Dautzenberg 1909: 165.
Oophana daedaleus—Richardson 1988: 234. Schileyko 2011: 23.
Oophana daedaleus major—Richardson 1988: 234.

Material examined. Syntype of *Streptaxis daedaleus* var. *major* MNHN-IM 200030871 (Fig. 5B). Tonkin: NHMUK 1909.6.9.118-9 (2 shells). Pac-Kha, Tonkin: NHMUK

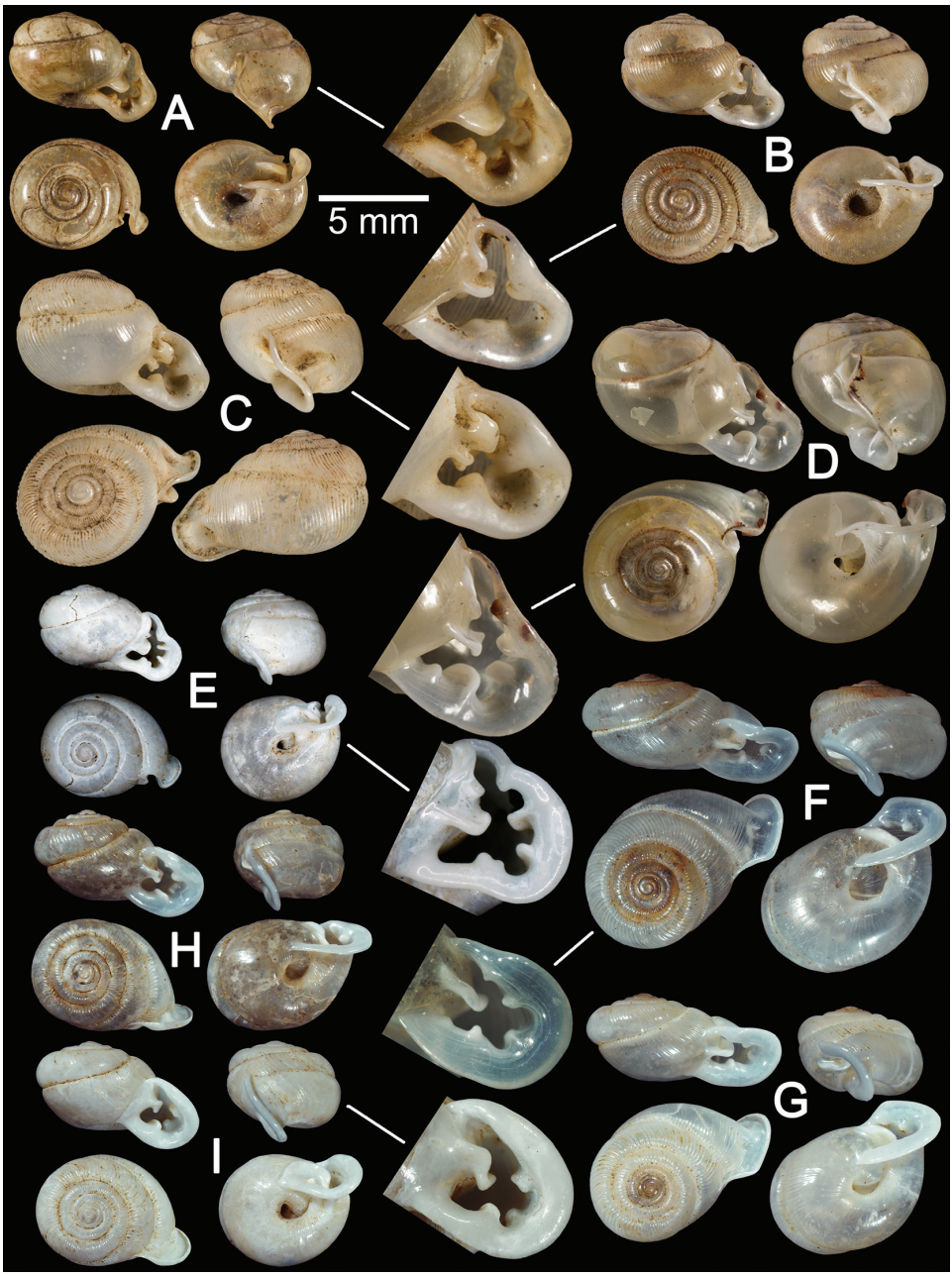


Figure 5. Shells of *Perrottetia* spp. **A** *Perrottetia dugasti* lectotype MNHN-IM 200030867 **B** *Perrottetia mabillei* syntype MNHN-IM 200030874 **C** *Perrottetia daedaleus* var. *major* syntype MNHN-IM 200030871 **D** *Perrottetia messageri* syntype MNHN-IM 200030875 **E** *Perrottetia aquilonaria* specimen CUMZ 6278 from Xayabouly with apertural dentition **F, G** *Perrottetia unidentata* sp. n. **F** holotype CUMZ 6281 with apertural dentition and **G** paratype CUMZ 6282 **H, I** *Perrottetia unidentata* sp. n. specimens from Tam Than Kaisone **H** specimen with upper palatal CUMZ 6284 and **I** specimen without upper palatal CUMZ 6285.

1909.7.9.15-6 (2 shells), NHMUK Preston coll. date 7.4.09 (2 shells), Rolle coll. date 27.11.09 (3 shells). Long Ping, Tonkin: NHMUK Rolle coll. date 27.1.09 (2 shells).

Remarks. Shell suboblique-heliciform with a convex spire and 6 whorls. Shell surface with strong transverse ridges running continuously to umbilicus. Embryonic shell with thin transverse ridges and following whorl regularly expanded. Last whorl rounded, axially deflected, longitudinal furrows present. Aperture triangular; peristome discontinuous, thickened, broadly expanded and sinulus absent. Apertural dentition with two parietal lamellae (lower one small; upper one large and close to sinulus), one angular lamella, one palatal lamella (located far inside aperture) and one columellar lamella.

This species is superficially similar to *P. mabillei* (Bavay & Dautzenberg, 1903) in having strong transverse ridge over the entire shell, but *P. daedaleus* has a large upper parietal lamella, a palatal lamella located inside the aperture, and strong columellar lamellae, while *P. mabillei* (Fig. 5B) has a large lower parietal lamella and bifid columellar lamellae.

Perrottetia aquilonaria Siriboon & Panha, 2013

Figs 1, 5E

Perrottetia aquilonaria Siriboon et al. 2013: 50–52, figs 3D–H, 4D–F: Type locality: Wat Tam Pha Plong, Chiangdao, Chiangmai, Thailand.

Material examined. Holotype CUMZ 5003, paratypes CUMZ 5004 (4 shells). Ban Namone, Xayabouly, Laos: CUMZ 6278 (2 shells; Fig. 5E), CUMZ 6279 (1 specimen in ethanol). Ban Bo Khoun, Boun Neua, Phongsaly, Laos: CUMZ 6280 (1 shell).

Remarks. *Perrottetia aquilonaria* was described from several localities in the northern part of Thailand with a complete information on shell, radula and genitalia. The specimens collected from limestone outcrops in Borkeo and Phongsaly of Laos have both shells and genitalia that match very well with this species. Laos specimens seem to differ only in the slightly smaller shell, therefore we treated them as the same species.

Perrottetia aquilonaria can be distinguished from *P. dugasti* and *P. messageri* from Vietnam by having a depressed spire, shouldered last whorl, thin parietal callus and upper-parietal lamella separated at a right angle. In contrast, *P. dugasti* has a rounded last whorl and a small upper-parietal lamella located deeper inside the aperture, and *P. messageri* has paralleled parietal lamellae, a small supercolumellar lamella is present, and the left side of the penultimate whorl extended beyond the diameter of the last whorl (Fig. 5A, D).

Perrottetia unidentata Inkhavilay & Panha, sp. n.

http://zoobank.org/B47C107D-B7A5-4D70-8640-47F10AE13AC7

Figs 1, 5F–I, 7E, F, 10A–F, I; Table 1

Type material. Holotype CUMZ 6281 (Fig. 5F). Measurement: shell height 5.3 mm, shell width 9.7 mm and 6½ whorls. Paratypes CUMZ 6282 (4 shells; Fig. 5G), CUMZ 6283 (1 specimen in ethanol; Figs 7E, F, 9A–F, I), NHMUK 20160250 (2 shells).

Other material examined. Tam Than Kaisone, Viengxay, Houaphanh, Laos: CUMZ 6284 (5 shell; Fig. 5I), CUMZ 6285 (2 shells; Fig. 5H).

Type locality. The limestone outcrop at Ban Nawit, Viengxay, Houaphanh, Laos (20°22'37.3"N, 104°16'43.2"E) about 700 meters above mean sea level.

Diagnosis. This new species differs from *P. daedaleus*, *P. aquilonaria*, *P. dugasti* and *P. messageri* from Vietnam in having an oblique shell, a single parietal lamella, widely expanded lip, the last whorl strongly axially deflected, the left side of penultimate whorl well extended beyond the diameter of last whorl, and the distal end of penis with a wing-like structure. The other four species have two parietal lamellae, the last whorl little axially deflected and the left side of penultimate whorl not extended beyond the diameter of the last whorl. For further comparison, *P. daedaleus* has an elevated spire, transverse ridges over the entire shell and a basal lamella located deep inside aperture (Fig. 5C); *P. aquilonaria* has a smaller shell, elevated spire, bifid columellar lamella, and genitalia with atrial pores and vaginal hooks absent (Fig. 5E); *P. dugasti* and *P. messageri* have a smooth shell surface, a bifid collumella lamella and a supracolumellar lamella (Fig. 5A, D). *Perrottetia gudei* from north Vietnam differs from the new species in having an elevated spire, in being less deviated from the vertical axis, and in having thin transverse ridges (see Siriboon et al. 2013).

Description. Shell. Shell oblique-heliciform, semi-transparent; whorls 6½, spire weakly convex with distinct suture. Shell surface glossy with strong transverse ridges on upper shell surface. Embryonic shell large, about 2½ whorls, with a smooth surface; following whorls regularly coiled. Shell periphery shouldered; last whorl axially deflected; two deep longitudinal furrows present. Aperture semi-ovate; peristome discontinuous; parietal callus thin; lip thickened, broadly expanded and slightly reflected. Apertural dentition with one large, strong and sinuous parietal lamella, one small upper palatal lamella, one palatal lamella, one large basal lamella, one strong columellar lamella, and one small supracolumellar lamella. Umbilicus widely open and shallow (Fig. 5F–I).

Radula. Each row consists of 26–38 teeth with formula (13-19)-1-(13-19). Central tooth small and triangular, with pointed cusp. Lateral and marginal teeth undifferentiated, lanceolate, unicuspid. Latero-marginal teeth gradually reduce in size, with outermost teeth much smaller and shorter than inner teeth (Fig. 10I).

Genital organs. Atrium (at) short. Proximal penis (p) long and slender; distal part near retractor muscle with an expanded wing-like structure (a flat blade on either side of the penis, each about one-tenth of penis length). Penial sheath (ps) thin and extending about one-third of penis length; penial sheath retractor muscle (psr) very thin, originating at atrium and inserting distally on penial sheath (Fig. 7E). Vas deferens (vd) passes through about one-third of penial sheath length before entering into penis apically (Fig. 7F). Penial retractor muscle (pr) thin and long, inserted at penis and vas deferens junction.

Internal wall of atrium generally smooth (Fig. 10A). Penial wall densely covered with light brown penial hooks, about 20 hooks/200 μm^2; hooks located on low elliptical penial papillae. Penial hooks small (< 0.1 mm in length), slender, expanded at base, tips pointed and curved towards genital orifice (Fig. 10B–D).

Vagina (v) short, about one-tenth of penis length. Gametolytic duct (gd) a long tube extending as far as albumin gland; gametolytic sac (gs) ovate. Free oviduct (fo) long and cylindrical with equivalent diameter to vagina, tapering distally. Oviduct (ov) enlarged and folded; prostate gland inconspicuous and bound to oviduct. Talon (ta) very small, short and club shape. Hermaphroditic duct (hd) bearing very short and thin seminal vesicle (sv) about one and half times longer than the length from talon to branching point of seminal vesicle (Fig. 7E).

Vaginal wall with transparent vaginal hooks (about 10 hooks/200 μm^2). Hooks located on low conical vaginal papillae. Vaginal hooks small (< 0.1 mm in length), short and expanded at base; tips pointed and straight to slightly curving away from genital orifice (Fig. 10E, F).

Etymology. The specific epithet "*unidentata*" derived from the Latin words "*unus*" meaning "one" and "*dens*" meaning "tooth". It referred to a single parietal lamella (or teeth) of the new species.

Distribution. This species is known only from the type locality, Houaphanh, a limestone karst area.

Remarks. Shell variation is evident from specimens from Tam Than Kaisone, about 20 km west of the type locality (Fig. 5H, I; CUMZ 6284, 6285). They are smaller, with a sinuous parietal lamella, and sometimes lack the upper palatal lamella (Table 1). However, only five shells and no living specimens were collected, so we provisionally identifying them as the same species.

Perrottetia megadentata **Inkhavilay & Panha, sp. n.**
http://zoobank.org/3DE66E56-8480-4993-91C8-47E885EE2C4D
Figs 1, 6A, B; Table 1

Type material. Holotype CUMZ 6286 (Fig. 6A). Measurement: shell height 7.1 mm, shell width 8.2 mm, and with 6 whorls. Paratypes: CUMZ 6287 (31 shells; Fig. 6B), CUMZ 6288 (2 shells), NHMUK (2 shells), NUOL 20160251 (2 shells), SMF (2 shells).

Type locality. The limestone outcrop at Ban Phone Can, Yommalat, Khammouan, Laos (17°31'35.6"N, 105°9'40.7"E)

Diagnosis. The characters distinguishing *Perrottetia megadentata* sp. n. from *P. daedaleus*, *P. aquilonaria*, *P. dugasti* and *P. mabillei* are a single large parietal lamella, the absence of a palatal lamella absent and the presence of an infra-columellar lamella. The other four species have two parietal lamellae and a palatal lamella. Furthermore, *P. dugasti* and *P. aquilonaria* have a smooth shell, slightly depressed spire, and a bifid columellar lamella (Fig. 5A, E). *Perrottetia daedaleus* and *P. mabillei* have strong transverse ridges over the entire shell, a palatal lamella, and a bifid basal lamella, a columellar lamella is absent in *P. mabillei* (Fig. 5B), while one basal and one columellar lamella are present in *P. daedaleus* (Fig. 5C). The new species differs from *P. unidentata* sp. n. in its ovate shape, smooth shell surface, thicker shell, in the absence of a palatal lamella, and in having infra- and supra-columellar lamellae.

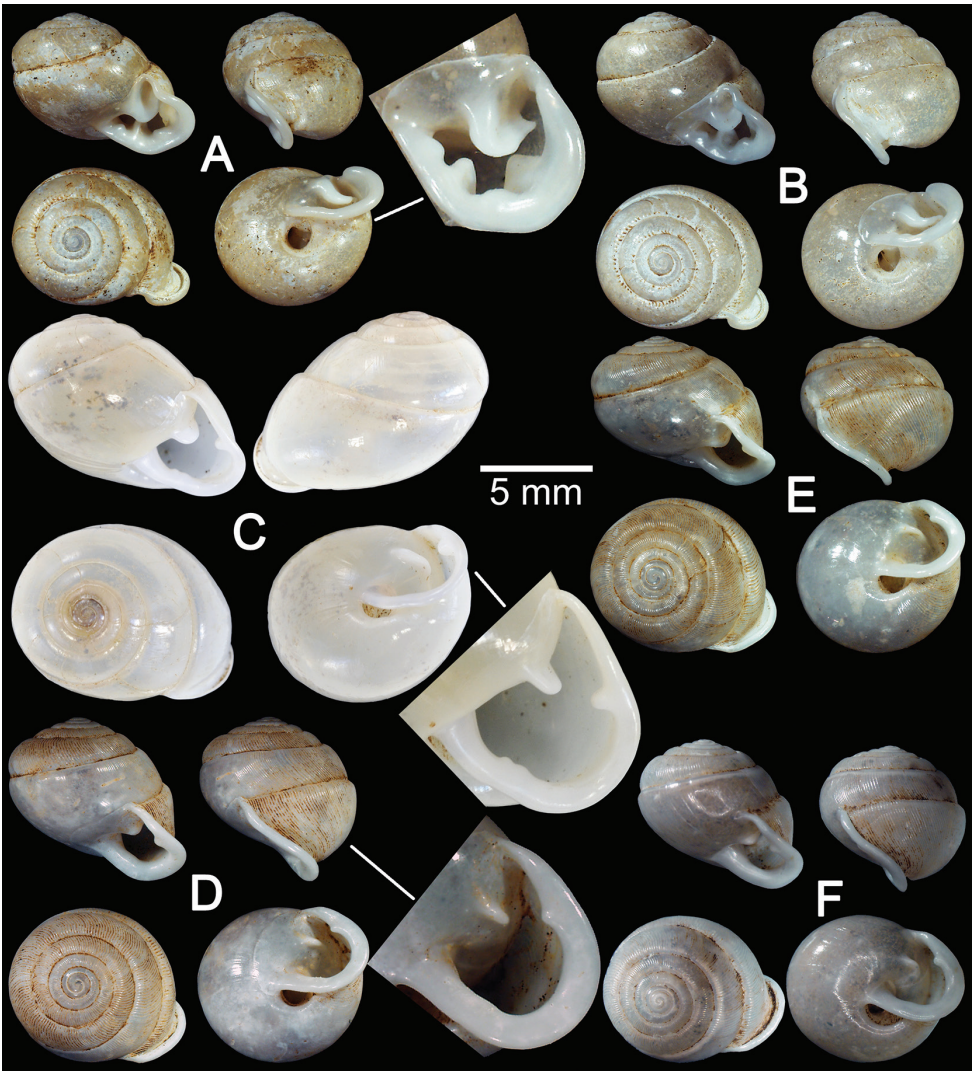


Figure 6. Shells of *Perrottetia* and *Indoartemon* spp. **A, B** *Perrottetia megadentata* sp. n. **A** holotype CUMZ 6286 with apertural dentition, and **B** paratype CUMZ 6287. **C** *Indoartemon tridens* holotype SMF 108507/1 with apertural dentition **D–F** *Indoartemon diodonta* sp. n. **D** holotype CUMZ 6289 with apertural dentition **E** paratypes CUMZ 6290, and **F** specimen from Tam Nang Ann, Tha Khek, Khammouan CUMZ 2691.

The new species is superficially similar to *P. dermapyrrhosa* Siriboon & Panha, 2013, but is distinguished by having a single and large parietal lamella, and in the absence of a palatal lamella.

Description. Shell oblique-ovate, white and translucent; whorls 6, spire conical, with distinct suture. Shell surface glossy with transverse ridges near suture. Embryonic shell large, about 2½ whorls, with a smooth surface; following whorls regularly

coiled. Shell periphery rounded; last whorl axially deflected; two shallow and short longitudinal furrows present. Aperture subcircular, peristome continuous; parietal callus thickened; lip thickened, expanded and reflected; short sinulus present. Apertural dentition with very large and strong sinuous parietal, one large basal lamella located deep inside aperture, one small infracolumellar lamella, one large columellar lamella, and one small supracolumellar lamella. Umbilicus widely open and deep (Fig. 6A, B)

Etymology. The specific epithet "*megadentata*" is derived from the Greek word "*mega*" meaning "large" and the Latin word "*dens*" meaning "tooth". It referred to the single large parietal lamella of the new species.

Distribution. This species is known only from the type locality in central Laos.

Remarks. To date no living specimens have been collected.

Genus *Indoartemon* Forcart, 1946

Oophana (*Indoartemon*) Forcart 1946: 215. Benthem Jutting 1954: 95.
Indoartemon—Zilch 1960: 562. Richardson 1988: 223. Schileyko 2000: 776, 777. Siriboon et al. 2014b: 162.

Type species. *Streptaxis eburnea* Pfeiffer, 1861, by original designation.

Remarks. The genus *Indoartemon* can be recognized by the dentition, which consists of one parietal and one palatal lamella (a basal lamella is also present in some species). The penis is long, with a thin penial sheath extending about half of the penis length, through which the vas deferens does not pass. Penial hooks are present (Siriboon et al. 2014b).

Currently, ten species are recognized, of which seven were reported from Indochina south of China and Hainan. Only one species, *I. tridens* (Möllendorff, 1898) has previously been recorded from Laos (Richardson 1988, Schileyko 2000, Siriboon et al. 2014b); here we describe another.

Indoartemon tridens (Möllendorff, 1898)

Figs 1, 6C

Streptaxis tridens Möllendorff 1898: 67. Type locality: Boloven, Laos [=Boloven Plateau, Paksong, Champasak, Laos]. Gude 1903: 220.
Odontartemon tridens—Kobelt 1905: 94, 95, pl. 58, figs 19, 20.
Indoartemon tridens—Zilch 1961: 85, pl. 5, fig. 15. Richardson 1988: 225. Schileyko 2011: 23.

Material examined. Holotype SMF 108507 (Fig. 6C).

Remarks. Shell oblique-ovate with 5½ whorls, semi-transparent, spire slightly convex, with distinct sutures. Shell surface glossy white with thin growth lines; following whorls regularly coiled. Last whorl axially deflected. Aperture triangular; peristome con-

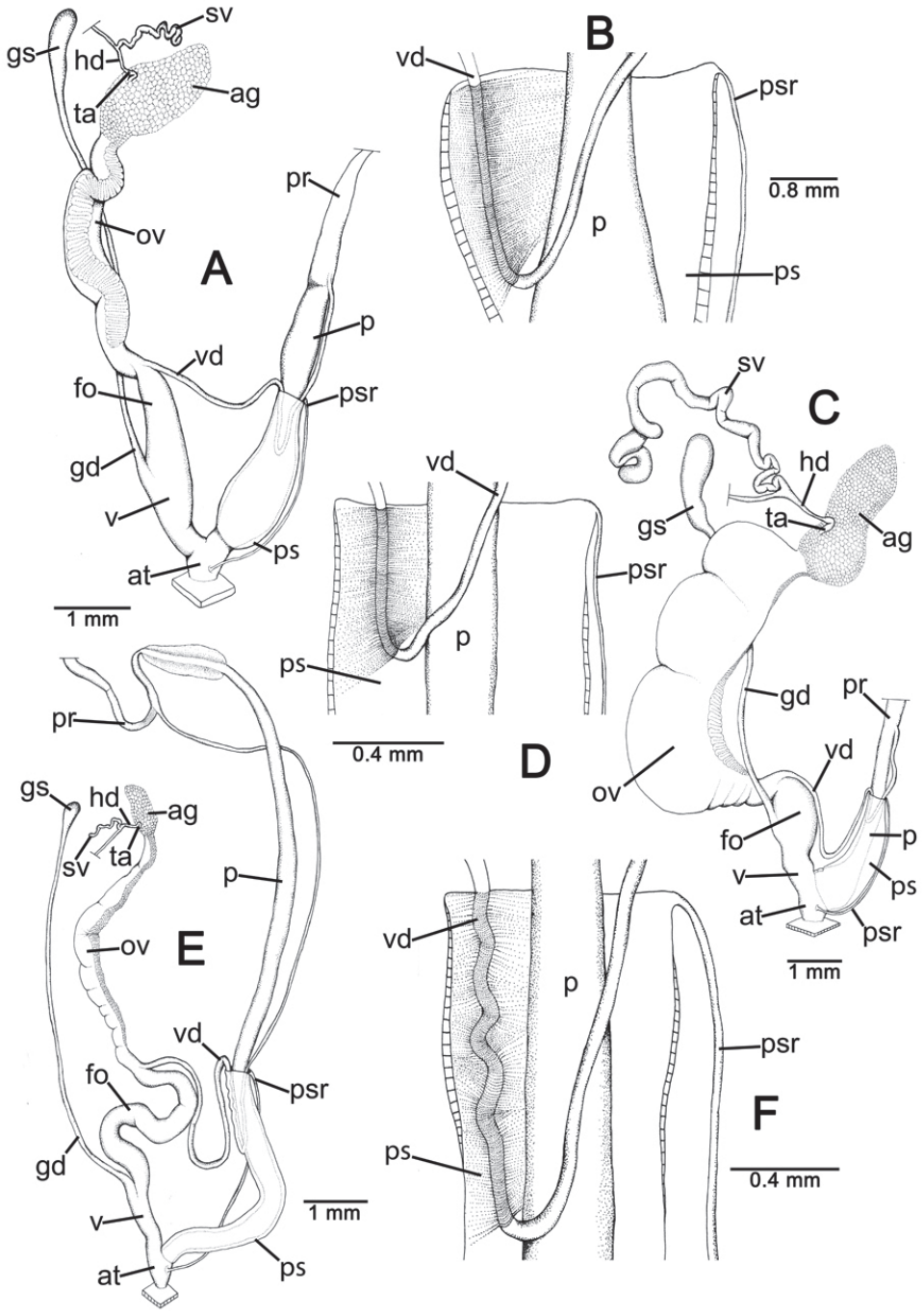


Figure 7. Genitalia of *Haploptychius* and *Perrottetia* species. **A, B** *Haploptychius pellucens* CUMZ 2670 **A** reproductive system, and **B** insertion of vas deferens into penial sheath **C, D** *Haploptychius porrectus* CUMZ 6274 **C** reproductive system, and **D** insertion of vas deferens into penial sheath **E, F** *Perrottetia unidentata* sp. n. CUMZ 6283 **E** reproductive system, and **F** insertion of vas deferens into penial sheath.

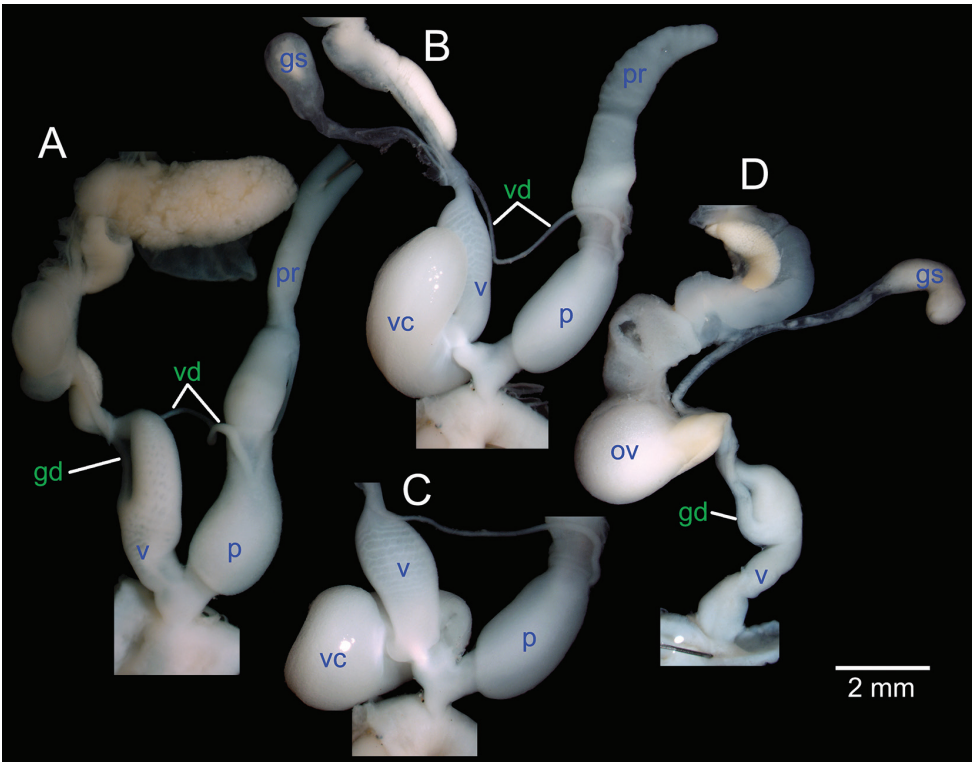


Figure 8. Genitalia of *Haploptychius pellucens* **A** completed reproductive system CUMZ 2670 **B, C** completed reproductive system with "vaginal caecum" CUMZ 6265, and **D** aphallic reproductive system CUMZ 6265.

tinuous; lip thickened, little expanded and slightly reflected. Apertural dentition with one large parietal lamella, one palatal lamella, and one small bifid columellar lamella.

Only the type specimen was examined. *Indoartemon tridens* differs from *I. eburneus*, *I. prestoni* (Gude, 1903) and *I. medius* Siriboon & Panha, 2014 from Thailand by having a bifid columellar lamella, an ovate-heliciform shape, its smooth shell surface, narrow umbilicus, and having the left side of penultimate whorl extended beyond the diameter of last whorl. For comparison, *I. eburneus* and *I. prestoni* have a less deviated last whorl, transverse ridges on the shell, and a widely open umbilicus; *I. medius* has an angular penultimate whorl and strong transverse ridges.

***Indoartemon diodonta* Inkhavilay & Panha, sp. n.**
http://zoobank.org/64F31C73-88D6-4A6B-BD0C-09FE2663E28F
Figs 1, 6D–F; Table 1

Type material. Holotype CUMZ 6289 (Fig. 6D). Measurement: shell height 7.5 mm, shell width 8.3 mm, and with 7 whorls. Paratypes: CUMZ 6290 (44 shells; Fig. 6E), NHMUK 20160252 (2 shells), NUOL (2 shells), SMF (2 shells).

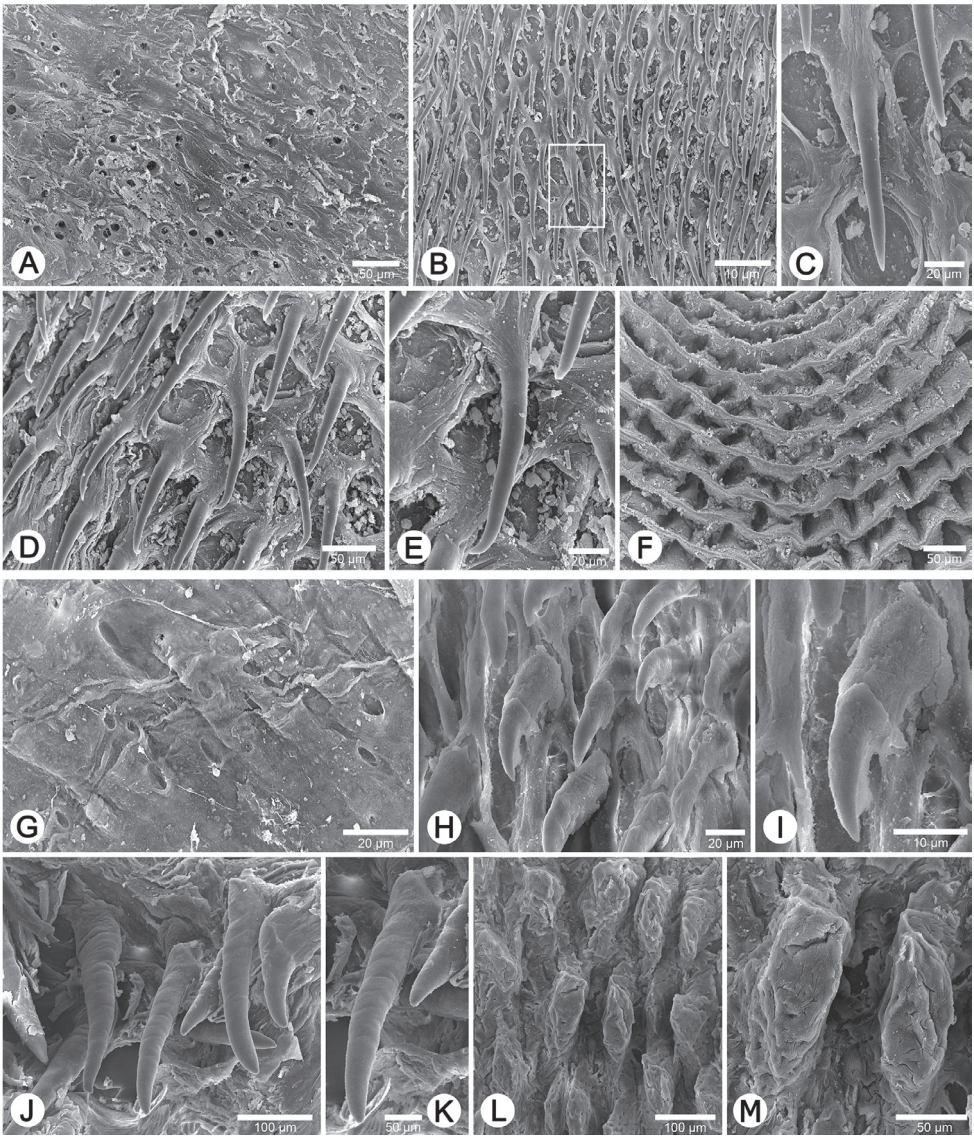


Figure 9. Internal sculpture of genitalia of *Haploptychius* spp. **A–F** *Haploptychius pellucens*, CUMZ 2670 **A** details of atrium surface **B** arrangement of penial hooks **C** top view of penial hook (from white square in **B**) **D** arrangement of penial hooks **E** lateral view of penial hook, and **F** arrangement of undulated parallel vaginal folds. **G–M** *Haploptychius porrectus* specimen CUMZ 6275 **G** details of atrium surface **H** arrangement of penial hooks in distal area **I** lateral view of penial hook in distal area **J** arrangement of penial hooks in proximal area **K** lateral view of penial hook in proximal area **L** arrangement of papillae and vaginal folds, and **M** arrangement of vaginal folds.

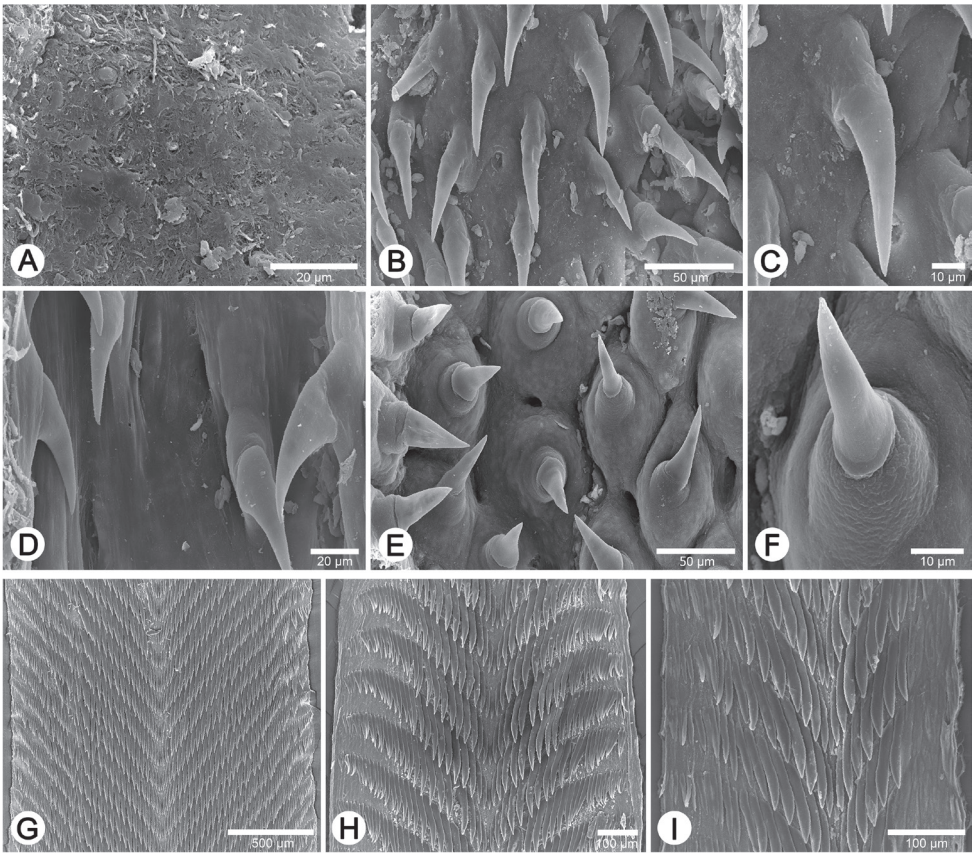


Figure 10. Internal sculpture of genitalia of **A–F** *Perrottetia unidentata* sp. n. paratype CUMZ 6283 **A** details of atrium surface **B** arrangement of penial hooks **C** top view of penial hook **D** lateral view of penial hooks **E** arrangement of vaginal hooks, and **F** top view of vaginal hook. Radula morphology of **G** *Haploptychius pellucens* specimen CUMZ 2670 **H** *Haploptychius porrectus* specimen CUMZ 6275, and **I** *Perrottetia unidentata* sp. n. paratype CUMZ 6283.

Other material examined. Tam Nang Ann, Tha Khek, Khammouan, Laos: CUMZ 6291 (7 shells, Fig. 6F). Tam Xieng Lieb, Tha Khek, Khammouan, Laos: CUMZ 6292 (15 shells).

Type locality. Tam Xang, Tha Khek, Khammouan, Laos, 17°25'44.0"N, 104°51'49.1"E.

Diagnosis. This new species superficially resembles *I. eburneus* and *I. prestoni* from Thailand, but it differs in having a much smaller shell, an oblique-heliciform shape, open umbilicus, and the last whorl is strongly deviated from the vertical axis. This species differs from *I. medius* from Thailand in its smaller shells, angular penultimate whorl and thin transverse ridges. *Indoartemon diodonta* sp. n. differs from *I. bidens* (Möllendorff, 1883) from Hainan and *I. tridens* by having fine transverse ridges on the upper periphery, and the last whorl is less deviated from the vertical axis. These two

species also have a smooth shell surface and a more strongly deviated last whorl, and a bifid columellar lamella is present in *I. tridens*.

Description. Shell. Shell oblique-heliciform, white and translucent; whorls 6½–7, spire conical, with distinct suture. Shell surface dull, with fine transverse ridges that diminish below the periphery. Embryonic shell large, about 2½ whorls, with smooth surface; following whorls regularly coiled. Last whorl shouldered, axially deflected, and not expanded. Aperture subcircular; peristome continuous, parietal callus thickened; lip thickened, expanded and little reflected. Apertural dentition with one large and strong parietal and one small palatal lamellae. Umbilicus narrow and deep (Fig. 6D–F).

Etymology. The specific epithet "*diodonta*" is derived from the Greek words "*di*" meaning "two" and "*odontos*" meaning "tooth", referring to the dentition of the new species.

Distribution. This species is known from limestone karst in Khammouan Province, central Laos. The animals can be found at altitudes up to 140 meters above mean sea level.

Remarks. To date no living specimens have been collected.

Discussion

This study increases the number of streptaxid species recorded from Laos to twelve, three of which are new. Streptaxids occur in both limestone and non-limestone areas in the central and northern parts of Laos. The fauna apparently remains less diverse than that of Thailand and Vietnam (Panha 1996, Hemmen and Hemmen 2001, Siriboon et al. 2013, 2014a, b, Schileyko 2011). The highly modified habitats of southern and some central areas of Laos may harbour a lower species diversity. For example, *Indoartemon tridens* was recorded in 1898 by Möllendorff from its type locality at Boloven plateau, Paksong, Champasak, Laos, but our surveys yielded no specimens collected from this locality.

The species can be separated by geography, shell morphology, and (where available) genital anatomy. Two species from the genus *Haploptychius*; *H. pellucens* and *H. porrectus* were described from Laos by Pfeiffer (1863). From our results living and shells specimens of *H. pellucens* and *H. porrectus* were collected from nine sampling sites in six provinces such as Louang Namtha, Oudomxay, Louang Phrabang, Xayabouly, Bolikhmaxay and Xieng Khaung. Shell morphology and genitalia anatomy were compared between the two species. The two can be separated by having different shell size and shape, as well as differences in the penial sheath, penial hooks, and vaginal wall. The southernmost population of *H. pellucens* is particularly small. Most records of *Haploptychius* species are from northern Laos, latitude 18°-21°.

Perrottetia unidentata sp. n. and *P. megadentata* sp. n. are the first two species of the genus recorded in Laos, and are geographically and altitudinally separated. *Perrottetia unidentata* sp. n. occurs in northern Laos close to the Lao-Vietnam border at over 700 m above sea level, while *P. megadentata* sp. n. occurs far to the south and lower than 200 m above sea level (Fig. 1). The two species can be separated by shell morphology. *Perrotettia* has been collected from central to northern Laos, latitude 18°–22°.

Indoartemon diodonta sp. n. is the second species of this genus recorded from Laos after *I. tridens* (Möllendorff 1898). The new species was found in central Laos, while the first was found in southern Laos, at over 1000 m above sea level. In Laos, *Indoartemon* has now been recorded between latitude 14°–18°.

Acknowledgements

The authors are grateful to members of the Animal Systematics Research Unit, Chulalongkorn University (ASRU) members for kind help during field collecting. For accommodation and technical supports during this study we cordially thank all staff in the Department of Biology, Faculty of Science, Chulalongkorn University. Special thanks are offered to the Faculty of Natural Science, National University of Laos for the kind preparation of permission documents during surveys and data collection in Laos. The authors would like to express our gratitude for the comments from anonymous referees that encourage to improve the manuscript. We are also indebted to the M. Caballer project E-RECOLNAT: ANR-11-INBS-0004 for their support with the type material database. This project was mainly funded by the TRF Senior Research Scholar (RTA 5880002) to SP, and additionally supported from Chulalongkorn University Graduate School Postdoctoral Project to TS and CU-ASEAN scholarship to KI.

References

Ancey CF (1884) Sur les divisions proposées dans le genre *Streptaxis*. Le Naturaliste 50: 399.

Bavay A, Dautzenberg P (1903) Description de coquilles nouvelles de l'Indo-Chine. Journal de Conchyliologie 51: 201–236.

Bavay A, Dauzenberg P (1908) Molluscorum terrestrium tonkinorum diagnoses. Journal de Conchyliologie 56: 229–251.

Benthem Jutting WSS van (1954) The Malayan Streptaxidae of the genera *Discartemon* and *Oophana*. Bulletin of the Raffles Museum 25: 71–106.

Berry AJ (1963) The anatomy of two Malayan limestone hill Streptaxidae, *Sinoennea kanchingensis* Tomlin and *Oophana diaphanopepla* van Benthem Jutting with special reference to the genital system. Proceedings of the Malacological Society of London 35: 139–150.

Berry AJ (1965) The genital systems of *Discartemon stenostomus* van Benthem Jutting and *Huttonella bicolor* (Hutton) (Pulmonata, Streptaxidae) from Malaya. Proceedings of the Malacological Society of London 36: 221–228.

Blanford WT, Godwin-Austen HH (1908) Mollusca: Testacellidae and Zonitidae. In: Bingham CT (Ed.) The Fauna of British India including Ceylon and Burma. Taylor and Francis, London, 311 pp. doi: 10.5962/bhl.title.55244

Bruggen AC van (1967) An introduction to the pulmonate family Streptaxidae. Journal of Conchology 26: 181–188.

Dautzenberg PH (1893) Mollusques nouveaux recueillis au Tonkin par M. le capitaine Em. Dorr. Journal de Conchyliologie 41[1894]: 157–165. [Published in parts, dates follow Winckworth (1936)]

Dautzenberg PH, Fischer H (1905) Liste des mollusques récoltés par M. le Frégate Blaise au Tonkin, et description d'espèces nouvelles. Journal de Conchyliologie 53: 85–234.

Do DS, Do VN (2015) The terrestrial snail family Streptaxidae J. Gray, 1860 (Gastropoda: Pulmonata) from Son La, Vietnam, with description of two new species. Ruthenica 25: 37–43.

Duncan FM (1937) On the dates of publication of the Society's 'Proceedings'1859–1926, with an appendix containing the dates of publication 1830–1858, compiled by F.H. Waterhouse; also of the 'Transactions' 1833–1869 by Henry Peavot, originally published in P.Z.S. 1893, 1913. Proceedings of the Zoological Society of London, Series A 107: 71–84.

Fischer P (1891) Catalogue et distribution gèographique des Mollusques terrestres, fluviatilies & marins d'une partie de l'Indo-Chine (Siam, Laos, Cambodge, Cochinchine, Annam, Tonkin). Bulletin de la Société d'Histoire Naturelle d'Autun 4: 87–276.

Forcart P (1946) *Indoartemon* subgen. nov. for *Odonartemon* Kobelt, 1905 (non Pfeiffer, 1856); Streptaxidae. Journal of Conchology 22: 215.

Gray JE (1860) On the arrangement of the land pulmoniferous Mollusca into families. Annals and Magazine of Natural History (Series 3) 6: 267–269.

Gude GK (1903) A synopsis of the genus *Streptaxis* and its allies. Proceedings of the Malacological Society of London 5: 201–275.

Johnson RI (1969) Pfeiffer's Novitates Conchologicae, Series I, Land Mollusca, 1854–1879, and Dunker's Novitates Conchologicae, Series II, Marine Mollusca, 1862–1882. A complete collation. Journal of the Society for the Bibliography of Natural History 5: 136–239. doi: 10.3366/jsbnh.1969.5.3.236

Kemp M (2011) Roads to destruction: ADB Contradictory Roads, Biodiversity and Plantations Activities in Lao PDR or How did you know we wanted ecocide? NGO forum on ADB, Quezon City, Philippines, 29 pp.

Kobelt W (1905–1906) Die Raublungenschnecken (Agnatha). Zweite Abtheilung: Streptaxidae und Daudebardiidae. Systematisches Conchylien-Cabinet von Martini und Chemnitz 1(12b) (2): 1–96, pls 42–59 [1905]; 97–211, pls 60–71 [1906].

Mabille MJ (1887) Molluques terrestres marquies (Polynesie). Bulletins de la Société Malacologique de France 4: 73–164.

Martens E von (1867) Die Landschnecken. Die Preussische Expedition nach Ost-Asien. Zoologischer Theil 2: 85.

Möllendorff OF (1881) Beiträge zur Mollusken fauna von Südchina. Jahrbucher der Deutschen Malakozoologischen Gesellschaft 8: 302–312.

Möllendorff OF von (1883) Diagnosen neuer Arten. Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft 1883: 65–67.

Möllendorff OF von (1898) Die Binnenmollusken Annams. Nachrichtsblatt der Deutschen Malakozoologischen Gesellschaft 30: 65–85.

Morelet A (1866) Description d'espèces appartenant à la faune malacologique de l'Indo-Chine. Journal de Conchyliologie 14: 62–64.

Morlet L (1883) Description ďespeces nouvelles de coquilles recueillies, par M. Pavie, au Cambodge. Journal de Conchyliologie 31: 104–110.

Morlet L (1892) Diagnoses Molluscorum novorum, in Indo-Chinâ. Journal de Conchyliologie 40[1892]: 82–86. [Published in parts, dated follow Winckworth (1936)]

Morlet L (1893) Description ďespèces nouvelles provenant de l'Indo-Chine. Journal de Conchyliologie 40[1892]: 315–329. [Published in parts, dates follow Winckworth (1936)]

Páll-Gergely B, Hunyadi A, Varga A, Rowson B (2015) Anatomy and taxonomic position of *Elma* H. Adams, 1866: a high-spired Southeast-Asian genus of Streptaxidae (Gastropoda: Eupulmonata). Folia Malacologica 23: 155–164. doi: 10.12657/folmal.023.013

Panha S (1996) A checklist and classification of the terrestrial pulmonate snails of Thailand. Walkerana 8: 31–40.

Pfeiffer L (1853) Description of fifty-four new species of helicea, from the collection of Hugh Cuming, Esq. Proceedings of the Zoological Society of London 19[1851]: 252–263. [Published in parts, dates follow Duncan (1937)]

Pfeiffer L (1856) Versuch einer anordnung der Heliceen nach natürlichen gruppen. Malakozoologische Blätter 3: 112–185.

Pfeiffer L (1861) Description of forty-seven new species of land shells, from the collection of H. Cuming, Esq. Proceedings of the Zoological Society of London 29[1861]: 20–29. [Published in parts, dates follow Duncan (1937)]

Pfeiffer L (1863) Description of thirty-six new land shells, from the collection of H. Cuming, Esq. Proceedings of the Zoological Society of London 30[1862]: 268–278. [Published in parts, dates follow Duncan (1937)]

Pfeiffer L (1868) Monographia Heliceorum Viventium (Volume 5). Backhaus FA, Lipsiae, 565 pp.

Pfeiffer L (1870–1876) Novitates Conchologicae. Series Prima. Mollusca Extramarina. Band 4. Verlag von Theodor Fischer, Cassel, 1–171, pls. 109–137. [p. 25–40, pls. 118–125 [1871]. Published in parts, dates follow Johnson (1969)]

Richardson L (1988) Streptaxacea: Catalog of species, Part I, Streptaxidae. Tryonia 16: 1–326.

Rowson B, Tattersfield P, Symondson WOC (2010a) Phylogeny and biogeography of tropical carnivorous land-snails (Pulmonata: Streptaxoidea) with particular reference to East Africa and the Indian Ocean. Zoologica Scripta 40: 85–98. doi: 10.1111/j.1463-6409.2010.00456.x

Rowson B, Warren BH, Ngereza CF (2010b) Terrestrial molluscs of Pemba Island, Zanzibar, Tanzania, and its status as an "oceanic" island. ZooKeys 70: 1–39. doi: 10.3897/zookeys.70.762

Rowson B, Tattersfield P (2013) Revision of *Dadagulella* gen. nov., the "*Gulella radius* group" (Gastropoda: Streptaxidae) of the eastern Afrotropics, including six new species and three new subspecies. European Journal of Taxonomy 37: 1–46. doi: 10.5852/ejt.2013.37

Schileyko AA (2000) Treatise on recent terrestrial pulmonate molluscs: Rhytididae; Chlamydephoridae; Systrophiidae; Haplotrematidae; Streptaxidae; Spiraxidae; Oleacinidae; Testacellidae. Ruthenica Supplement 2 Part 6: 731–880.

Schileyko AA (2011) Check-list of land pulmonate molluscs of Vietnam (Gastropoda: Stylommatophora). Ruthenica 21: 1–68.

Siriboon T, Sutcharit C, Naggs F, Panha S (2013) Three new species of the carnivorous snail genus *Perrottetia* Kobelt, 1905 from Thailand (Pulmonata, Streptaxidae). ZooKeys 287: 41–57. doi: 10.3897/zookeys.287.4572

Siriboon T, Sutcharit C, Naggs F, Rowson B, Panha S (2014a) Revision of the carnivorous snail genus *Discartemon* Pfeiffer, 1856, with description of twelve new species (Pulmonata, Streptaxidae). ZooKeys 401: 45–107. doi: 10.3897/zookeys.401.7075

Siriboon T, Sutcharit C, Naggs F, Rowson B, Panha S (2014b) Revision of the carnivorous snail genus *Indoartemon* Forcart, 1946 and a new genus *Carinartemis* from Thailand (Pulmonata: Streptaxidae). Raffles Bulletin of Zoology 62: 161–174.

Stoliczka F (1871) Notes on the terrestrial mollusca from the neighbourhood of Moulmein, with descriptions of new species. Journal of the Asiatic Society of Bengal 40: 143–177.

Sutcharit C, Naggs F, Wade CM, Fontanilla I, Panha S (2010) The new family Diapheridae, a new species of *Diaphera* Albers from Thailand and the position of the Diapheridae within a molecular phylogeny of the Streptaxoidea (Pulmonata: Stylommatophora). Zoological Journal of the Linnean Society 160: 1–16. doi: 10.1111/j.1096-3642.2009.00598.x

Thiele J (1929–1935) Handbuch der Systematischen Weichtierkunde. Gustav Fischer, Jena, 1134 pp. [p. 1–376 (1929); p. 377–778 (1931); p. 779–1022 (1934); p. 1023–1134 (1935)]

Tryon GW Jr (1885) Manual of Conchology, Structure and Systematic, with Illustrations of the Species. Volume 1. The Academy of Natural Science of Philadelphia, 364 pp. doi: 10.5962/bhl.title.6534

Winckworth R (1936) Journal de Conchyliologie: dates of publication. Proceedings of the Zoological Society of London 106: 153–156.

Zilch A (1960) Gastropoda, Euthyneura. In: Schinderwolf OH (Ed.) Handbuch der Paläozoologie. Gebrüder Borntraeger, Berlin, 6: 401–834.

Zilch A (1961) Die Typen und Typoide des Natur-Museums Senckenberg 24: Mollusca, Streptaxidae. Archiv für Molluskenkunde 90: 79–120.

ZooKeys 589: 55–69 (2016)
doi: 10.3897/zookeys.589.7637
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

The mitochondrial genome of the land snail *Cernuella virgata* (Da Costa, 1778): the first complete sequence in the family Hygromiidae (Pulmonata, Stylommatophora)

Jun-Hong Lin[1], Wei-Chuan Zhou[2], Hong-Li Ding[1], Pei Wang[2], Hong-Mu Ai[1]

1 *College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China*
2 *Key Laboratory of Molluscan Quarantine and Identification of AQSIQ, Fujian Entry-Exit Inspection & Quarantine Bureau, Fuzhou, Fujian 350001, China*

Corresponding authors: *Pei Wang* (wangpei601@126.com); *Hong-Mu Ai* (aihongmu@yahoo.com.cn)

Academic editor: *F. Köhler* | Received 27 December 2015 | Accepted 25 April 2016 | Published 16 May 2016

http://zoobank.org/C251453E-ED89-4EDC-BD2D-7D37F5DAA5D1

Citation: Lin J-H, Zhou W-C, Ding H-L, Wang P, Ai H-M (2016) The mitochondrial genome of the land snail *Cernuella virgata* (Da Costa, 1778): the first complete sequence in the family Hygromiidae (Pulmonata, Stylommatophora). ZooKeys 589: 55–69. doi: 10.3897/zookeys.589.7637

Abstract

The land snail *Cernuella virgata* (da Costa, 1778) is widely considered as a pest to be quarantined in most countries. In this study, the complete mitochondrial genome of *C. virgata* is published. The mitochondrial genome has a length of 14,147 bp a DNA base composition of 29.07% A, 36.88% T, 15.59% C and 18.46% G, encoding 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes and two ribosomal RNA (rRNA) genes. The complete nucleotide composition was biased toward adenine and thymine, A+T accounting for 69.80%. Nine PCGs and 14 tRNA genes are encoded on the J strand, and the other four PCGs and eight tRNA genes are encoded on the N strand. The genome also includes 16 intergenic spacers. All PCGs start strictly with ATN, and have conventional stop codons (TAA and TAG). All tRNAs fold into the classic cloverleaf structure, except $tRNA^{Arg}$, $tRNA^{Ser(UCN)}$, $tRNA^{Ser(AGN)}$ and $tRNA^{Pro}$. The first three lack the dihydrouridine arm while the last lacks the TψC arm. There are 502 bp long noncoding regions and 418bp long gene overlaps in the whole mitochondrial genome, accounting for 3.54% and 2.95% of the total length respectively. Phylogenetic analyses based on the sequences of the protein coding genes revealed a sister group relationship between the Hygromiidae and the Helicidae.

Keywords

DNA sequencing, phylogeny, plant quarantine, secondary structure, white snail

Introduction

The land snail *Cernuella virgata* (da Costa, 1778), also known as the Mediterranean white snail or Common white snail, is endemic to the Mediterranean and western Europe, and has been introduced to America, Australia and Morocco (Barker 2004). The snail is omnivorous, feeding on detritus and plant matter, such as bark, stems and leaves of various green plants. Not only does it destroy agricultural crops, such as beans, cereal, various fruits and vegetables, it also can spread zoonotic food-borne parasitic diseases. For example, the species acts as intermediate host for the terrestrial trematode parasite *Brachylaima cribbi* (Kerney and Cameron 1979; Butcher and Grove 2006). Because of its remarkable adaptability and the severe damage it causes to agriculture, the natural environment and humans, the snail is considered a serious pest in the USA, Australia, Japan, Chile and other countries (Dennis 1996; Barker 2004; USDA 2008; MOA and AQSIQ 2012). One ship carrying barley from Australia was refused entry and berthing by Chile because of the presence of this snail causing huge economic losses (USDA 2008). It is also one of the more important quarantine terrestrial mollusks in America. To prevent invasion and proliferation, the U.S. government has invested considerable human and financial resources to eradicate the snails in Washington, Michigan and North Carolina (USDA 2008). Recently, Chinese ports have intercepted snails in barley, rapeseed and other consignments from abroad. Owing to its great harm, the snail was listed in "The People's Republic of China entry plant quarantine pest list" by the government in 2012 to prevent its introduction (MOA and AQSIQ 2012).

The metazoan mitochondrial (mt) genome usually comprise 37 genes and some noncoding regions, such as 13 protein coding genes (PCGs) (*COI–COIII*, *Cytb*, *ND1–ND6*, *ND4L*, *ATP6* and *ATP8*), two ribosomal RNA (rRNA) genes, 22 transfer RNA (tRNA) genes and the AT-rich region or control region (Wolstenholme 1992; Boore 1999). It has been extensively used to study the origin of species, phylogeography and population genetic structure and so on due to its small genome size, fast evolution, uniparental inheritance and lack of extensive recombination (Saccone et al. 1999; Elmerot et al. 2002). To date, only nine species from the order Stylommatophora have been determined as dispersing in Helicidae (Terrett et al. 1995; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013), Bradybaenidae (Yamazaki et al. 1997; Deng et al. 2014), Clausiliidae (Hatzoglou et al. 1995), Succineidae (White et al. 2011), Achatinidae (He et al. 2014) and Camaenidae (Wang et al. 2014). However, there are no reports on the mt genome of the family Hygromiidae. In this work, the complete mt genome of the snail *C. virgata* was obtained firstly using primer walking and shotgun sequencing techniques based on PCR. Studying the mitochondrial genome of *C. virgata* can not only offer more worthwhile information for phylogeny but also be applied to molecular alignment and identification in international plant quarantine measures.

Materials and methods

Specimen collection and DNA isolation

Adult snail was intercepted from barley shipments imported to China from southern Australia on 1 March 2012 and stored at -20 °C in the Key Laboratory of Molluscan Quarantine and Identification of AQSIQ, Fujian Entry-Exit Inspection & Quarantine Bureau, Fuzhou, Fujian, China (FJIQBC). Voucher specimens (FJIQBC000123) were deposited in FJIQBC. Total genomic DNA was obtained from approximately 50 mg fresh foot tissue, using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions.

DNA sequencing

The entire genome was successfully amplified by polymerase chain reaction (PCR) in overlapping fragments with four pairs of mitochondrial universal primers chosen from previous works (Palumbi et al. 1991; Folmer et al. 1994; Merritt et al. 1998; Hugall et al. 2002) and four pairs of perfectly matched primers designed from sequenced short fragments with Primer Premier 5.0 (Table 1). Short PCRs (< 2 kb) were performed using Takara Taq DNA polymerase (TaKaRa, Dalian, China), with the following cycling conditions: 30s at 94°C, followed by 35 cycles of 10s at 94°C, 50s at 40°C or 45°C, and 1 min at 72°C. The final elongation step was continued for 10 min at 72°C. Long range PCRs (> 4 kb) were performed using Takara Long Taq DNA polymerase (TaKaRa, Dalian, China) under the following cycling conditions: 1 min at 94°C, followed by 40 cycles of 10s at 98°C, 50s at 60°C, 4–8 min at 68°C, and the final elongation step at 72°C for 6 min. The PCR products were checked by spectrophotometry and 1.0% agarose gel electrophoresis.

The BigDye Terminator Sequencing Kit (Applied Biosystems, San Francisco, CA, USA) and the ABI PRIMER™ 3730XL DNA Analyzer (PE Applied Biosystems) were used to sequence short fragments from both directions after purification. For the long fragments, the shotgun libraries of *C. virgata* were constructed, and the positive clones were then sequenced using the above kit and sequenator with vector-specific primers *Bca*Best primer M13-47 and *Bca*Best Primer RV-M.

Genome annotation and inference of secondary structure

To control sequencing errors, each partial sequence was evaluated at least twice. Annotations and editing procedures of the mitochondrial genomes of *C. virgata* were performed in MEGA5.0. Mitochondrial PCGs and rRNA genes were identified by

Table 1. Primer pairs used for PCR amplification.

No. of fragment	Primer name	Nucleotide sequence (5' – 3') and location	Size (bp)	Reference
1	LCO-1490	GGTCAACAAATCATAAAGATATTGG		Folmer et al. 1994
	HCO-2198	TAAACTTCAGGGTGACCAAAAAATCA		Folmer et al. 1994
2	F1231	GAACGGGTTAGTTTGTTTGTCT(490–511)	1763	Present study
	R1231	TAGGGTCTTCTCGTCTATTATGGT(2229–2252)		Present study
3	16Sar-L	CGCCTGTTTATCAAAAACAT		Palumbi et al. 1991
	16Sbr-H	CCGGTCTGAACTCAGATCACGT		Palumbi et al. 1991
4	123F116	TGTAACCATAATAGACGAGAAGACC(2225–2249)	4545	Present study
	123R1b	TAGGAGCAAAAAATACTACCAGAAA(6745–6769)		Present study
5	144F	TGAGSNCARATGTCNTWYTG		Merritt et al. 1998
	272R	GCRAANAGRAARTACCAYTC		Merritt et al. 1998
6	123Fb	CTTTTCACCCCTACTTTAC(6683–6701)	1044	Present study
	123RII	ACTCCCTTTCAGGTGTTAT(7708–7726)		Present study
7	FCOII	AAATAATGCTATTTCATGAYCAYG		Hugall et al. 2002
	RCOII	GCTCCGCAAATCTCTGARCAYTG		Hugall et al. 2002
8	F1233	AGTTACATTGGCCCTCCCTAGTCTTCGC(7560–7587)	6930	Present study
	R1233	GTAAACGGTTCAACCTGTACCAGCTCCC(315–342)		Present study

BLAST searches at NCBI against other Eupulmonata sequences (Wang et al. 2014; He et al. 2014; Deng et al. 2014; Yang et al. 2014). The limits of both protein coding and rRNA genes were adjusted manually based on location of adjacent genes, and the presence of start and stop codons. The tRNA genes were located using DOGMA (Wyman et al. 2004) and tRNAscan-SE v.1.21(Lowe and Eddy 1997), while others that could not be determined by DOGMA and tRNAscan-SE were identified by comparison with other land snails (Terrett et al. 1995; Yamazaki et al. 1997; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013; Wang et al. 2014).

The base composition and codon usage were analyzed with MEGA 5.0 (Tamura et al. 2007). AT skew and GC skew were used to describe strand asymmetry according to the formulae AT = [A–T]/[A+T] and GC = [G–C]/[G+C] (Perna and Kocher 1995).

Phylogenetic analyses

Phylogenetic analyses were performed based on 15 complete mt genomes of gastropods from GenBank (Table 2) using maximum likelihood (ML) method. Two species from Basommatophora and Opisthobranchia were selected as outgroups. A DNA alignment with 10,362 bp length was inferred from the amino acid alignment of 13 PCGs using MEGA 5.0 (Tamura et al. 2007). The selection of best-fit-substitution model for ML estimation was performed using MEGA 5.0 with corrected Akaike information criterion (AIC). Node supports for ML analyses were calculated through 1000 bootstrap replicates. All other settings were kept as default.

Table 2. Summary of samples used in this study.

Subclass /order	Family	Species	Accession number	Reference
Stylommatophora	Hygromiidae	*Cernuella virgata*	KR736333	Present study
	Camaenidae	*Camaena cicatricosa*	KM365408	Wang et al. 2014
		Camaena sp.	KT001074	Ding et al. 2015 (submitted)
	Bradybaenidae	*Euhadra herklotsi*	Z71693 – Z71701	Yamazaki et al. 1997
		Mastigeulota kiangsinensis	KM083123	Deng et al. 2014
		Aegista diversifamilia	KR002567.1	Huang et al.2015
		Dolicheulota formosensis	KR338956.1	Huang et al.2015
	Helicidae	*Cornu aspersum*	JQ417195	Gaitán-Espitia et al. 2013
		Cepaea nemoralis	CMU23045	Terrett et al. 1995
		Cylindrus obtusus	JN107636	Groenenberg et al. 2012
	Succineidae	*Succinea putris*	JN627206	White et al. 2011
	Clausiliidae	*Albinaria caerulea*	X83390	White et al. 2011
	Achatinidae	*Achatina fulica*	NC024601	He et al. 2014
Basommatophora	Lymnaeidae	*Galba pervia*	JN564796	Liu et al. 2012
Opisthobranchia	Aplysiidae	*Aplysia californica*	AY569552	Knudsen et al. 2006

Results

Genome structural features

The entire circular genome was 14,147 bp in length (GenBank: KR736333), containing 13 PCGs, 22 tRNA genes and two rRNA genes (Figure 1). Twenty-four genes were encoded on the majority coding strand (J strand) while 13 genes were encoded on the minority coding strand (N strand) (*tRNA*^{Gln}, *tRNA*$^{Leu(UUR)}$, *tRNA*^{Asn}, *tRNA*^{Arg}, *tRNA*^{Glu}, *tRNA*^{Met}, *tRNA*$^{Ser(UCN)}$, *tRNA*^{Thr}, *ATP6*, *ATP8*, *ND3*, *COIII* and *SrRNA*) (Table 3). The nucleotide composition of the whole genome was biased toward adenine and thymine, accounting for 69.80% of base composition (Table 4). Gene overlaps with a total of 418 bp have been found at 14 gene junctions; the longest overlap (85 bp) existed between *ND5* and *ND1*. In addition, 502 nucleotides were dispersed in 16 intergenic spacers, the largest of which was 149 bp long between *tRNA*^{Trp} and *tRNA*^{Gly}. Additionally, two long spacers of 77 bp and 76 bp each were found between *ND4L* and *ND1*, *tRNA*$^{Ser(UCN)}$and *tRNA*$^{Ser(AGN)}$, respectively. There were seven close gene junctions with no intergenic spacers or overlap (Table 3).

Protein coding genes

The total length of all PCGs was 10, 977 bp, accounting for 77.59% of the entire mt genome (Table 4). All PCGs started strictly with the Start Codon ATN (four with ATG, five with ATT, and four with ATA) and ended with the conventional stop codons TAA or TAG. (Table 3).

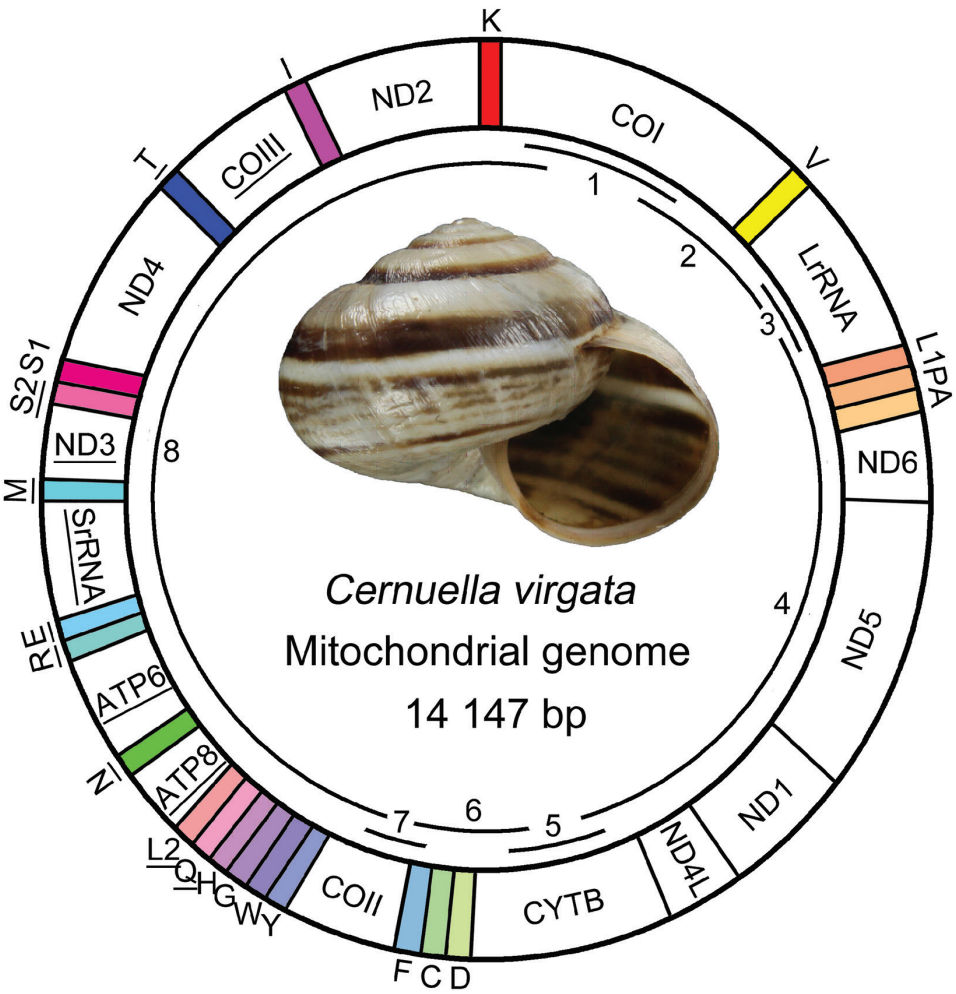


Figure 1. The mt genome of *Cernuella virgata*. The tRNA genes are labeled based on the IUPACIUB single letter amino acid codes. Genes with underline illustrate the direction of transcription from 3' to 5', and without underline revealing from 5' to 3'. Numbers and overlapping lines within the circle indicate PCR fragments amplified for sequencing (see Table 1).

Codon usage could reveal nucleotide bias. NNA and NNU as codons were used frequently in most PCGs. Additionally, the codons TTT (phenylalanine), TTA (leucine) and ATT (isoleucine) composing A and T were used widely (Figure 2).

Transfer RNA genes

The length of tRNA genes ranged from 53 to 69 bp.The 22 tRNA genes typically found in metazoan mt genomes were also discovered in *C. virgata*; eleven of them

Table 3. Organization of the *Cernuella virgata* mt genome.

Gene	Direction	Location	Size (bp)	Anticodon	Start codon	Stop codon	Intergenic nucleotides
COI	F	1–1497	1497		ATT	TAA	26
tRNA^{Val}	F	1494–1554	61	1524–1526 TAC			–4
lrRNA	F	1555–2567	1013				0
tRNA$^{Leu(CUN)}$	F	2568–2628	61	2597–2599 TAG			0
tRNA^{Pro}	F	2629–2685	57	2655–2657 TGG			0
tRNA^{Ala}	F	2687–2748	62	2718–2720 TGC			1
ND6	F	2767–3222	456		ATA	TAA	18
ND5	F	3227–4888	1662		ATT	TAA	4
ND1	F	4804–5769	966		ATG	TAG	–85
ND4L	F	5847–6215	369		ATT	TAA	77
CytB	F	6151–7167	1017		ATA	TAG	–65
tRNA^{Asp}	F	7157–7214	58	7188–7190 GTC			–11
tRNA^{Cys}	F	7215–7276	62	7245–7247 GCA			0
tRNA^{Phe}	F	7283–7341	59	7313–7315 GAA			6
COII	F	7387–8031	645		ATT	TAA	45
tRNA^{Tyr}	F	8015–8083	69	8046–8048 GTA			–17
tRNA^{Trp}	F	8071–8132	62	8102–8104 TCA			–13
tRNA^{Gly}	F	8282–8341	60	8311–8313 TCC			149
tRNA^{His}	F	8338–8398	61	8369–8371 GTG			–4
tRNA^{Gln}	R	8400–8457	58	8427–8429 TTG			1
tRNA$^{Leu(UUR)}$	R	8457–8513	57	8485–8487 TAA			–1
ATP8	R	8485–8754	270		ATG	TAA	–29
tRNA^{Asn}	R	8743–8804	62	8771–8773 GTT			–12
ATP6	R	8807–9472	666		ATG	TAA	2
tRNA^{Arg}	R	9458–9517	60	9489–9491 TCG			–15
tRNA^{Glu}	R	9518–9578	61	9547–9549 TTC			0
SrRNA	R	9579–10277	699				0
tRNA^{Met}	R	10278–10343	66	10306–10308 CAT			0
ND3	R	10304–10735	432		ATA	TAA	–40
tRNA$^{Ser(UCN)}$	R	10691–10743	53	10723–10725 TGA			–45
tRNA$^{Ser(AGN)}$	F	10820–10880	61	10844–10846 GCT			76
ND4	F	10904–12178	1275		ATT	TAG	23
tRNA^{Thr}	R	12182–12246	65	12210–12212 TGT			3
COIII	R	12170–13051	882		ATG	TAA	–77
tRNA^{Ile}	F	13068–13127	60	13096–13098 GAT			16
ND2	F	13182–14060	879		ATA	TAG	54
tRNA^{Lys}	F	14062–14121	60	14090–14092 TTT			1

Note: Negative numbers indicate adjacent gene overlap.

were determined by tRNAscan-SE and eight of them were determined by DOGMA. Another three tRNA genes that could not be detected by the above two programs were identified and passed through comparisons with known patterns of previous research

Table 4. Nucleotide composition and skewness of the *Cernuella virgata* mt genome.

	Proportion of nucleotides							
Feature	**%A**	**%T**	**%G**	**%C**	**%A+T**	**AT Skew**	**GC Skew**	**No. of nucleotides**
Whole genome	29.07	36.88	18.46	15.59	69.80	–0.12	0.08	14147
Protein coding genes	26.39	39.31	18.43	15.87	69.26	–0.20	0.07	10977
Protein coding genes (J)	26.08	39.96	18.70	15.26	69.17	–0.21	0.10	8739
Protein coding genes (N)	27.61	36.77	17.38	18.23	69.67	–0.14	–0.02	2034
tRNA genes	31.46	34.23	18.73	15.58	71.41	–0.04	0.09	1335
tRNA genes (J)	29.82	34.77	20.30	15.10	70.77	–0.08	0.15	788
tRNA genes (N)	33.82	33.46	16.45	16.27	72.54	0.01	0.01	547
rRNA genes	32.83	35.63	17.00	14.54	72.42	–0.04	0.08	1712

Figure 2. Relative synonymous codon usage (RSCU) in the *Cernuella virgata* mt genome. Codon families are provided on the x axis.

Fourteen tRNA genes were encoded on the J strand and the remainder on the N strand. Most tRNA genes could be folded into classic clover leaf structures except for $tRNA^{Arg}$, $tRNA^{Ser(UCN)}$and $tRNA^{Ser(AGN)}$, which lack the dihydrouridine arm. The gene $tRNA^{Pro}$ has a loop in its TψC arm (Figure 3).

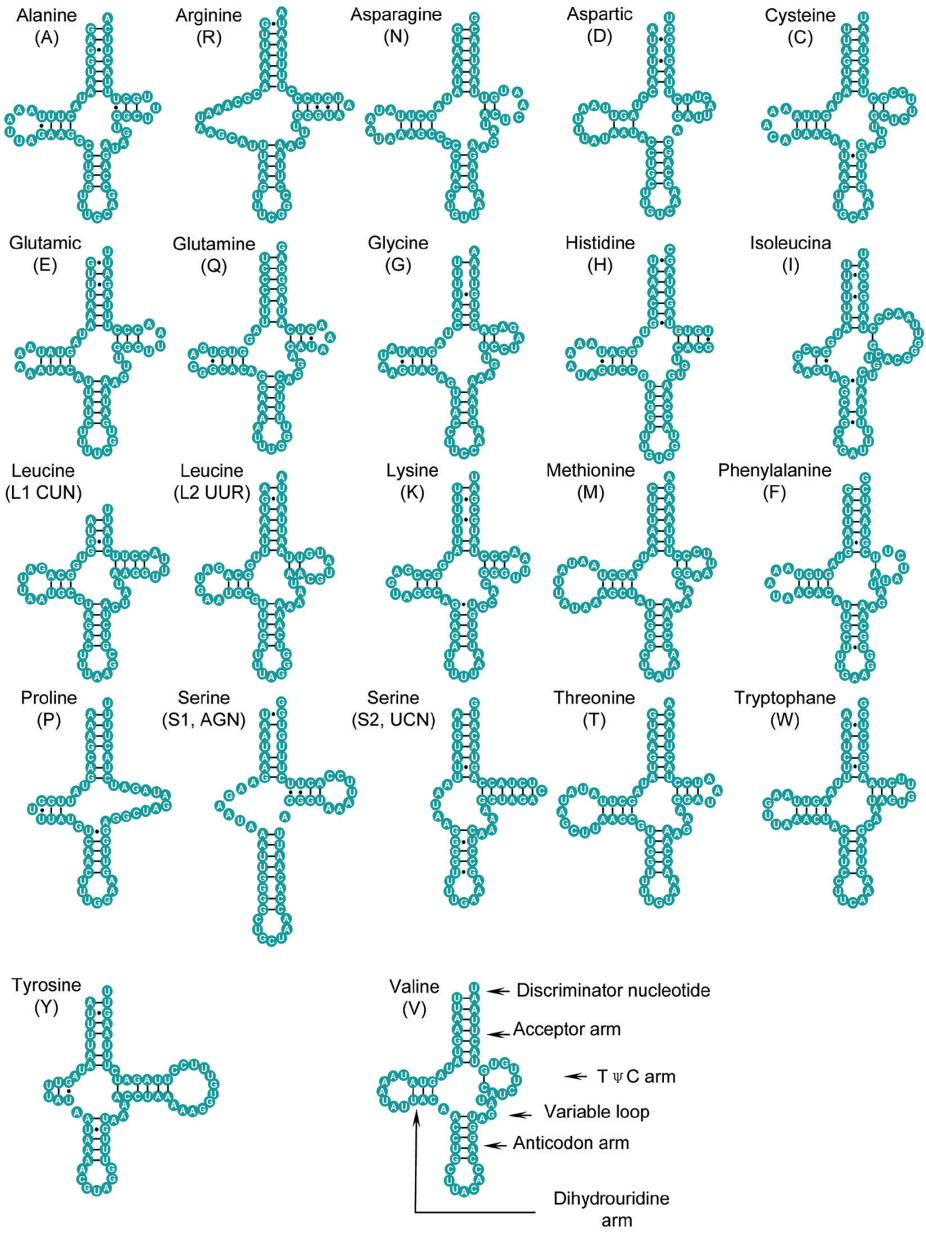


Figure 3. Inferred secondary structures of 22 tRNA genes in *Cernuella virgata*. Dashes (-) indicate Watson-Crick base pairing and bullets (•) indicate G-U base pairing.

In some tRNA genes, non-Watson-Crick matches and aberrant loops had been found. For example, a total of 41 unmatched base pairs existed in some tRNAs, and 18 of them were G-U non-classical pairs, most of which existed in Discriminator nucleotide, anticodon arm and Dihydrouridine arm (Figure 3).

Ribosomal RNA genes

The rRNA genes of *C. virgata* encompassed the *lrRNA* and *srRNA* genes with a length of 1,013 bp and 699 bp, repsectively. The former was situated between $tRNA^{Val}$ and $tRNA^{Leu(CUN)}$ and the latter was located between $tRNA^{Glu}$ and $tRNA^{Met}$ (Table 3).

Noncoding regions

In the mitochondrial genome of *C. virgata*, there are 16 noncoding regions with total 502 bp length, accounting for 3.54%. The longest was 149bp, between $tRNA^{Trp}$ and $tRNA^{Gly}$. The shortest was 1 bp existing three regions, respectively locating $tRNA^{Pro}$ and $tRNA^{Ala}$, $tRNA^{His}$ and $tRNA^{Gln}$, *ND2* and $tRNA^{Lys}$ (Table 3).

Phylogenetic reconstruction

The ML tree (Figure 4) presented nine major clades containing the families Helicidae, Hygromiidae, Camaenidae, Bradybaenidae, Succineidae, Clausiliidae, Achatinidae, Lymnaeidae and Aplysiidae. The four bradybaenid species and three helicid species each formed a clade and a sister pair. In addition, we found that Camaenidae and Bradybaenidae each were monophyletic and also in a sister group relationship with each other.

Discussion

The length of mt genome of *C. virgata* was 304 bp longer than *Camaena cicatricosa* and 97 bp longer than *Cornu aspersum*. All gene directions showed similarity to the sequenced mt genome of *C. cicatricosa*, but gene order was different, especially with respect to the positions between *CYTB* and *ATP8* genes (Gaitán-Espitia et al. 2013; Wang et al. 2014). The overall mt genome of *C. virgata* was loose particularly, with more and longer intergenic spacers.

In the study of mt genome of *C. cicatricosa*, GTG is the start codon of the *COII* gene, and *COI* and *ND6* genes of *C. aspersum* start with TTG (Gaitán-Espitia et al. 2013; Wang et al. 2014). From previous studies we can see that most start signals of land snails were consistent with *C. virgata* factually, but ATC, TTA, TTG, CTT, TCG and CGA as start signals have been found (Raay and Crease 1994; Crease 1999; Yamazaki et al. 1997; Yu et al. 2007; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013; Wang et al. 2014). Conventional stop codons TAA and TAG have been found in all PCGs of *C. virgata*, which corresponds to *C. cicatricosa* (Wang et al. 2014). However, *COII*, *CYTB*, *ND3* and *ATP8* genes of *C. aspersum* from the family Helicidae ended with T, and this phenomenon has also been discovered in other snails as

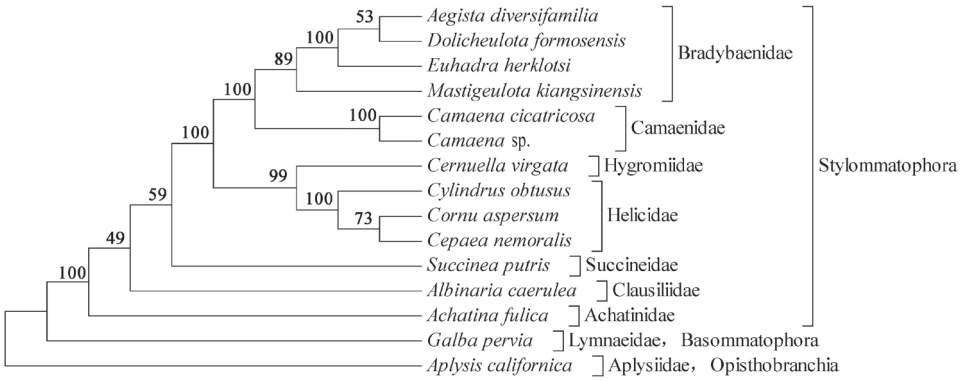


Figure 4. Phylogenetic tree inferred by maximum likelihood (ML) method based on 13 protein genes. The tree is rooted with *Aplysis californica* and *Galba pervia*. Numbers on the nodes represent bootstrap values.

well (Terrett et al. 1995; Hatzoglou et al. 1995; Yamazaki et al. 1997; White et al. 2011; Groenenberg et al. 2012; Wang et al. 2012; Gaitán-Espitia et al. 2013). Some authors suggested that this nucleotide exchange was caused by post-transcriptional polyadenylation (Ojala et al. 1981; Cha et al. 2007).

Usually, in the tRNA, the Acceptor arm (7 bp) and Anticodon arm (5 bp) were conservative in size (Kinouchi et al. 2000). However, the length of Acceptor arm of $tRNA^{Leu(CUN)}$ in *C. virgata* was distinctive, with only 4 bp in size. The Anticodon arm of $tRNA^{Ser(AGN)}$ (8 bp) and all Anticodon loops (7 nucleotides) was coincident with the snail *C. cicatricosa* (Wang et al. 2014). The remaining arms and loops changed apparently in size comparing to that of other land snails (Hatzoglou et al. 1995; Groenenberg et al. 2012; Wang et al. 2014). Some non-Watson-Crick matches existed in all tRNA, including G-U pairs, A-C mismatch, U-C mismatch etc. Tomita et al. (2001) raised that these mismatches may can be rectified by post-transcriptional RNA-editing mechanism to hold tRNA function.

Noncoding regions are assumed to splice recognition sites during the process of transcription (He et al. 2005). In the previous sequenced complete mt genome of the order Stylommatophora, the noncoding regions range from 1 bp to 65 bp (Hatzoglou et al. 1995; Terrett et al. 1995; Yamazaki et al. 1997; White et al. 2011; Groenenberg et al. 2012; Gaitán-Espitia et al. 2013; Deng et al. 2014; Wang et al. 2014) except *Achatina fulica* with 551 bp length (He et al. 2014). In metazoan mt genomes, these noncoding regions are normal. The longest one can be called control region or AT-rich region (Boore 1999). Usually, changes in length of the whole mt genome are mainly caused by difference of the control region (Zhang and Hewitt 1997). However, the control region may not be aligned accurately in gastropods (Groenenberg et al. 2012) except in *A. fulica* which included a 551 bp putative control region (POR) between *COI* and $tRNA^{Val}$ (He et al. 2014). Another ten sequenced stylommatophoran species may possess short putative control region located in different places (Hatzoglou et al. 1995; Terrett et al. 1995; Yamazaki et al. 1997; White et al. 2011; Groenenberg et

al. 2012; Gaitán-Espitia et al. 2013; Deng et al. 2014; Wang et al. 2014; Huang et al. 2015; 2015). The PORs of *C. virgata*, *Mastigeulota kiangsinensis* and *Dolicheulota formosensis* are situated adjacent to *tRNA^{Trp}*, at 149 bp, 216 bp and 245 bp respectively. The PORs of *C. cicatricosa* (29 bp) and *Succinea putris* (48 bp) were located between *COIII* and *tRNA^{Ile}*. Two other helicid species had PORs located between *COIII* and *tRNA^{Ser}* with lengths of 158–186 bp, whereas the PORs of *Albinaria caerulea* (65 bp), *Aegista diversifamilia* (93 bp), *Cylindrus obtusus* (395 bp) and *Euhadra herklotsi* (78 bp) were specific, respectively between *ND3* and *tRNA^{Ser}*, *tRNA^{Met} and tRNA^{Ser}*, *ND5* and *tRNA^{Ala}*, *tRNA$^{Se\,r\,(UCN)}$* and *tRNA$^{Ser(AGN)}$*. The absence of a control region was consistent with other gastropods (Deng et al. 2014; Wang et al. 2014; Yang et al. 2014). In the present study, the longest noncoding region was 149 bp, which was the second longest one by far.

Three species in the Helicidae were sister groups and consistent with previous works (Gaitán-Espitia et al. 2013). However, the systematics of Camaenidae, Helicidae and Bradybaenidae are complicated and have not been fully resolved; systematic and phylogenetic studies based on analyses of morphological and molecular markers have produced inconsistent results (Scott 1996; Cuezzo 2003; Wade et al. 2007; Hirano et al. 2014). More complete taxon sampling need to be prepared to assess the phylogenetic relationship of these three families.

Acknowledgments

We sincerely thank professor De-Niu Chen of Institute of Zoology, Chinese Academy of Science for helpful comments on the manuscript. We thank the reviewers and the subject editor for their very helpful suggestions on the manuscript. This research is supported by National Natural Science Foundation of China (31372162), Natural Science Foundation in Fujian Province (2015J05076) and Public Science and Technology Research Funds Projects of General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (201410076, 2015IK042).

References

Barker GM (2004) Natural Enemies of Terrestrial Molluscs. CABI Publishing, London, 644 pp. doi: 10.1079/9780851993195.0000

Boore JL (1999) Animal mitochondrial genomes. Nucleic Acids Research 27(8): 1767–1780. doi: 10.1093/nar/27.8.1767

Boore JL (2006) The complete sequence of the mitochondrial genome of *Nautilus macromphalus* (Mollusca: Cephalopoda). BMC Genomics 7: 182. doi: 10.1186/1471-2164-7-182

Butcher AR, Grove DI (2006) Seasonal variation in rates of sporocyst and metacercarial infection by *Brachylaima cribbi* in helicid and hygromiid land snails on the Yorke Peninsula, South Australia. Australian Journal of Zoology 53(6): 375–382. doi: 10.1071/ZO05054

Cha SY, Yoon HJ, Lee EM, Yoon MH, Hwang JS, Jin BR, Han YS, Kim I (2007) The complete nucleotide sequence and gene organization of the mitochondrial genome of the bumblebee, *Bombus ignitus* (Hymenoptera: Apidae). Gene 392: 206–220. doi: 10.1016/j.gene.2006.12.031

Crease TJ (1999) The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). Gene 233: 89–99. doi: 10.1016/S0378-1119(99)00151-1

Deng PJ, Wang WM, Huang XC, Wu XP, Xie GL, Ouyang S (2016) The complete mitochondrial genome of Chinese land snail *Mastigeulota kiangsinensis* (Gastropoda: Pulmonata: Bradybaenidae). Mitochondrial DNA 27(2): 1084–1085. doi: 10.3109/19401736.2014.953083

Dennis H (1996) Snails: A Quarantine Concern for Export Citrus to the USA. Entomology Unit, SARDI, Australia.

Elmerot C, Arnason U, Gojobori T (2002) The mitochondrial genome of the pufferfish, Fugu rubripes, and ordinal teleostean relationships. Gene 295(2): 163–172. doi: 10.1016/s0378-1119(02)00688-1

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Gaitán-Espitia JD, Scheihing R, Poulin E, Artacho P, Nespolo RF (2013) Mitochondrial phylogeography of the land snail *Cornu aspersum*: tracing population history and the impact of human-mediated invasion in austral South America. Evolutionary Ecology Research 15: 1–18.

Grande C, Templado J, Cervera JL, Zardoya R (2002) The complete mitochondrial genome of the nudibranch *Roboastra europaea* (Mollusca: Gastropoda) supports the monophyly of opisthobranchs. Molecular Biology and Evolution 19(10): 1672–1685. doi: 10.1093/oxfordjournals.molbev.a003990

Groenenberg DSJ, Pirovano W, Gittenberger E, Schilthuizen M (2012) The complete mitogenome of *Cylindrus obtusus* (Helicidae, Ariantinae) using Illumina next generation sequencing. BMC Genomics 13: 114. doi: 10.1186/1471-2164-13-114

Hatzoglou E, Rodakis GC, Lecanidou R (1995) Complete sequence and gene organization of the mitochondrial genome of the land snail *Albinaria coerulea*. Genetics 140(4): 1353–1366.

He Y, Jones J, Armstrong M, Lamberti F, Moens M (2005) The mitochondrial genome of *Xiphinema americanum sensu stricto* (Nematoda: Enoplea): Considerable economization in the length and structural features of encoded genes. Journal of Molecular Evolution 61: 819–833. doi: 10.1007/s00239-005-0102-7

He ZP, Dai XB, Zhang S, Zhi TT, Lun ZR, Wu ZD, Yang TB (2014) Complete mitochondrial genome of the giant African snail, *Achatina fulica* (Mollusca: Achatinidae): a novel location of putative control regions (CR) in the mitogenome within Pulmonate species. Mitochondrial DNA 27(2): 1–2.

Hirano T, Kameda Y, Kimura K, Chiba S (2014) Substantial incongruence among the morphology, taxonomy, and molecular phylogeny of the land snails *Aegista*, *Landouria*, *Trishoplita*, and *Pseudobuliminus* (Pulmonata: Bradybaenidae) occurring in East Asia. Molecular Phylogenetics and Evolution 70: 171–181. doi: 10.1016/j.ympev.2013.09.020

Hugall A, Moritz C, Moussalli A, Stanisic J (2002) Reconciling paleodistribution models and comparative phylogeography in the Wet Tropics rainforest land snail *Gnarosophia bellendenkerensis* (Brazier 1875). PNAS 99(9): 6112–6117. doi: 10.1073/pnas.092538699

Kerney MP, Cameron RAD (1979) A field guide to the land snails of Britain and northwestern Europe. London, Collins, 288 pp.

Kinouchi M, Kanaya S, Ikemura T, Kudo Y (2000) Detection of tRNA based on the cloverleaf secondary structure. Genome Information 11: 301–302.

Lowe TM, Eddy SR (1997) TRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Research 25: 955–964. doi: 10.1093/nar/25.5.0955

Merritt TJS, Shi L, Chase MC, Rex MA, Etter RJ, Quattro JM (1998) Universal cytochrome b primers facilitate intraspecific studies in molluscan taxa. Molecular Marine Biology and Biotechnology 7: 7–11.

MOA, AQSIQ (2012) The List of Quarantine Pests of the People's Republic of China of the Entry of Plants. Beijing, China.

Ojala D, Montoya J, Attardi G (1981) tRNA punctuation model of RNA processing in human mitochondrial. Nature 290(5806): 470–474. doi: 10.1038/290470a0

Palumbi S, Martin A, Romano S, Mcmillan WO, Stice L, Grabowwski G (1991) The Simple Fool's Guide to PCR. Department of Zoology, University of Hawaii, Honolulu.

Perna NT, Kocher TD (1995) Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. Journal of Molecular Evolution 41: 353–358. doi: 10.1007/BF01215182

Raay TJ, Crease TJ (1994) Partial mitochondrial DNA sequence of the crustacean *Daphnia pulex*. Current Genetics 25: 66–72. doi: 10.1007/BF00712970

Saccone C, De GC, Gissi C, Pesole G, Reyes A (1999) Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. Gene 238(1): 195–209. doi: 10.1016/S0378-1119(99)00270-X

Scott B (1996) Phylogenetic relationships of the Camaenidae. Journal of Molluscan Studies 62: 65–73. doi: 10.1093/mollus/62.1.65

Steinke D, Albrecht C, Pfenninger M (2004) Molecular phylogeny and character evolution in the Western Palearctic Helicidae s.l. (Gastropoda: Stylommatyophora). Molecular Phylogenetics and Evolution 32: 724–734. doi: 10.1016/j.ympev.2004.03.004

Tamura K, Peterson D, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739. doi: 10.1093/molbev/msr121

Terrett JA, Miles S, Thomas RH (1995) Complete DNA sequence of the mitochondrial genome of *Cepaea nemoralis* (Gastropoda: Pulmonata). Journal of Molecular Evolution 42: 160–168. doi: 10.1007/BF02198842

USDA (2008) Port of Tacoma *Cernuella virgata* (*C. virgata*) Eradication Program in Pierce County, Washington, 12 pp.

Wade CM, Hudelot C, Davison A, Naggs F, Mordan PB (2007) Molecular phylogeny of the helicoid land snails (Pulmonata: Stylommatophora: Helicoidea), with special emphasis on the Camaenidae. Journal of Molluscan Studies 73: 411–415. doi: 10.1093/mollus/eym030

Wang JP, Nie XP, Cao TW, Zhang M, Guo YP, Ma EB, Zhang XN (2012) Analysis of complete mitochondrial genome of *Sasakia charonda coreana* (Lepidoptera, Nymphalida). Acta Zootaxonomica Sinica 37(1): 1–9.

Wang P, Yang HF, Zhou WC, Hwang CC, Zhang WH, Qian ZX (2014) The mitochondrial genome of the land snail *Camaena cicatricosa* (Müller, 1774) (Stylommatophora: Camaenidae): Presently known as the first in Camaenidae. ZooKeys 451: 33–48. doi: 10.3897/zookeys.451.8537

White TR, Conrad MM, Tseng R, Balayan S, Golding R, Martins AM, Dayrat BA (2011) Ten new complete mitochondrial genomes of pulmonates (Mollusca: Gastropoda) and their impact on phylogenetic relationships. BMC Evolutionary Biology 11(1): 295. doi: 10.1186/1471-2148-11-295

Wyman SK, Jansen RK, Boore JL (2004) Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20(17): 3252–3255. doi: 10.1093/bioinformatics/bth352

Yamazaki N, Ueshima R, Terrett JA, Yokobori S, Kaifu M, Segawa R, Kobayashi T, Numachi K, Ueda T, Nishikawa K, Watanabe K, Thomas RH (1997) Evolution of Pulmonate Gastropod mitochondrial genomes: comparisons of gene organizations of *Euhadra*, *Cepaea* and *Albinaria* and implications of unusual tRNA secondary structures. Genetics 145: 749–758.

Yang HR, Zhang JE, Luo H, Luo MZ, Guo J, Deng ZX, Zhao BL (2014) The complete mitochondrial genome of the mudsnail *Cipangopaludina cathayensis* (Gastropoda: Viviparidae). Mitochondrial DNA 16: 1–3.

Yu DJ, Xu L, Nardi F, Li JG, Zhang RJ (2007) The complete nucleotide sequence of the mitochondrial genome of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). Gene 396: 66–74. doi: 10.1016/j.gene.2007.02.023

Zhang DX, Hewitt GM (1997) Insect mitochondrial control region: A review of its structure, evolution and usefulness in evolutionary studies. Biochemical Systematics and Ecology 25: 99–120. doi: 10.1016/S0305-1978(96)00042-7

ZooKeys 589: 71–96 (2016)
doi: 10.3897/zookeys.589.7951
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Three new species of *Misionella* from northern Brazil (Araneae, Haplogynae, Filistatidae)

Antonio D. Brescovit[1], Ivan L. F. Magalhaes[2], Igor Cizauskas[1]

1 *Laboratório Especial de Coleções Zoológicas, Instituto Butantan, Av. Vital Brasil, 1500, 05503-900, São Paulo, SP, Brasil* **2** *División Aracnología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Av. Angel Gallardo 470, C1405DJR, Buenos Aires, Argentina*

Corresponding author: *Antonio D. Brescovit* (antonio.brescovit@butantan.gov.br)

Academic editor: *Cor Vink* | Received 27 January 2016 | Accepted 18 April 2016 | Published 16 May 2016

http://zoobank.org/4C75D8FC-6DAB-4706-9077-0922FABDD800

Citation: Brescovit AD, Magalhaes ILF, Cizauskas I (2016) Three new species of *Misionella* from northern Brazil (Araneae, Haplogynae, Filistatidae). ZooKeys 589: 71–96. doi: 10.3897/zookeys.589.7951

Abstract

Three new species of the genus *Misionella* are described from Brazil: *M. carajas* **sp. n.** and *M. aikewara* **sp. n.** from caves in the states of Pará and Tocantins and *M. pallida* **sp. n.** from natural and synanthropic dry areas in the states of Piauí, Maranhão, Rio Grande do Norte and Bahia. These species seem to belong to a distinct group within the genus; the males have an elongate palpal tibia and bulb, a pair of characteristic and hirsute macrosetae in the second metatarsus and the females have internal genitalia with only one pair of spermathecae, with relatively short ducts, lacking the auxiliary receptacles. Their phylogenetic placement and geographic distribution are briefly discussed.

Keywords

Caves, Caatinga, endemics, Prithinae, spiders, taxonomy

Introduction

The genus *Misionella* Ramírez & Grismado, 1997 was established to include the species *Filistata mendensis* described by Mello-Leitão (1920) from Mendes, state of Rio de Janeiro, Brazil. This species is very common in southeastern Brazil and northeastern Argentina, where populations have synanthropic habits, living in the brick walls in houses or on tree bark near buildings or urban squares. Grismado and Ramírez (2000) described *M. jaminawa*, a second species from the state of Acre, in Brazilian Amazonia.

The species of the genus *Misionella* resemble those of *Pikelinia* Mello-Leitão in having enlarged male palpal tibia and second male metatarsi retrolaterally excavated, with short spinules (Figs 1–2) or with excavation absent, but having at least a pair of macrosetae (Fig. 1H). According to Ramírez and Grismado (2000) species of these genera differ by the absence of any projection in the male palpal tibia (Ramírez and Grismado 1997, figs 100–102) and by having two pairs of spermathecae placed side by side in the internal female genitalia (see Ramírez and Grismado 1997, Fig. 103) in Misionella. Following the original diagnosis presented by Ramírez and Grismado (2000) the species here described seem to belong to a distinct group in the genus Misionella. The males of these species lack an apophysis on the palpal tibia, have a characteristic pair of hirsute spines in the second metatarsus (Figs 1G–I) and the females have internal genitalia with only one pair of spermathecae with short ducts (Figs 8C, 11C).

In this paper, we describe these three new, morphologically deviant species of *Misionella* from Brazil, two from caves in the states of Pará and Tocantins and a third from dry areas in the states of Piauí, Maranhão, Rio Grande do Norte and Bahia.

Material and methods

The material examined belongs to the following institutions:

CHNUFPI Coleção de História Natural, Universidade Federal do Piauí, Floriano (L.S. Carvalho);
IBSP Instituto Butantan, São Paulo (A.D. Brescovit);
ISLA Zoology Collection, Seção de Invertebrados Subterrâneos da Universidade Federal de Lavras, Lavras (R.L. Ferreira);
MPEG Museu Paraense Emílio Goeldi, Belém (A.B. Bonaldo);
MZSP Museu de Zoologia da Universidade de São Paulo, São Paulo (R. Pinto da Rocha);
UFMG Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte (A.J. Santos).

Descriptions follow Ramírez and Grismado (1997). Measurements are expressed in millimeters. Leg segment lengths were measured laterally. The illustrations were made in a stereomicroscope with *camera lucida*. For the illustration of female genitalia, we used dissected organs immersed in clove oil, following Levi (1965). Image stacks were obtained with a Leica M165 C stereoscopic microscope and extended focus images were generated using Helicon Focus 6 (www.heliconsoft.com); female spermathecae were digested with pancreatin and photographed in temporary mounts without a coverslip in lactic acid on an Olympus BH-2 compound microscope. Material for SEM was either air-dried or dehydrated using an ethanol series followed by immersion in HDMS, and sputter-coated with 10 nm of gold or gold-palladium. Micrographs were taken with a Quanta 250 the electron scanning microscope at Laboratório de Biologia

Celular Instituto Butantan, LEO 1450VP of the Museu Paraense Emílio Goeldi, or a Philips FEI XL30 TMP at Museo Argentino de Ciencias Naturales "Bernardino Rivadavia".

Taxonomy

Family Filistatidae Ausserer, 1867
Subfamily Prithinae Gray, 1995

Misionella Ramírez & Grismado, 1997

Misionella Ramírez & Grismado, 1997: 342, type species *Misionella mendensis* (Mello-Leitão, 1920), by monotypy and original designation.

Diagnosis. Males of *Misionella* have the cymbium fused to the tegulum, as do *Pikelinia* and *Lihuelistata*; they can be distinguished from *Pikelinia* by the lack of an apophysis on the palpal tibia, and from *Lihuelistata* by the modified second metatarsi (Ramírez and Grismado 1997). Females of *Misionella* can be distinguished from *Kukulcania* Lehtinen by having a three-rowed calamistrum, from *Pikelinia* by having spermathecae either paired side by side or unilobulate, and from *Lihuelistata* by lacking pores on the ducts of the spermathecae (Ramírez and Grismado 1997).

Misionella carajas sp. n.

http://zoobank.org/37090EE0-9A6D-4A39-8E2D-5F79135D97B9
Figs 1A–L, 2A–D, 3A–F, 11A–C, 12A, 13A–D, 14

Types. Male holotype from Cave N4E_0024 (06°02'01"S, 50°10'07"W), FLONA Carajás, Parauapebas, Pará, Brazil, 20/IV–04/V/10, D. B. Pedroso col. (IBSP 161396). Paratypes: female paratype from Cave N5S_0059 (06°06'29"S, 50°07'57"W), FLONA Carajás, Parauapebas, Pará, Brazil, 25/VIII–03/IX/2009, I. Cizauskas col. (IBSP 161395); two males and two females from Cave N4E_0070 (06°01'56"S, 50°09'10"W), 24-30/VII/2009, I. Cizauskas (MPEG 24927); two males and two females from Cave N4E_0079 (06°01'59"S, 50°09'05"W), 24/VII/2009–04/III/2010, I. Cizauskas, D.B. Pedroso, J.B. Verdiani & J. Mascarenhas (MZSP 68015)

Additional material examined. BRAZIL. *Pará*: Parauapebas, Flona Carajás, Cave GEM-1797 (06°06'25"S, 50°08'07"W), 3♂ 3♀, 23/VIII/2010, R. Zampaulo col. (IBSP 165001); Cave N1_0002 (06°02'24"S, 50°16'12"W), 2♀, 11/VI–02/VII/2014 (IBSP 181113; 191159); Cave N1_0023 (06°01'13"S, 50°16'40"W), 2♂, 11/VI–02/VII/2014 (IBSP 181114; 191160); Cave N1_0059 (06°01'11"S, 50°16'44"W), 2♂ 2♀, 11/VI-02/VII/2014 (IBSP 181115–181116, 191161–191162); Cave N1_0063 (06°01'07"S, 50°16'45"W), 4♂ 5♀, 11/VI–02/

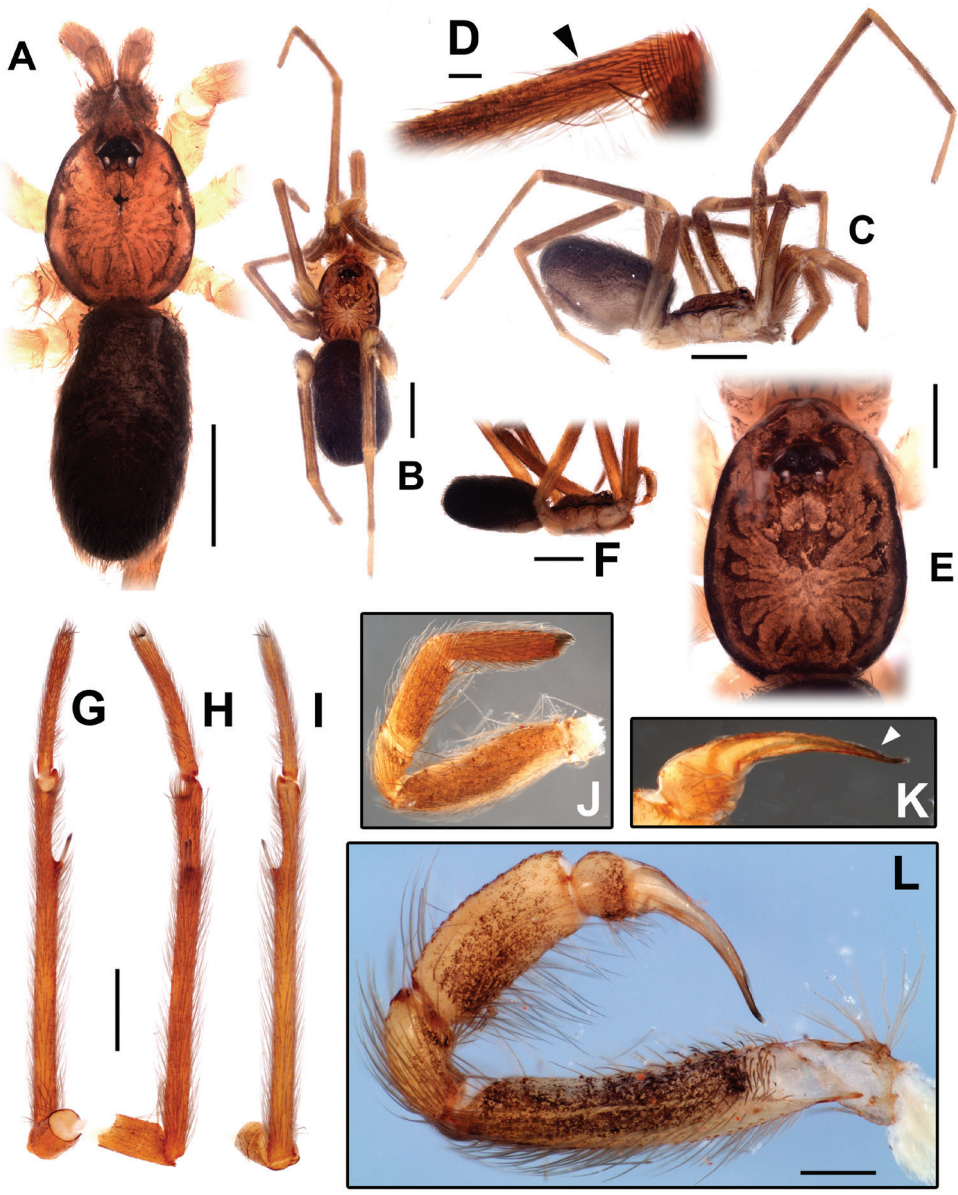


Figure 1. Female from Cave N4E_0079, Flona Carajás, Parauapebas, Pará (IBSP 166200) (**B–E, J**) male from the same locality (IBSP 166199) (**A, F–I, K-L**). **A–B** habitus, dorsal view **C** habitus, lateral view **D** left metatarsus IV retrolateral showing calamistrum (indicated by an arrow) **E** carapace, dorsal view **F** habitus, lateral view **G** left metatarsus II, ventral view **H** same, retrolateral view **I** same, dorsal view **J** right pedipalp **K** left palp, prolateral view, white arrow indicates the paraembolic lamina **L** same, complete male palp, prolateral view. Scale bars: 1 mm, except **G–I** (0.5 mm) and **D, L** (0.1 mm).

VII/2014 (IBSP 181117–18119; 191163–191165); Cave N1_0075 (06°01'14"S, 50°16'49"W), 2♂ 1♀, 11/VI–02/VII/2014 (IBSP 181120; 191166); Cave N1_0077 (06°01'14"S, 50°16'52"W), 2♀, 11/VI–02/VII/2014 (IBSP 191167–191168); Cave N1_0080 (06°01'11"S, 50°16'48"W), 2♀, 11/VI–-02/VII/2014 (IBSP 191169); Cave N1_0081 (06°01'13"S, 50°16'47"W), 1♀, 11/VI–02/VII/2014 (IBSP 191170); Cave N1_0083 (06°01'20"S, 50°16'47"W), 1♂ 1♀, 11/VI–02/VII/2014 (IBSP 191171); Cave N1_0087 (06°01'09"S, 50°16'59"W), 1♂ 1♀, 11/VI–02/VII/2014 (IBSP 191172); Cave N1_0094 (06°01'11"S, 50°16'57"W), 2♂ 3♀, 11/VI–02/VII/2014 (IBSP 191173–191174); Cave N1_0096 (06°01'09"S 50°16'59"W), 1♀, 11/VI–02/VII/2014 (IBSP 191175); Cave N1_0098 (06°01'09"S, 50°17'05"W), 2♀ 2 imm, 28/X–03/XI/2007 (IBSP 97883); Cave N1_0123 (06°01'09"S, 50°16'45"W), 1♀, 11/VI–02/VII/2014 (IBSP 191176); Cave N1_0185 (06°02'35"S, 50°16'33"W), 1♂ 1♀, 16/VII–06/VIII/2014 (IBSP 191187); Cave N1_0224 (06°01'16"S 50°16'19"W), 3♂ 3♀, 11/VI–02/VII/2014 (IBSP 191178-191181); Cave N1_0234 (06°01'15"S, 50°16'24"W), 2♀, 11/VI–02/VII/2014 (IBSP 191182-191183); Cave N1_0236 (06°01'15"S, 50°16'25"W), 1♂, 11/VI–02/VII/2014 (IBSP 191184); Cave N1_0239 (06°01'19"S, 50°16'26"W), 2♀, 11/VI–02/VII/2014 (IBSP 191185–191186); Cave N2_0002 (06°03'13"S 50°14'32"W), 1♂ 3 imm, 26/IX–17/X/2012 (IBSP 191200); Cave N3_0031 (06°02'38"S, 50°13'11"W), 2♀, 03–17/IV/2013 (IBSP 191201); Cave N3_0053 (06°02'27"S, 50°13'45"W), 2♀, 02–23/VIII/2013 (IBSP 191202); Cave N3_0054 (06°02'27"S 50°13'43"W), 1♂, 02–23/VIII/2013 (IBSP 191203); Cave N3_0063 (06°02'33"S, 50°13'37"W), 1♀, 02–23/VIII/2013 (IBSP 191204); Cave N3_0066 (06°02'32"S, 50°13'36"W), 2♂, 02–23/VIII/2013 (IBSP 191205); Cave N3_0069 (06°02'30"S, 50°13'37"W), 2♀, 02–23/VIII/2013 (IBSP 191206), all collected by Equipe Carste; Cave N4E_0004 (06°02'26"S, 50°09'39"W), 2 imm, 20/X–01/XI/2006 (IBSP 97837); Cave N4E_0009 (06°02'21"S, 50°09'36"W), 1♀ 1 imm, 20/X–01/XI/2006 (IBSP 97855); Cave N4E_0012 (06°02'16"S, 50°09'37"W), 1♀ 2 imm, 20/X–01/XI/2006 (IBSP 97836), Cave N4E_0013 (06°02'18"S, 50°09'38"W), 1♀, 20/X–01/XI/2006 (IBSP 97848), all collected by R. Andrade et al.; Cave N4E_0015 (06°02'10"S, 50°09'35"W), 1♀, 20/X–01/XI/2006 R. Andrade (IBSP 97854); 1♀, 20/IV–04/V/10, J. Mascarenhas (IBSP 191142); Cave N4E_0020 (06°02'02"S, 50°09'35"W), 2♂ 1♀ 4 imm, 20/X–01/XI/2006, R. Andrade & I. Cizauskas (IBSP 97841, SEM; IBSP 191157); Cave N4E_0022 (06°02'02"S, 50°10'04"W), 2♀, 20/X–01/XI/2006, R. Andrade et al. (IBSP 97852); Cave N4E_0024 (06°02'01"S, 50°10'07"W), 10♀ 5 imm, 20/IV–04/V/10, D. B. Pedroso, R. Andrade & I. Cizauskas col. (IBSP 97831, SEM; IBSP 161036, IBSP 191143); Cave N4E_0026 (06°02'14"S, 50°10'03"W), 2♂ 4♀ 1 imm, 18/VIII–03/IX/2009, I. Cizauskas & J. Mascarenhas col. (IBSP 191127–191128; IBSP 191151, MPEG 24925); Cave N4E_0031 (06°02'24"S, 50°09'39"W), 1♀, 08–12/II/2007, R. Andrade et al. (IBSP 97748); Cave N4E_0035 (06°02'19"S, 50°09'38"W), 1♂, 18/VIII–03/IX/2009, J. B. Verdiani (IBSP 191152); Cave N4E_0036 (06°02'08"S, 50°09'36"W), 1 imm., 08–12/II/2007, R. Andrade et al. (IBSP 97740); Cave

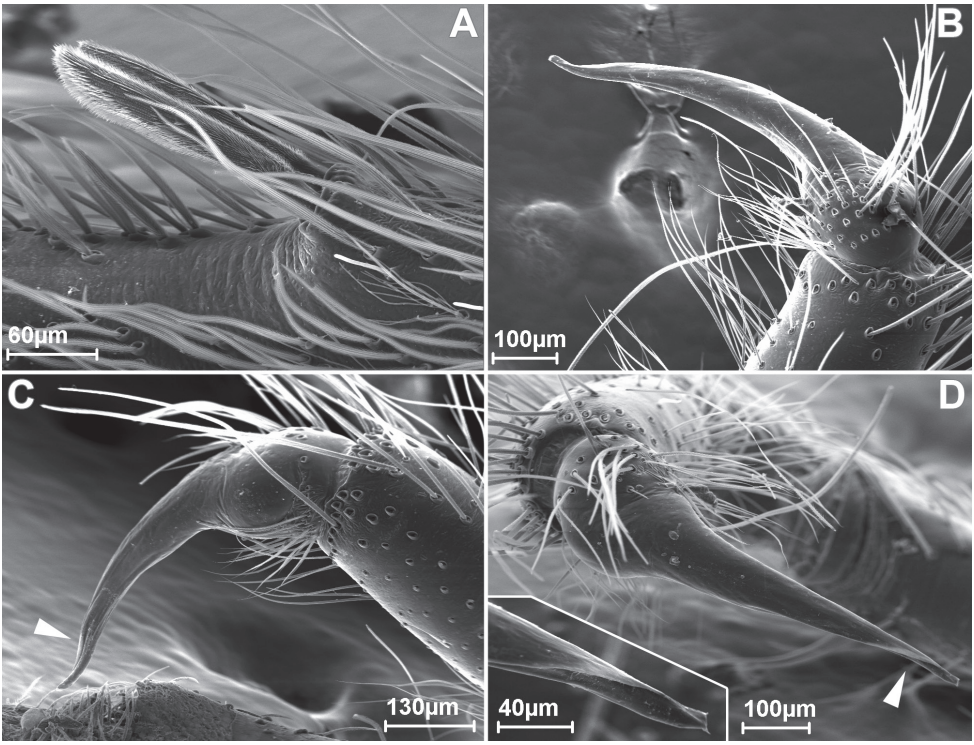


Figure 2. SEM images of *Misionella carajas* sp. n., male from Cave N4E-0020, Flona Carajás, Parauapebas, Pará (IBSP 97841) (**A–D**), **A** male metatarsus II, retrolateral view **B–C** male left palp, prolateral **D** same, dorsal view (inset: detail of embolus tip). White arrows indicate the paraembolic lamina.

N4E_0037 (06°02'07"S, 50°09'37"W), 2♀, 08–12/II/2007, R. Andrade et al. (IBSP 97754); Cave N4E_0038 (06°02'05"S, 50°09'37"W), 4♂ 10♀ 10 imm., 08–12/II/2007, R. Andrade, D. Bebiano & J. B. Verdiani (IBSP 97762; IBSP 191130–191131); Cave N4E_0045 (06°02'25"S, 50°09'40"W), 2♂ 1♀, 1 imm., 19/II–04/III/2010, I. Cizauskas (MPEG 24926; IBSP 191154–191155); Cave N4E_0049 (06°02'14"S, 50°09'36"W), 1♂, 3 imm., 18/VIII–03/IX/2009, D. Bebiano (IBSP 191153); Cave N4E_0050 (06°02'09"S, 50°09'36"W), 1♂, 1♀ 1 imm., 18/VIII–03/IX/2009, D. B. Pedroso (IBSP 191132); Cave N4E_0052 (06°02'02"S, 50°09'37"W), 2♂ 1♀, 24–30/VII/2009, R. Andrade (IBSP 191133); Cave N4E_0053 (06°02'03"S, 50°10'02"W), 1♂, 1 imm., 24–30/VII/2009, D. Bebiano (IBSP 191156); Cave N4E_0056 (06°01'58"S, 50°09'41"W), 1♀ 3 imm., 19/II–04/III/2010, C.A.R. Souza (IBSP 191135); Cave N4E_0063 (06°02'01"S, 50°09'15"W), 1♀, 24–30/VII/09, J. Mascarenhas (MZSP 68013); Cave N4E_0066 (06°01'52"S, 50°09'03"W), 3♂ 2♀, 17 imm., 24-30/VII/09–19/II-04/III/2010, I. Cizauskas, D.B. Pedroso & J.B. Verdiani (IBSP 191116; IBSP 191136; IBSP 191147–191149); Cave N4E_0079 (06°01'59"S, 50°09'05"W), 14♂ 20♀ 19 imm., 24/VII/2009–04/III/2010, I. Cizauskas, D.B. Pedroso, J.B. Verdiani & J. Mascarenhas (IBSP 166199–166200,

IBSP 191119, IBSP 191121–191123, IBSP 191137–191140, MZSP 68014); Cave N4E_0092 (06°02'22"S, 50°09'31"W), 5♂ 3♀ 2 imm., 24–30/VII/2009, D. B. Pedroso R. Andrade C. A. R. Souza & J. B. Verdiani (IBSP 191125–191126; IBSP 191141; IBSP 191150); Cave N4WS_0006 (06°04'36"S, 50°11'36"W), 1♀, 18/XI–01/XII/2010, L. Tunes (IBSP 164958); Cave N4WS_0015 (06°03'59"S, 50°11'22"W), 1♀, 20/X–01/XI/2006, R. Andrade et al. (IBSP 97741); Cave N4WS_0019 (06°04'35"S, 50°11'37"W), 1♀, 18/XI–01/XII/2010, L. Tunes col. (IBSP 164960), Cave N4WS_0021 (06°03'59"S, 50°11'24"W), 1♀, 18/XI–01/XII/2010, D. Bebiano (IBSP 164985); Cave N4WS_0024 (06°03'47"S, 50°11'29"W), 2♀, 18/XI–01/XII/2010, J. B. Verdiani (IBSP 164959); Cave N4WS_0029 (06°03'48"S, 50°11'28"W), 1 imm., 18/XI–01/XII/2010, L. Tunes (IBSP 164988); Cave N4WS_0033 (06°03'58"S, 50°11'23"W), 2♀, 18/XI–01/XII/2010, C.A.R. Souza (IBSP 164984); Cave N4WS_0050/51 (06°04'43"S, 50°11'34"W), 5♀ 1 imm., XI-XII/2010–09/VI/2011, D. Bebiano, C.A.R. Souza & I. Cizauskas col. (IBSP 164957, IBSP 164990, IBSP 164992); Cave N4WS_0054 (06°05'13"S, 50°11'40"W), 1♀, 18/XI–01/XII/2010, B. F. Takano (IBSP 164989), Cave N4WS_0057 (06°04'33"S, 50°11'28"W), 2♀ 1 imm., 10–19/V/2011, C.A.R. Souza (IBSP 164956); Cave N4WS_0066 (06°04'S 50°11'30"W), 3♂ 1♀ 1 imm., 18/XI–01/XII/2010–10-19/V/2011, C.A.R. Souza & I. Cizauskas (IBSP 164954–164955); Cave N4WS_0073 (06°04'25"S, 50°11'37"W), 1♀, 18/XI-01/XII/2010, D. Bebiano (IBSP 164987); Cave N4WS_0074 (06°04'19"S, 50°11'22"W), 3♀, 18/XI–01/XII/2010, D. Bebiano (IBSP 164962); Cave N4WS_0077 (06°04'28"S, 50°11'18"W), 1♀, 10–19/V/2011, C.A.R. Souza col. (BSP 164963); Cave N4WS_0078 (06°04'20"S, 50°11'22"W), 4♀, 18/XI–01/XII/2010-10-19/V/2011, L. Tunes, D. Bebiano & F. P. Franco (IBSP 164961; IBSP 164986; IBSP 164991); Cave N5S_0005 (06°06'21"S, 50°08'01"W), 5♀, 4 imm., 14–23/X/2009, I. Cizauskas & J. B. Verdiani (IBSP 161038–161039; IBSP 161050); Cave N5S_0008 (06°06'21"S, 50°07'57"W), 3♀ 5 imm., 14–23/X/2009, J. B. Verdiani & D. B. Pedroso (IBSP 161042, IBSP 161046); Cave N5S_0013 (06°06'19"S, 50°08'02"W), 2♀ 3 imm., 14–23/X/2009, D. B. Pedroso (IBSP 191145); Cave N5S_0015 (06°06'20"S, 50°08'W), 13♀ 15 imm., 14-23/X/2009, R. Andrade, I. Cizauskas & J.B. Verdiani (IBSP 161033, IBSP 161035, IBSP 161044, IBSP 161048, IBSP 191146); Cave N5S_0017 (06°05'15"S, 50°07'11"W), 2♀ 1 imm., 25/VIII–03/IX/2009, I. Cizauskas & D.B. Pedroso (IBSP 161045, IBSP 161047); Cave N5S_0022 (06°05'16"S, 50°07'33"W), 1♀ 2 imm., 25/VIII–03/IX/2009, J. Mascarenhas (IBSP 161034); Cave N5S_0026 (06°05'15"S, 50°07'38"W), 1♀, 10–19/V/2011, I. Cizauskas (IBSP 165000); Cave N5S_0058 (06°06'29"S, 50°07'57"W), 1♀, 14/III–04/IV/2010, J. B. Verdiani (IBSP 161041); Cave N5S_0059 (06°06'29"S, 50°07'57"W), 1♂ 1♀ 1 imm., 25/VIII-03/IX/2009, D. Bebiano (IBSP 161037); Cave N5S_0059 (06°06'29"S, 50°07'57"W), 1♂ 1♀, 25/VIII–03/IX/2009, I. Cizauskas & J.B. Verdiani (IBSP 161040, IBSP 191158); Cave N5S_0063/64/65 (06°06'12"S, 50°08'07"W), 2♀ 1 imm., 15–21/IX/2009, I. Cizauskas (IBSP 191144); Cave N5S_0067 (06°06'10"S, 50°08'07"W), 1♀ 2 imm.,

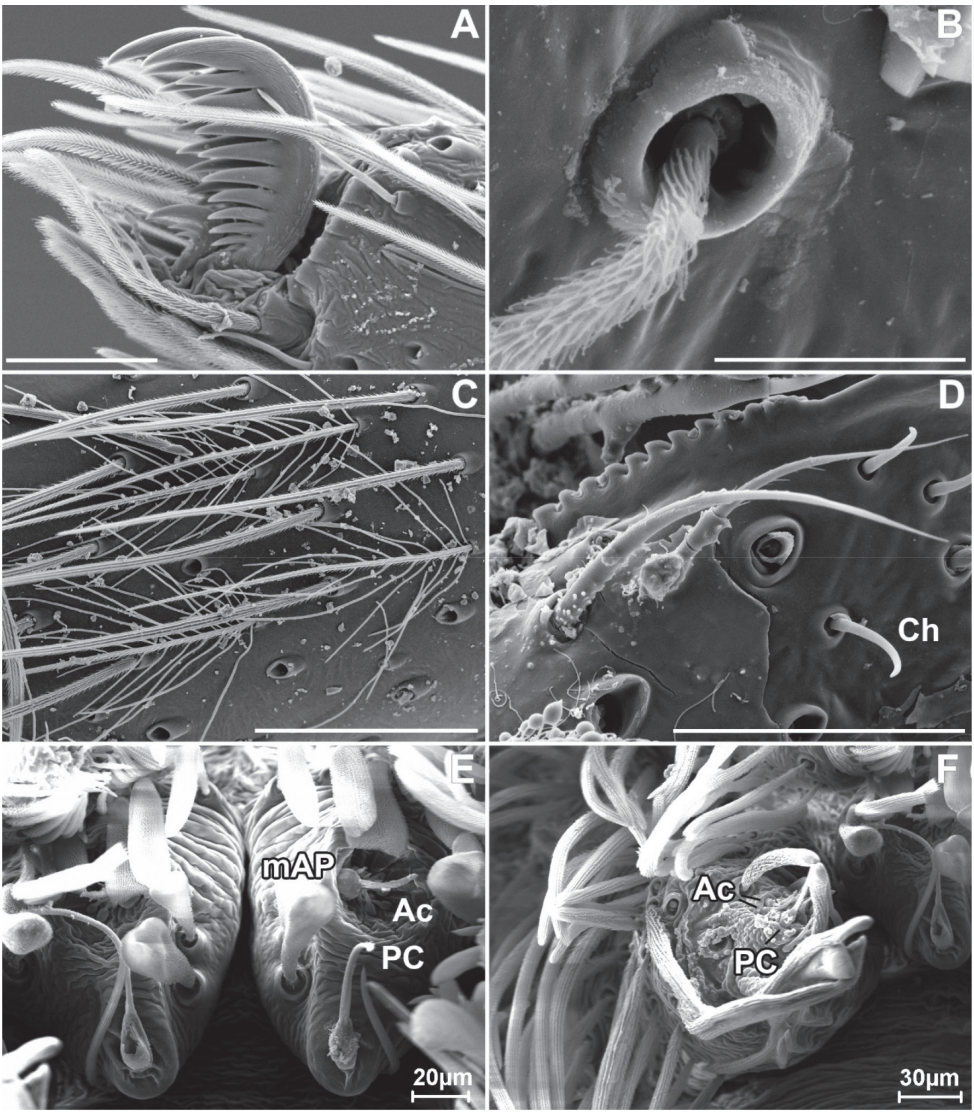


Figure 3. SEM images of *Misionella carajas* sp. n., female from Cave N4E-0024, Flona Carajás, Parauapebas, Pará (IBSP 97831) **A** female right leg I, tarsal claws **B** female right leg III, tarsus, dorsal, trichobothria **C** same, retrolateral, detail of plumose setae **D** endite, serrula and chemosensory setae **E** PMS, ventral view **F** PLS, ventral view. Abbreviations: Ac = aciniform gland spigots, Ch = chemosensory setae, mAP = minor ampullate gland spigot, PC = paracribellar gland spigot. Scale bars: 0.05 mm (**A, D**), 0.01 mm (**B**), 0.1 mm (**C**).

14/III–04/IV/2010, J. B. Verdiani (IBSP 161043); Cave N6_0005 (06°07'22"S, 50°10'28"W), 2♂, 16/VII-06/VIII/2014, Equipe Carste (IBSP 191188); Cave N8_0007 (06°10'05"S, 50°09'34"W), 1♂ 2♀, 16/VII–06/VIII/2014, Equipe Carste (IBSP 191189–191191); Cave N8_0019 (06°10'10"S, 50°09'25"W), 1♂ 1♀, 16/

VII–06/VIII/2014, Equipe Carste (IBSP 191192-191193); Canaã dos Carajás, Flona Carajás, Cave CAV_0039 (06°24'53"S, 50°22'23"W), 1♀ 3 imm., 22–31/V/2010-22–28/IX/2010, D. B. Pedroso, C.A.R. Souza & J. Mascarenhas (IBSP 164964–164965, IBSP 191114); Cave GEM-1342 (06°16'02"S, 49°57'05"W), 1♀, 05–15/III/2012 (IBSP 191208); Cave GEM-1475 (06°16'38"S, 49°55'05"W), 1♀ 1 imm., 17/I–02/II/2012 (IBSP 191207); Cave GEM-1483 (06°16'35"S, 49°55'05"W), 1♀, 29/VIII–27/IX/2012 (IBSP 191210); Cave GEM-1508 (06°18'55"S, 49°57'23"W),1♀, 29/VIII–27/IX/2012 (IBSP 191209); Cave GEM-1517 (06°15'50"S, 49°58'38"W), 1♀, 10–31/I/2013, (IBSP 191211), all collected by C.A.R. Souza & J. Mascarenhas et al.; Cave NV_0004 (06°28'42"S, 49°54'08"W), 1♀ 1 imm., 22–28/II/2005, R. Andrade & I. Amoni (IBSP 55364); Cave S11_0002 (06°26'21"S, 50°16'50"W), 1♂ 3♀ 4 imm., 24/II–19/VIII/2010, I. Cizauskas & V. Felice (IBSP 164993, IBSP 164995, IBSP 164997-164998); Cave S11A_0003 (06°21'S 50°27'02"W), 1 imm., 23/VIII–02/IX/2007, R. Andrade (IBSP 97825); Cave S11_0016 (06°25'10"S, 50°15'03"W), 3♂ 1♀ 2 imm., 24/II–04/III/2010–19/VIII/2010, I. Cizauskas & D. Bebiano (IBSP 164994, IBSP 164996, IBSP 164999); Cave S11A_0020 (06°19'04"S, 50°26'23"W), 2♂ 1♀ 3 imm., 23/VIII-02/IX/2007, R. Andrade et al. (IBSP 97818); Cave S11B_0014 (06°20'59"S, 50°24'10"W), 1♀ 5 imm., 23/VIII–02/IX/2007, R. Andrade et al. (IBSP 97701); Cave S11B_0016 (06°20'53"S, 50°24'24"W), 1 imm., 23/VIII–02/IX/2007, R. Andrade et al. (IBSP 97968); Cave S11C_0020 (06°24'03"S, 50°22'50"W), 1♂ 2♀ 1 imm., 23/VIII–02/IX/2007, R. Andrade et al. (IBSP 97687, IBSP 97863); Cave S11D_0003 (06°24'02"S, 50°21'W), 1♀, 01–14/VII/2010, I. Cizauskas (IBSP 164973); Cave S11D_0012 (06°23'46"S, 50°21'34"W), 1♀, 23/VIII–02/IX/2007, R. Andrade et al. (IBSP 97801); Cave S11D_0049 (06°24'25"S, 50°19'14"W), 1♀, 13–30/I/2010, D. Bebiano (IBSP 164971); Cave S11D_0054 (06°24'22"S, 50°19'13"W), 2 imm., 13–30/I/2010, R. Andrade (IBSP 164969); Cave S11D_0055 (06°24'23"S, 50°19'12"W), 2♂ 4♀, 01–14/VII/2010, I. Cizauskas (IBSP 164968, IBSP 191113); Cave S11D_0061 (06°23'33"S, 50°18'47"W), 1♂ 1♀ 1 imm., 13/I/2010–14/VII/2010, I. Cizauskas & J. Mascarenhas (IBSP 164967, IBSP 164975, IBSP 164977); Cave S11D_0064 (06°23'31"S, 50°18'48"W), 1♀, 23/VIII–02/IX/2007, R. Andrade et al. (IBSP 97803); Cave S11D_0067 (06°23'34"S, 50°18'53"W), 1 imm., 13–30/I/2010, J. B. Verdiani (IBSP 164981); Cave S11D_0072 (06°23'33"S, 50°19'09"W), 1 imm., 13–30/I/2010, I. Cizauskas (IBSP 164974); Cave S11D_0076 (06°23'33"S, 50°19'W), 1♀ 1 imm., 13-30/I/2010, R. Andrade (IBSP 164982); Cave S11D_0079 (06°23'33"S, 50°18'56"W), 1♂, 01-14/VII/2010, V. Felice (IBSP 164970); Cave S11D_0083 (06°23'48"S, 50°19'25"W), 1♀, 13–30/I/2010, I. Cizauskas (IBSP 164979); Cave S11D_0083 (06°23'48"S, 50°19'25"W), 3♂ 5♀, 23/VIII/2007–14/VII/2010, R. Andrade & I. Cizauskas et al. (IBSP 97793, IBSP 164976, IBSP 164978); Cave S11D_0085 (06°23'47"S, 50°19'24"W), 1 imm., 13–30/I/2010, J. Mascarenhas (IBSP 164980); Cave S11D_0093 (06°23'41"S, 50°19'18"W), 1♀ 3 imm., 01-14/VII/2010, V. Felice & J. Mascarenhas (IBSP 164966, IBSP 164972); Cave S11D_0098 (06°23'46"S,

50°20'27"W), 1 imm., 03–19/VIII/2010, D.B. Pedroso (IBSP 164983); Cave S11D_0107 (06°23'35"S, 50°18'45"W), 1♀, 30/VIII–02/IX/2011, D. Bebiano (IBSP 191115); Cave SB-140 (06°21'05"S, 49°48'34"W), 1♀, 10–31/I/2013 (IBSP 191213); Cave SB-149 (06°21'04"S, 49°50'30"W), 1♀ 3 imm., 10–1/I/2013 (IBSP 191214); Cave SB-150 (06°21'04"S, 49°50'30"W), 2♀ 2 imm.,10–31/I/2013 (IBSP 191212); Cave SB-210 (06°20'22"S, 49°57'36"W), 1♀, 10–20/IX/2013, (IBSP 19215), all collected by C.A.R. Souza & J. Mascarenhas et al.; Cave N5SM2_0001 (06°08'33"S, 50°08'03"W), 3♀ 14 imm. (ISLA); Cave N5SM2_0005 (06°08'28"S, 50°08'10"W), 3 imm. (ISLA); Cave N5SM2_0013 (06°08'18"S, 50°08'12"W), 1 imm. (ISLA); Cave N5SM2_0014 (06°08'20"S, 50°08'02"W), 2 imm. (ISLA); Cave N5SM2_0020 (06°08'S 50°07'53"W), 1 imm. (ISLA); Cave N5SM2_0021 (06°08'S 50°07'53"W), 2♀ 9 imm. (ISLA); Cave N5SM2_0022 (06°08'09"S, 50°08'09"W), 6 imm. (ISLA); Cave N5SM2_0023 (06°08'08"S, 50°08'07"W), 2♀ 3 imm. (ISLA); Cave N5SM2_0025 (06°08'10"S, 50°08'07"W), 1♀ 6 imm. (ISLA); Cave N5SM2_0026 (06°08'10"S, 50°08'08"W), 1 imm. (ISLA); Cave N5SM2_0027 (06°08'06"S, 50°08'12"W), 1 imm. (ISLA); Cave N5SM2_0029 (06°08'06"S, 50°08'11"W), 1♀ 10 imm. (ISLA); Cave N5SM2_0030 (06°08'05"S, 50°08'11"W), 4 imm. (ISLA); Cave N5SM2_0031 (06°08'04"S, 50°08'10"W), 2 imm. (ISLA); Cave N5SM2_0032 (06°08'04"S, 50°08'10"W), 1 imm. (ISLA); Cave N5SM2_0033 (06°08'04"S, 50°08'10"W), 1♀ 2 imm. (ISLA); Cave N5SM2_0034 (06°08'03"S, 50°08'10"W), 1♀ 7 imm. (ISLA); Cave N5SM2_0035 (06°08'03"S, 50°08'07"W), 3♀ 3 imm. (ISLA); Cave N5SM2_0037 (6°08'S 50°08'07"W), 1 imm. (ISLA); Cave N5SM2_0038 (06°07'59"S, 50°08'07"W), 2 imm. (ISLA); Cave N5SM2_0039 (06°07'59"S, 50°08'08"W), 2 imm. (ISLA); Cave N5SM2_0040 (06°08'S 50°08'13"W), 5 imm. (ISLA); Cave N5SM2_0041 (06° 08'S 50°08'14"W), 1 imm. (ISLA); Cave N5SM2_0043 (06°07'57"S, 50°08'12"W), 2♀ 5 imm. (ISLA); Cave N5SM2_0047 (06°07'54"S, 50°08'10"W), 1♀ 4 imm. (ISLA); Cave N5SM2_0050 (06°07'52"S, 50°08'07"W), 1♀ 2 imm. (ISLA); Cave N5SM2_0052 (06°07'52"S, 50°08'07"W), 2♀ (ISLA); Cave N5SM2_0055 (06°07'48"S, 50°08'06"W), 6♀ 12 imm. (ISLA); Cave N5SM2_0056 (06°07'48"S, 50°08'06"W), 1 imm. (ISLA); Cave N5SM2_0057 (06°07'48"S, 50°08'07"W), 1♀ 1 imm. (ISLA); Cave N5SM2_0059 (06°07'47"S, 50°08'07"W), 2♀ 2 imm. (ISLA); Cave N5SM2_0060 (06°07'45"S, 50°08'10"W), 1♂ 1♀ 7 imm. (ISLA); Cave N5SM2_0061 (06°07'44"S, 50°08'08"W), 3 imm. (ISLA); Cave N5SM2_0062 (06°07'43"S, 50°08'07"W), 7 imm. (ISLA); Cave N5SM2_0066 (06°07'42"S, 50°08'09"W), 1♀ 7 imm. (ISLA); Cave N5SM2_0067 (06°07'41"S, 50°08'14"W), 1 imm. (ISLA); Cave N5SM2_0070 (06°07'33"S, 50°07'56"W), 4 imm. (ISLA); Cave N5SM2_0071 (06°07'32"S, 50°07'56"W), 3 imm. (ISLA); Cave N5SM2_0072 (06°07'32"S, 50°07'56"W), 13 imm. (ISLA); Cave N5SM2_0073 (06°07'34"S, 50°07'57"W), 2 imm. (ISLA); Cave N5SM2_0074 (06°07'33"S, 50°07'57"W), 1♀ 1 imm. (ISLA); Cave N5SM2_0075 (06°07'33"S, 50°07'56"W), 9 imm. (ISLA); Cave N5SM2_0075 (06°07'33"S, 50°07'56"W), 4 imm. (ISLA); Cave N5SM2_0076

(06°07'32"S, 50°07'56"W); 1 imm. (ISLA); Cave N5SM2_0077 (06°07'30"S, 50°07'54"W), 2♀ 2 imm. (ISLA); Cave N5SM2_0078 (06°07'24"S, 50°07'50"W), 3 imm. (ISLA); Cave N5SM2_0078 (06°07'24"S, 50°07'50"W), 2 imm (ISLA); Cave N5SM2_0079 (06°07'24"S, 50°07'51"W), 4 imm. (ISLA); Cave N5SM2_0080 (06°07'21"S, 50°07'49"W), 7♀ 9 imm. (ISLA); Cave N5SM2_0081 (06°07'20"S, 50°07'46"W), 1♂ 5♀ 4 imm. (ISLA); Cave N5SM2_0082 (06°07'21"S, 50°07'44"W), 2♀ 11 imm. (ISLA); Cave N5SM2_0083 (06°07'22"S, 50°07'43"W), 5♀ 10 imm. (ISLA); Cave N5SM2_0084 (06°07'21"S, 50°07'42"W) 4 imm. (ISLA); Cave N5SM2_0086 (06°07'18"S, 50°07'49"W), 2 imm. (ISLA); Cave N5SM2_0090 (06°07'16"S, 50°07'47"W), 3♀ 7 imm. (ISLA); Cave N5SM2_0091 (06°07'16"S, 50°07'47"W), 10 imm. (ISLA); Cave N5SM2_0092 (06°07'19"S, 50°07'57"W), 1 imm. (ISLA); Cave N5SM2_0097 (06°07'43"S, 50°08'10"W), 5 imm. (ISLA); Cave N5SM2_0100 (06°07'19"S, 50°07'56"W), 1♀ 4 imm. (ISLA); Cave N5SM2_0101 (06°07'18"S, 50°07'56"W), 2 imm. (ISLA); Cave N5SM2_0102 (06°07'19"S, 50°07'54"W), 8♀ 20 imm (ISLA), 2007–2009, all collected by Equipe UFLA.

Etymology. The specific name is a noun in apposition taken from the type locality.

Diagnosis. Males of *Misionella carajas* can be distinguished from *M. aikewara* by the longer palpal tibia and shorter paraembolic lamina (Fig. 1K–L: arrow; 2B–D, 11A–B) and from *M. pallida* by the narrow paraembolic lamina (Figs 1K–L: arrow; 11A–B). Females can be recognized by the shorter and not curved spermathecae (Fig. 11C).

Description. Male (IBSP 161036). Carapace orange brown with lateral borders, thoracic groove and ocular area black. Sternum, chelicerae and labium orange. Legs and palp orange. Abdomen dorsally black and ventrally grayish (Figs 1A–C, E–F, 13B). Total length 4.1. Carapace 1.8 long, 1.3 wide. Eye diameters: PME 0.4, separated by about 2 diameters. Sternum with shallow longitudinal ventral sulcus, without sigillae. Palp: femur length 0.9, patella 0.4, tibia 1.1 long, 0.5 wide. Leg measurements: I: femur 3.8, patella 0.5, tibia 3.7, metatarsus 3.3, tarsus 1.8, total 13.1; II: 3.3, 0.5, 3.7, 2.4, 0.8, 10.7; III: 2.2, 0.4, 2.4, 2.6, 1.1, 8.7; IV: 3.1, 0.5, 3.0, 3.4, 1.4, 11.4. Metatarsus II with a pair of hirsute macrosetae (Figs 1G–I, 2A) on a cuticular outgrowth. Abdomen 2.2 long. Palp: tibia two times longer cymbium, with membranous area, cymbium short, and bulb globose and short, with elongated embolus (Figs 1K–L, 2B–D).

Female (IBSP 161040). Coloration as in male, but darker. Total length 5.6. Carapace 2.3, long, 1.5 wide. Serrula with 10−11 teeth (Fig. 3D). Sternum as in male. Eye diameters: PME 0.5, separated by about 2 diameters. Pedipalp: length 1.4, patella 0.7, tibia 1.1, tarsus 0.4. Leg measurements: I: femur 3.1, patella 0.7, tibia 3.5, metatarsus 3.2, tarsus 1.5, total 12.0; II: 2.1, 0.7, 3.4, 2.0, 0.9, 9.1; III: 1.9, 0.6, 1.6, 1.7, 0.8, 6.6; IV: 2.6, 0.6, 2.4, 2.4, 1.0, 9.0. Legs with plumose hairs (Fig. 3C), trichobothria elevated and smooth (Fig. 3B), paired claws with 9 teeth and unpaired claw with two teeth (Fig. 3A) and calamistrum in three rows (Fig. 1D). Pedipalp hirsute (Fig. 1J) Abdomen 3.2 long. Posterior median spinnerets with one paracribellar gland spigot,

along one minor ampullate gland and at least seven aciniform gland spigots (Fig. 3E). Posterior lateral spinnerets with large paracribellar gland spigots at the margin of spinning field and few aciniform gland spigots (Fig. 3F). Spermathecae with short ducts, close at base (Figs 11C, 12 A–D)

Variation. 10 males: total length 3−4.2; carapace 1.4−1.7; femur I 2.8−4.5. 10 females: total length 4.2−6.8; carapace 1.7−2.4; femur I 2.7−3.2.

Natural history. This species is very common in the Carajás area, where 352 adult specimens were collected. 101 males, 251 females and approximately 400 immature (only 164 included here) were sampled in 144 caves, between the years 2006–2010. The caves are formed in iron ore in areas of residual plateau, more specifically on the bases of outcrops of iron ore or 'canga'. The 'canga' are usually covered by open vegetation type called 'metalophylic vegetation', which is characterized by plants able to grow in soils rich in iron and other heavy metals (Pinto-da-Rocha and Andrade 2012; Carmo and Jacobi 2013). The specimens were found in lighter areas as well as in the darker areas of the interior of caves. They were always caught in the refuge of their irregular webs located on the ground and/or walls of the cavities (Figs 13A–C). Two types of refuges were found, one formed by tubes in the interior of the guano (Fig. 13C) and other, with irregular distribution of silk, in the walls or using the nest of wasps as substrate (Fig. 13D). The prey commonly observed were micro-Lepidoptera of the family Tineidae (Fig. 13A) and immature of Hemiptera of the genus *Zelurus* Silvestre (Reduviidae). Although the new species has been found only inside the caves, the specimens do not show any troglomorphism, except perhaps the elongate legs.

Distribution. The species seems to occur exclusively in caves in the region of the Flona of Carajás, in the municipalities of Parauapebas and Canaã dos Carajás (Fig. 14).

***Misionella aikewara* sp. n.**
http://zoobank.org/FA3E70CA-B8BC-4C93-ABBE-060356DCB449
Figs 4A–G, 11D–F, 13E, 14

Type material. Male holotype from Cave SI-07 (788310 9295476), São Geraldo do Araguaia, Pará, Brazil, 31.VIII–09.IX.2009, F. P. Franco et al., deposited in IBSP 191196; female paratype from Cave SI-04 (786471 9290451), Xambioá, Tocantins, 31.VIII–-09.IX.2010, F. P. Franco et al., deposited in IBSP 191194.

Additional material examined. BRAZIL. *Pará*: São Geraldo do Araguaia, Cave SI-30 (783442 9304748), 1♀, 31.VIII−09.IX.2010, F. P. Franco et al. (IBSP 191197); 2 imm., 22.II−02.III.2011, F. P. Franco et al. (IBSP 191199); *Tocantins*: Ananás, Cave SI-13 (785816 9310724), 1♀, 31.VIII−09.IX.2010, F. P. Franco et al. (IBSP 191195); Cave SI-13, 2♀, 27−31.I.2011 (IBSP 191198); *Tocantins*: Miracema do Tocantins (09°34'02"S, 48°23'30"W), 3♀ 1 imm., 17−25.IV.2005, I. Knysak & R. Martins, in a cave at night (IBSP 124517).

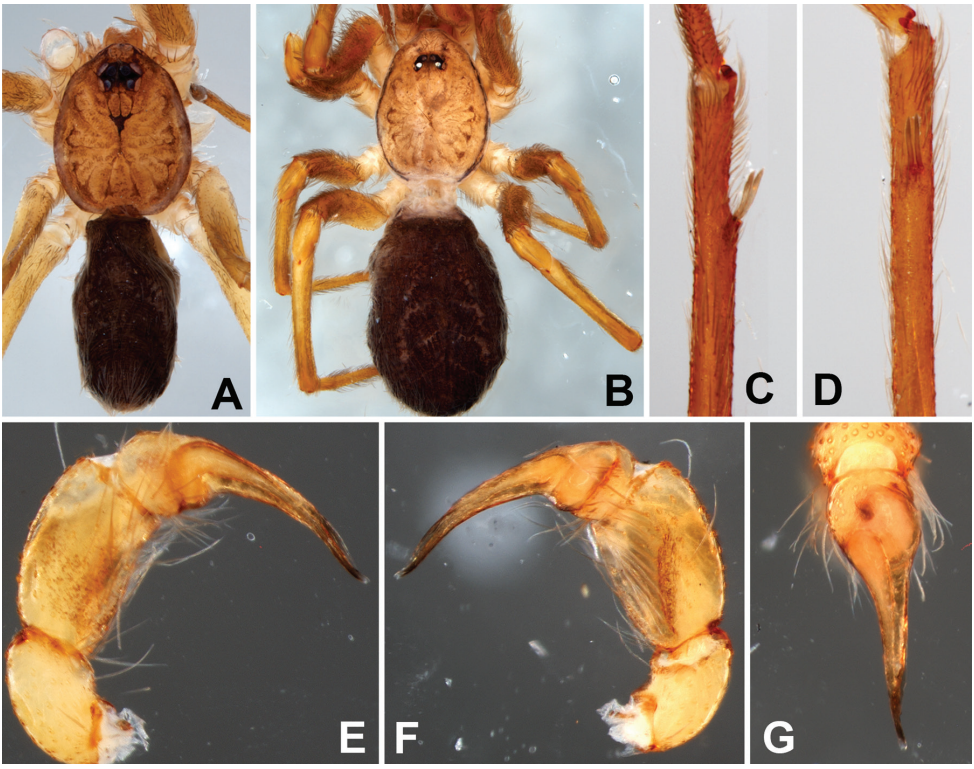


Figure 4. *Misionella aikewara* sp. n.. Male from São Geraldo do Araguaia, Pará (IBSP 191196) (**A, C–G**), female from Xambioá, Tocantins (IBSP 191194) (**B**). **A–B** habitus, dorsal view **C** male left metatarsus II, ventral view **D** male left metatarsus II, retrolateral **E** male left palp, prolateral view **F** same, retrolateral view **G** same, dorsal view.

Etymology. The specific name is a noun in apposition and refers to the ethnic group of the region of São Geraldo do Araguaia, where the type locality is located: the Tupi indigenous group Aikewará.

Diagnosis. Males of *Misionella aikewara* can be distinguished from *M. carajas* and *M. pallida* by the shorter palpal tibia and elongated paraembolic lamina (Figs 4E–G, 11D–E). Females can be recognized by the elongated receptacles curved distally and separated at the base (Fig. 11F).

Description. Male (holotype). Carapace orange with brown submarginal bands. Thoracic groove and ocular area black. Chelicerae orange. Labium and endites yellowish. Sternum yellowish with brown borders. Legs and palps orange. Abdomen dark brown (Fig. 4A). Total length 2.7. Carapace 1.2, long, 1.0 wide. Sternum with small and shallow sulcus, without sigillae. Eye diameters: PME 0.4, separated by your diameters. Palp: femur length 2.0, patella 1.0, tibia 1.2 long, 0.8 wide. Leg measurements: I: femur 2.8, patella 0.5, tibia 1.9, metatarsus 1.8, tarsus 0.9, total 7.9; II: 2.0, 0.4, 2.1, 1.3, 0.6, 6.4; III: 1.3, 0.5, 1.3, 1.3, 0.7, 5.1; IV: 2.0, 0.5, 1.9, 1.8, 0.9, 7.0. Metatarsus II with a pair

of hirsute macrosetae (Fig. 4C–D). Abdomen 1.5 long. Palp: tibia shorter, two times the length of cymbium, bulb globose (Fig. 4E–G).

Female (IBSP 191194, cave SI-04). Coloration pattern as in male (Fig. 4B), except endites orange and legs darker. Total length 5.6. Carapace 2.1 long, 1.6 wide. Sternum as in male. Eye diameters: PME 0.4, separated by 2 diameters. Palp: femur length 1.5, patella 0.7, tibia 0.8, tarsus 0.9. Leg measurements: I: femur 2.9, patella 0.9, tibia 3.0, metatarsus 2.8, tarsus 1.2, total 10.8; II: 2.1, 0.7, 2.0, 1.8, 0.9, 7.5; III: 1.8, 0.7, 1.4, 1.5, 0.8, 6.2; IV: 2.0, 0.8, 2.1, 2.0, 0.9, 7.8. Abdomen 3.2 long. Spermathecae with elongated ducts and curved apex (Fig. 11F).

Variation. 5 females: total length 3−4.5; carapace 1.4−2; femur I 1.7−2.2.

Natural history. Eleven specimens were collected, only one male, eight females and two immature, in four limestone caves located in municipalities very close to the border of the states of Pará and Tocantins (Fig. 14). In general, the walls of the caves had high humidity with pools and/or running water therein. These caves have high number of micro-habitats such as roots, guano and crevices. The specimens of *M. aikewara* sp. n. were located in lighter areas as well as in the darker areas of the interior of caves. The webs are irregular, as in *Misionella carajas* sp. n., and the capture was always performed in the refuge of their webs located on the walls and cracks in the cavity (Fig. 13E). All specimens were found inside caves and do not show any kind of troglomorphism.

Distribution. This species occurs only in the region of the State Park of Serra das Andorinhas, in states of Pará and Tocantins (Fig. 14).

Misionella pallida sp. n.

http://zoobank.org/39C0C8CD-8EF0-4507-96E4-CC643E479536
Figs 5A–H, 6A–D, 7A–E, 8A–D, 9A–F, 10A–F, 11G–I, 12B–D, 13E, 14

Type material. Male holotype from Bairro Morada do Sol (5°3'56"S, 42°46'1,02"W), Teresina, Piauí, Brazil, 30.I.2006, L.S. Carvalho col., deposited in MPEG 22760. Paratypes: two females from Parque Nacional de Sete Cidades (4°5'39,9"S, 41°43'53,3"W), Brasileira/Piracura, Piauí, Brazil, 3.XII.2006, L.S. Carvalho, D. Candiani & N.F.L. Man Hung col., deposited in MPEG 22748; male and female from Parque Municipal Pedra do Castelo (5°12'5,9"S, 41°41'14,2"W), Castelo do Piauí, Piauí, Brazil, 9.V.2004, L.S. Carvalho et al. col. deposited in CHNUFPI 604 and 605, respectively; male from Bairro Morada do Sol, Teresina, Piauí, Brazil (into a house), 5°3'56"S, 42°46'1"W, 15.X.2015, L.S. Carvalho col. (CHNUFPI 1624); male and female from Mossoró (5°11'16"S, 37°20'38"W), Rio Grande do Norte, Brazil, 29.X.2007, I.T. Rocha & D. Araujo col. (IBSP 91662, IBSP 91663).

Additional material examined. BRAZIL. *Piauí*: José de Freitas, Fazenda Nazareth (4°45'21"S, 42°34'33"W), 1♂ 1♀ 2 imm, 12.X.2003, J. Riceti (MPEG 22747); Francinópolis, Sítio Vigário (6°23'46"S, 42°15'44.82"W), 1♀, 7.IV.2007, E.B.O Marques (MPEG 22749); Oeiras (7°01'30"S, 42°07'51"W), 1♂ 2 imm, 3.VI.2008, Yamaguti

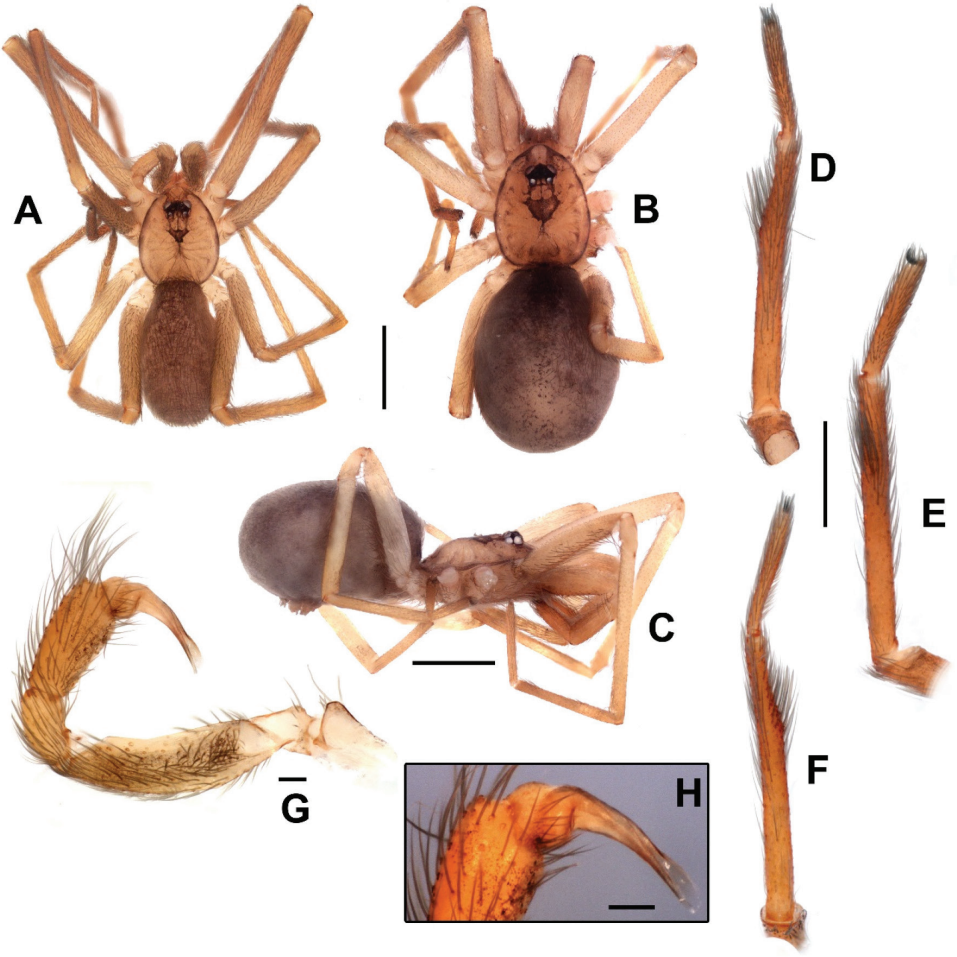


Figure 5. *Misionella pallida* sp. n.. **A, G–H** Male from Bairro Morada do Sol, Teresina, Piauí (UFMG 14827) **D–F** Sítio Ouro Verde, União, Piauí (CHNUFPI 1099) **B–C** female from Parque Nacional Serra da Capivara, Coronel José Dias, Piauí (UFMG 14829). **A–B** habitus, dorsal view **C** habitus, lateral view **D** male right leg II, ventral view **E** same, retrolateral view **F** same, dorsal view **G** male right palp, prolateral view **H** same, distal area (mirrored). Scale bars: 1 mm (**A–C**), 0.5 mm (**D–F**), 0.1 mm (**G–H**).

(MPEG 22755; MPEG 22751); Teresina, Campus UFPI, CCA (5°3'56"S, 42°46'W), 1♂, 2.III.2007, L.S. Carvalho (MPEG 22753); Teresina, Bairro São Joaquim, 7° DP (5°3'56"S, 42°46"W), 2♂, 22.I.2007, S.C. Carvalho (1 ♂, SEM; MPEG 22756); Teresina, Bairro Morada do Sol (5°3'56"S, 42°46'1,02"W), 1♀ 1 imm., 11.VII.2007, L.S. Carvalho (MPEG 22746); 1♂ 1♀, 4.X.2013, L.S. Carvalho (CHNUFPI 601); 1♂, I.2014, L.S. Carvalho (UFMG 14827; UFMG 14829); Brasileira/Piracura, Parque Nacional Sete Cidades (4°5'39,9"S, 41°43'53,3"W), 1 imm., 25.VI.2007 (MPEG 22757); 1♀, 03.XII.2006 (MPEG 22752); 1 imm., 31.I.2007 (MPEG 22761); 1 imm., 31.I.2007 (MPEG 22754), all collected by L.S. Carvalho, D. Candiani & N.F.

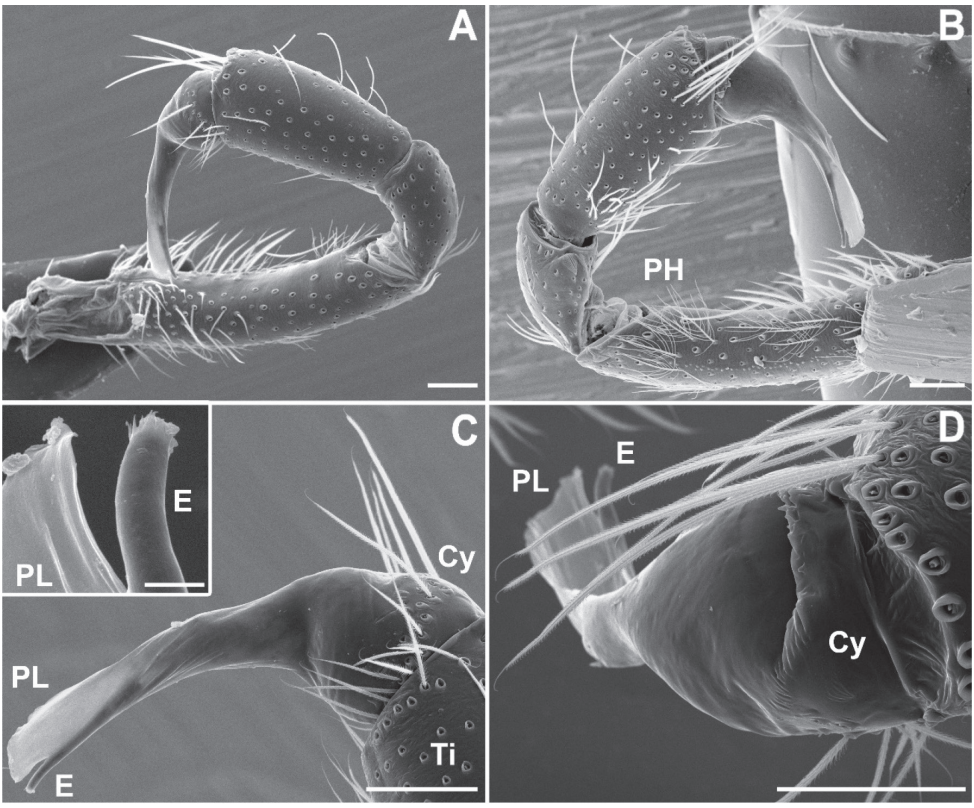


Figure 6. SEM images of *Misionella pallida* sp. n., male from Floriano, Piauí, Brasil (UFMG 14828) **A** male right palp, prolateral view **B** same, retrolateral view **C** same, prolateral view, detail of right bulb (inset embolus and PL, dorsal) **D** same, right cymbium, dorsal view. Abbreviations: Cy = cymbium, E = embolus, PH = plumose setae, PL = paraembolic lamina, Ti = palpal tibia. Scale bars: 0.1 mm, except inset, 0.01 mm.

Lo Man Hung; Coronel José Dias, Parque Nacional da Serra da Capivara (08°53'07"S, 42°33'12"W), 1♀, 07/VI/2012, L.S. Carvalho (UFMG 14829); Castelo do Piauí, Fazenda Bonito, E.C.B. Rochas Ornamentais (5°14'7,5"S, 41°41'16,3"W), 2♂, 13.VIII.2008, L.S. Carvalho (MPEG 22745; MPEG 22759); Castelo do Piauí, Parque Municipal Pedra do Castelo (5°12'5,9"S, 41°41'14,2"W), 2♂ 11♂ 1 imm., 9.V.2003, L.S. Carvalho et al. (UFMG 14384-14394; UFMG 14387; CHNUFPI 602; CHNUFPI 603); União, Sítio Ouro Verde (04°54'14"S, 42°47'21"W), 1♂, 25/V/2014, L.S. Carvalho (CHNUFPI 1099); Floriano, campus da UFPI (06°47'29"S, 43°2'50"W), 4/IX/2013, L.S. Carvalho, 1♂ (UFMG 14828). *Maranhão*: Caxias, Reserva Ecológica do Inhamum (04°53'30"S, 43°24'53"W), 30♂, 23–26.IV.2007, F.B. Lima-Lobato (IBSP 129097; IBSP 131101; IBSP 131029; IBSP 129092; IBSP 129088; IBSP 131024; IBSP 129093; IBSP 129095; IBSP 130967; IBSP 131022; IBSP 99121; IBSP 98671; IBSP 131027; IBSP 131025; IBSP 129090, IBSP 98670); *Rio Grande do Norte*: Mossoró (5°11'16"S, 37°20'38"W), 1♀, 29.X.2008, I.T. Rocha & D. Arau-

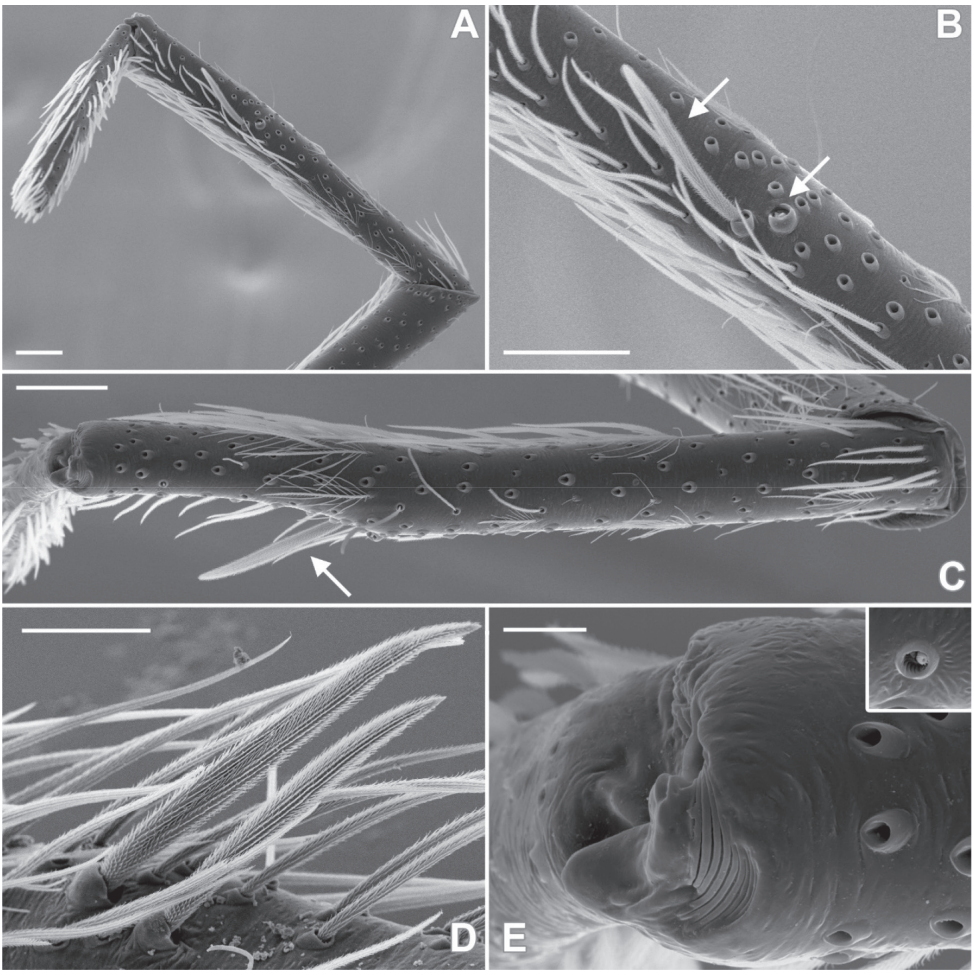


Figure 7. SEM images of *Misionella pallida* sp. n., male from Bairro Morada do Sol, Teresina, Piauí (UFMG 14827), **A** left leg II, retrolateral view **B** same, detail of macrosetae, retrolateral view. Arrow points to spines. **C** same, dorsal view. Arrow points to macrosetae **D** same, detail of macrosetae, subventral view **E** same**,** metatarsus stopper, dorsal view (inset, tricobothrial base). Scale bars: 0.1 mm (**A–C**), 0.05 mm (**D**), 0.02 mm (**E**).

jo (IBSP 91661). *Bahia*: Brumado, Magnesita (14°12'14"S, 41°39'55"W), 1♀, E.A. Araújo, 02–3/V/2014 (UFMG 15513).

Etymology. The name is an adjective referring to the pale coloration of the body in both males and females of this species.

Diagnosis. *Misionella pallida* can be distinguished from other *Misionella* species by the pale coloration of the body. Males are further distinguished by the large and flattened paraembolic lamina (Figs 5G–H, 6A–E: PL, 11G–H). Females can be recognized by the long, distally incrassate, and largely separated distal area of the spermathecae (Fig. 11I).

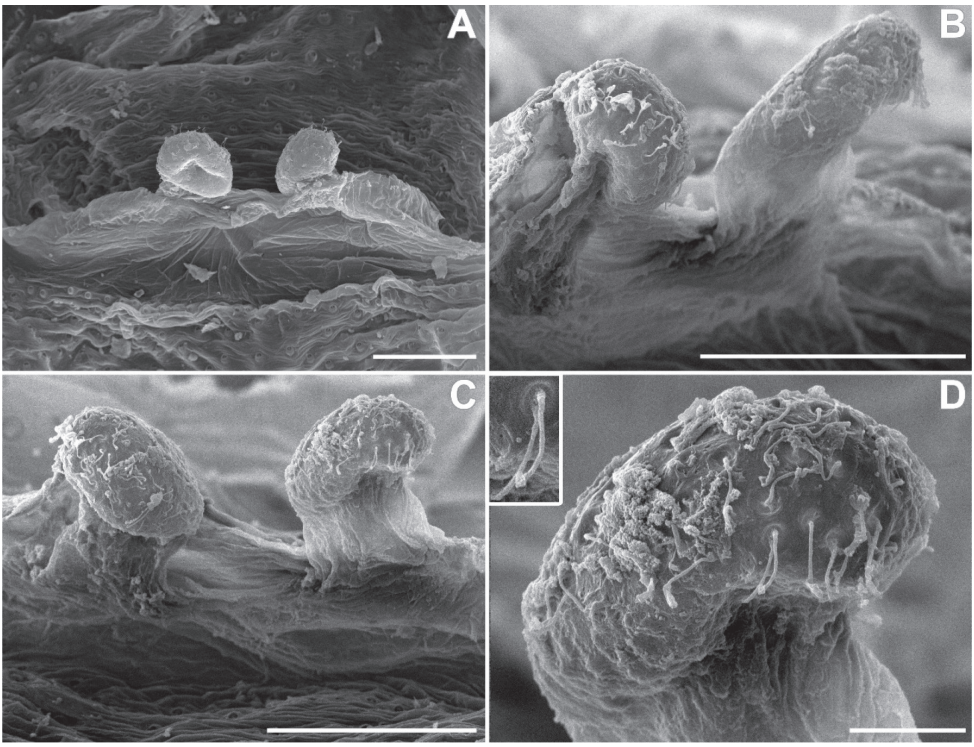


Figure 8. SEM images of *Misionella pallida* sp. n., female from Parque Municipal Pedra do Castelo, Castelo do Piauí, Piauí (UFMG 14385) **A** spermathecae, dorsal view **B** same, lateroventral view **C** same, anteroventral view **D** same, anteroventral (inset: detail of pores with filamentous gland). Scale bars: 0.1 mm (**A–C**), 0.02 mm (**D**).

Description. Male (MPEG 22756). Carapace orange with thoracic groove, lateral stripes and border black. Ocular area black. Chelicerae and labium orange. Endites and sternum cream. Legs and palps orange. Abdomen dorsally greyish, with grey stripes in the anterior border, and ventrally cream (Fig. 5A). Total length 3.0. Carapace 1.3 long, 1.0 wide. Sternum with small and shallow sulcus, without sigillae. Eye diameters: PME 0.4, separated by about four diameters. Pedipalp: femur length 0.9, patella 0.3, tibia 0.5 long, 0.3 wide. Leg measurements: I: femur 2.5, patella 1.0, tibia 3.0, metatarsus 2.3, tarsus 1.1, total 9.9; II: 1.8, 0.5, 2.0, 1.2, 0.5, 6.0; III: 1.3, 0.4, 1.0, 1.1, 0.4, 4.2; IV: 1.9, 1.0, 2.0, 1.7, 0.6, 7.2. Metatarsus II with a pair of hirsute macrosetae (Figs 5D–F, 7A–D). Abdomen 1.7 long, epiandrous area with at least 15 fusules (Fig. 9A). Cribellum divided and smooth (Fig. 9B). Spinnerets: ALS with one major ampullate gland spigot and at least 20 piriform gland spigots, PMS with one minor ampullate gland spigot, two aciniform gland spigots and one elongated paracribellar gland spigot, PLS with one paracribellar gland spigot and two aciniform gland spigots (Fig. 9B, D–F). Palp: tibia elongated, short cimbyum, bulb globose, with large and flattened paraembolic lamina (Figs 5G–H, 6A–E, 7A–E, 11G–H).

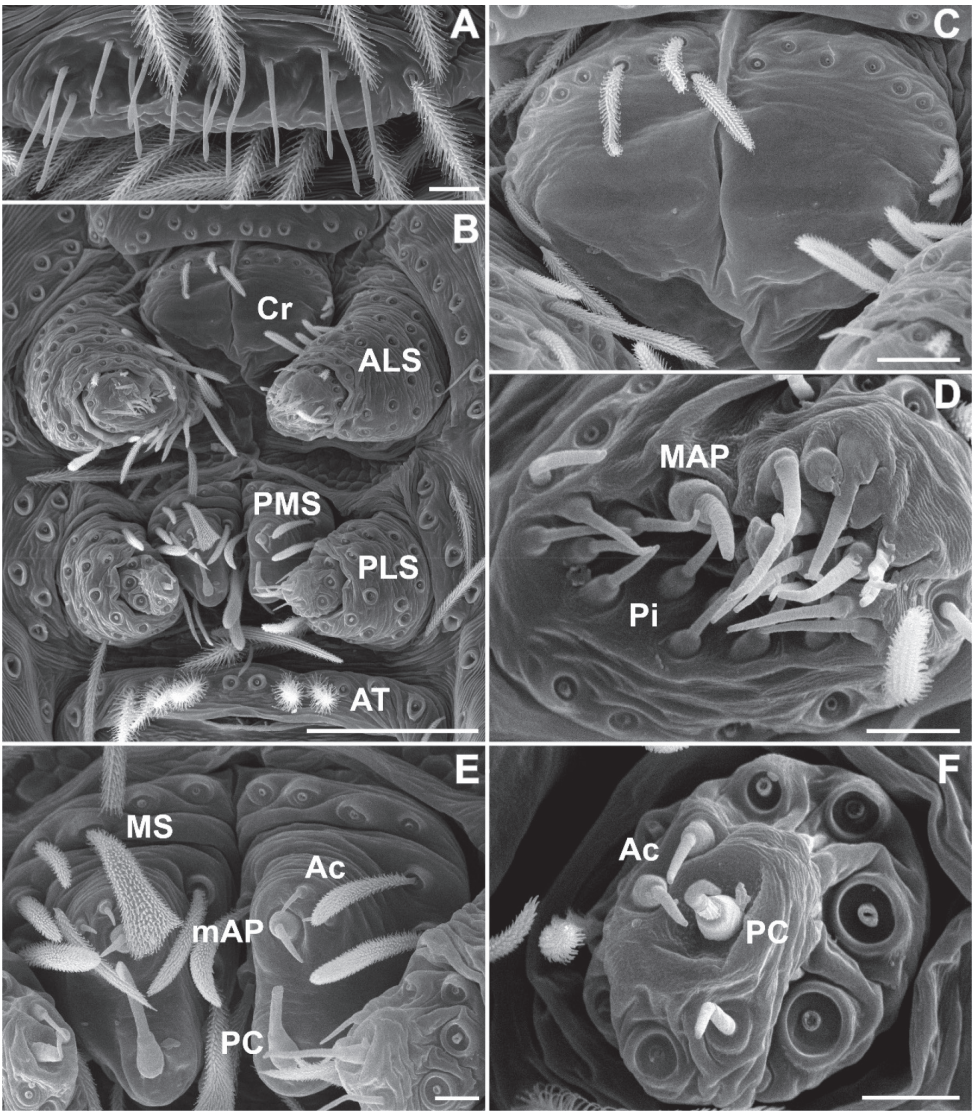


Figure 9. SEM images of *Misionella pallida* sp. n., male from Floriano, Piauí, Brasil (UFMG 14828) **A** epiandrium, ventral view **B** spinnerets, ventral view **C** cribellum, ventral view **D** spinnerets, left ALS, ventral view **E** PMS, ventral view **F** left PLS, ventral view. Abbreviations: Ac = aciniform gland spigots, ALS = anterior lateral spinnerets, AT = anal tubercle, Cr = cribellum, MAP = major ampullate gland spigot, mAP = minor ampullate gland spigot, PC = paracribellar gland spigot, Pi = piriform gland spigot, PLS = posterior lateral spinnerets, PMS = posterior median spinnerets. Scale bars: 0.02 mm (**A, C**), 0.1 mm (**B**), 0.01 mm (**D–F**).

Female (MPEG 22748). Coloration as in male, except pedipalp red brown and abdomen greenish gray with anterior dorsal border black (Fig. 5B–C). Total length 4.0. Carapace 1.7 long, 1.2 wide. Sternum as in male. Eye diameters: PME 0.4, separated by about 2 diameters. Pedipalp: femur length 1.1, patella 0.5, tibia 0.7, tarsus 0.3. Leg

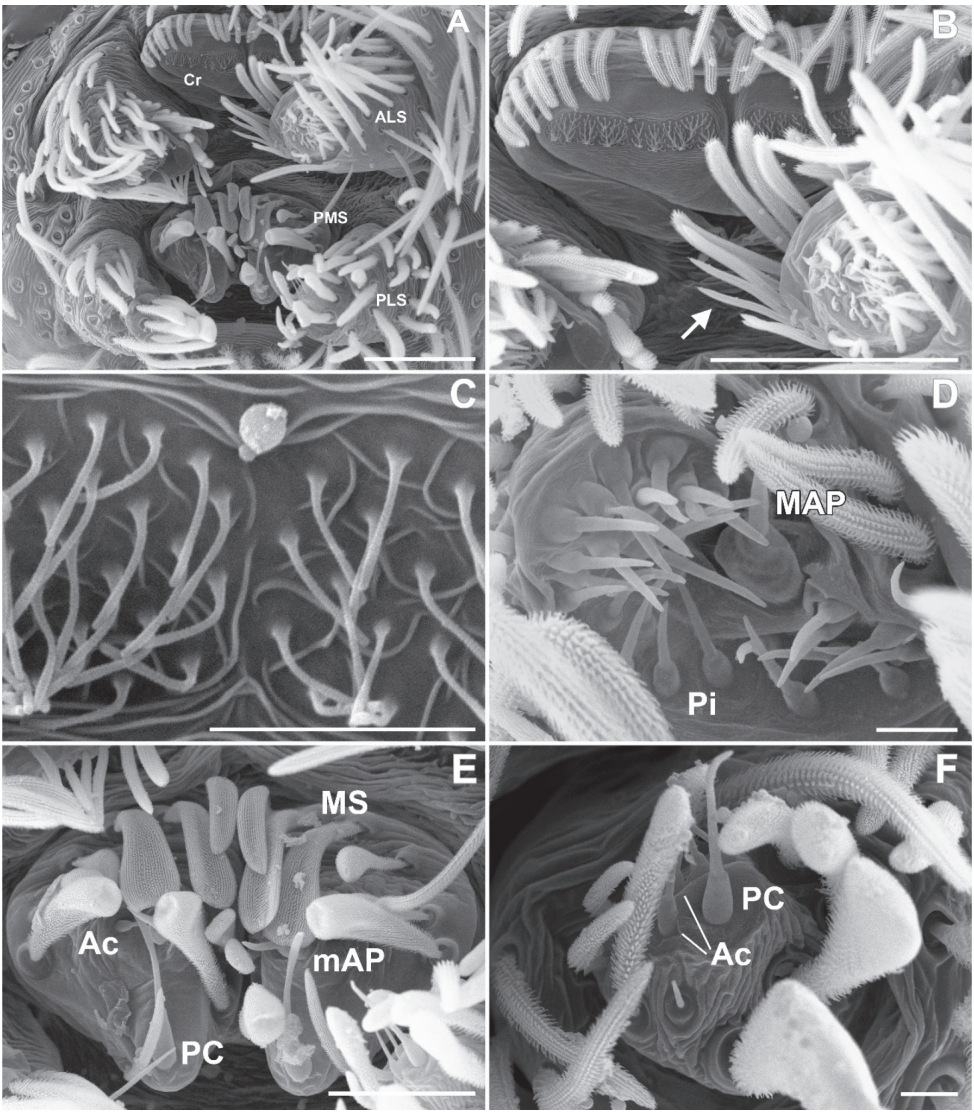


Figure 10. SEM images of *Misionella pallida* sp. n., female from Parque Municipal Pedra do Castelo, Castelo do Piauí, Piauí (UFMG 14385) **A** spinnerets, ventral view **B** same, cribellum and left ALS, ventral view. Arrow points to row of setae on ALS, characteristic of filistatids **C** same, cribellar spigots, ventral view **D** same, right ALS, ventral view **E** PMS, ventral view **F** right PLS, ventral view. Abbreviations: Ac = aciniform gland spigots, ALS = anterior lateral spinnerets, Cr = cribellum, MAP = major ampullate gland spigot, mAP = minor ampullate gland spigot, PC = paracribellar gland spigot, Pi = piriform gland spigot, PLS = posterior lateral spinnerets, PMS = posterior median spinnerets. Scale bars: 0.02 mm (**A–B**), 0.01 mm (**C–D, F**), 0.05 mm (**E**).

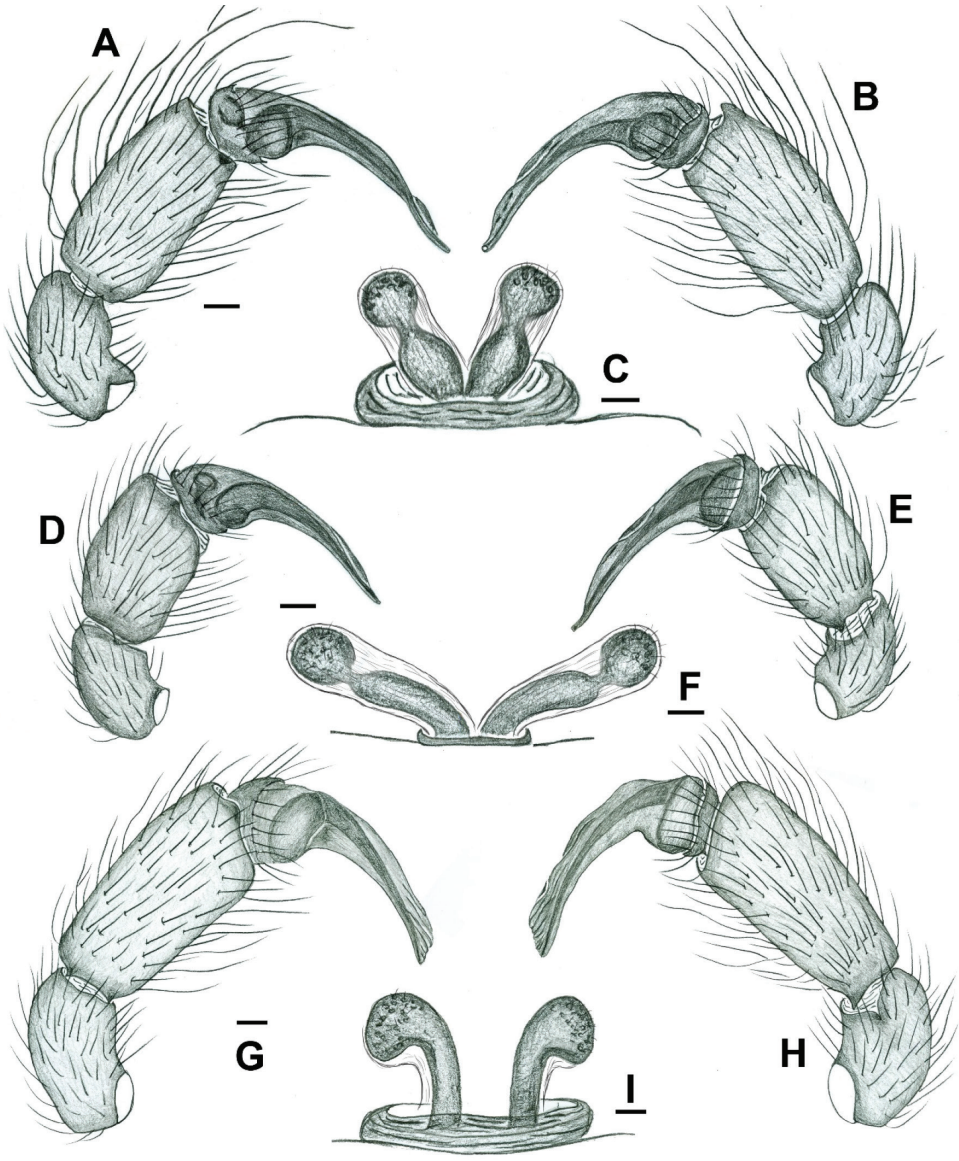


Figure 11. *Misionella carajas* sp. n., male and female from Cave N5S_0059, Flona Carajás, Parauapebas, Pará (IBSP 161040) (**A–C**) *Misionella* aikewara sp. n., male from São Geraldo do Araguaia, Pará (IBSP 191196) (**E**), female from Xambioá, Tocantins (IBSP 191194) (**F**). *Misionella pallida* sp. n. , male and female from Bairro São Joaquim, Teresina, Piauí (MPEG 22756) (**G–I**). **A–B** male palp. **A** prolateral view **B** retrolateral view **C** spermathecae, dorsal view **D–E** male palp, prolateral view **E** retrolateral view **F** spermathecae, dorsal view **G–H** male palp **G** prolateral view **H** retrolateral view **I** spermathecae, dorsal view. Scale bars: 0.1 mm(**A–B, D–E, G–H**), 0.02 mm (**C, F, I**).

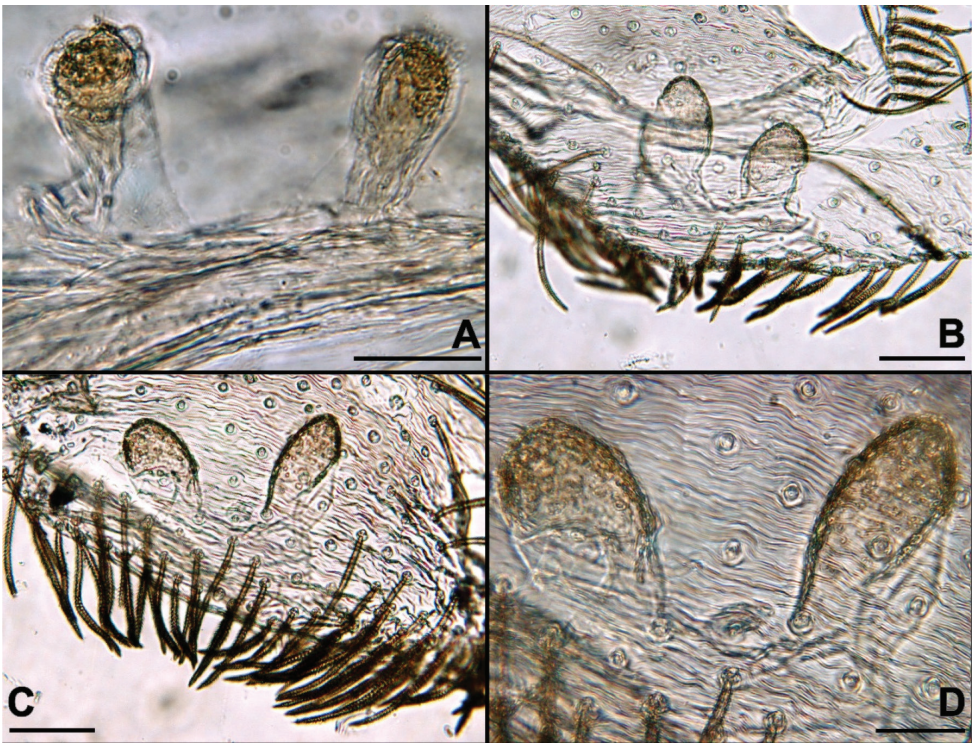


Figure 12. Spermathecae, dorsal view, latic acid cleared. **A** *Misionella carajas* sp. n., female from Cave N4E_0079, Flona Carajás, Parauapebas, Pará (IBSP 166200) **B** *Misionella pallida* sp. n., female from Parque Nacional das Sete Cidades, Piracuruca, Piauí (MPEG 22752) **C–D** same species, variation, female from Magnesita, Brumado, Bahia (UFMG 15513). Scale bars: 0.05 mm (**A, D**), 0.1 mm (**B–C**).

measurements: I: femur 1.8, patella 0.5, tibia 2.1, metatarsus 1.4, tarsus 1.0, total 6.8; II: 1.4, 0.5, 1.2, 1.0, 0.6, 4.7; III: 1.2, 0.4, 0.9, 1.0, 0.5, 4.0; IV: 1.7, 0.5, 1.5, 1.3, 0.6, 5.6. Abdomen 2.4 long. Cribellum as in male, but more numerous spigots (Fig. 10B–C). Spinnerets: ALS as in male, PMS with one minor ampullate gland spigot, at least one aciniform gland spigots and one elongated paracribellar gland spigot, PLS with one paracribellar gland spigot and four aciniform gland spigots (Fig. 10A, D–F). Spermathecae curved at tip with elongated ducts (Figs 8A–D, 11I).

Variation. 10♂: total length 1.9−2.8; carapace 1−1.3; femur I 1.4−2.6; 10♀: total length 3.8−4.2; carapace 1.6−1.8; femur I 1.8−2.1.

Natural history. This species has been collected several times in both natural and synanthropic habitats in northeastern Brazil. The species seems to naturally occur in Caatinga vegetation, a type of seasonally dry tropical forest. In synanthropic conditions, females can be found in their webs in the corners and cracks of windows and doors (L.S. Carvalho, pers. comm.). Males have been collected in pitfall traps in Caxias, in the state of Maranhão.

Distribution. Known from Northeastern Brazil (Fig. 14).

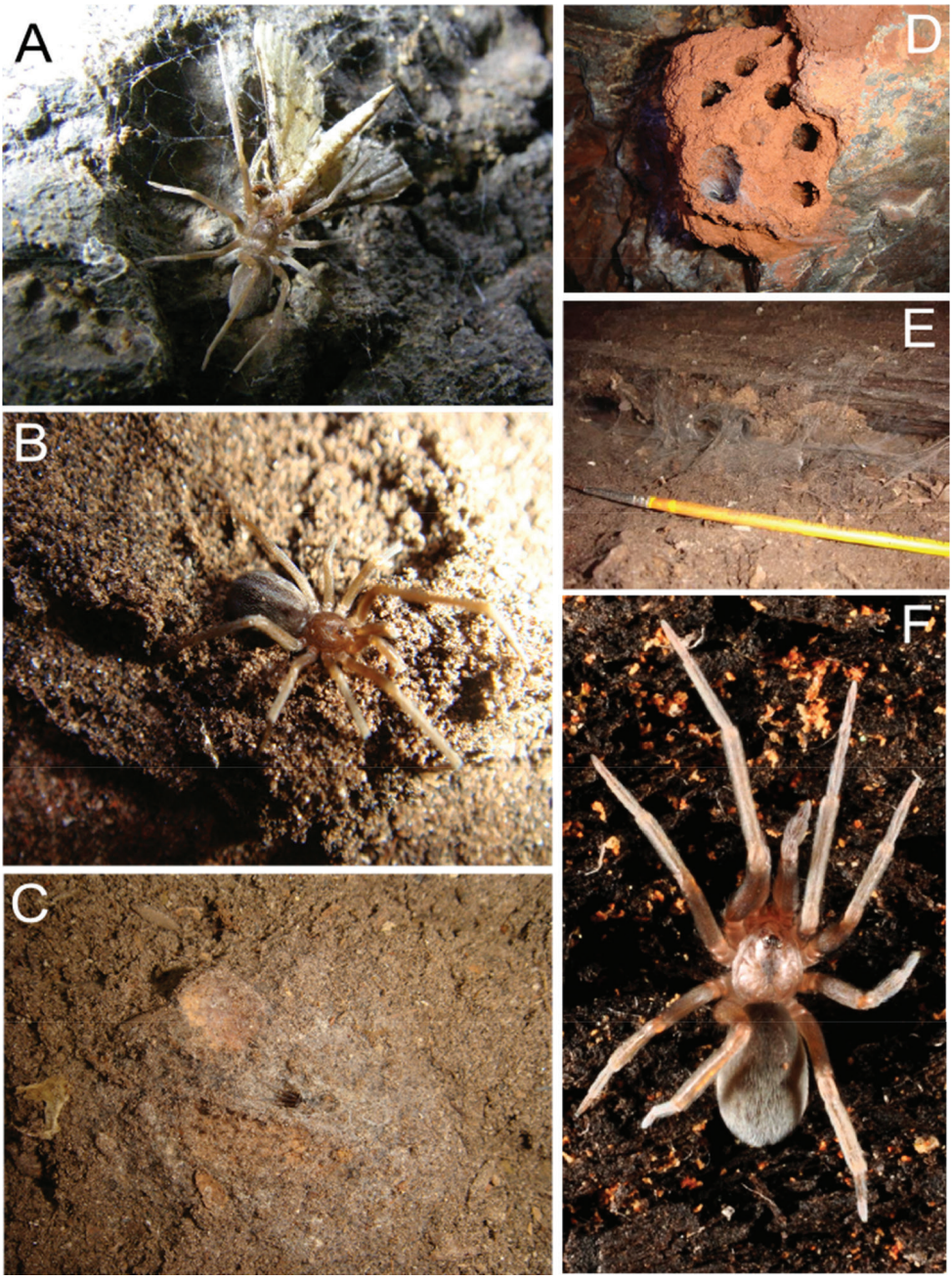


Figure 13. *Misionella carajas* sp. n. (**A–D**), **A** capturing a Tineidae (Lepidoptera) **B** male, dorsal view **C** soil web in bat guano **D** female living in nests of wasps (Hymenoptera); *M. aikewara* sp. n. **E** refuge formed the web in the wall of cave **F** *M. pallida* sp. n., female, dorsal view.

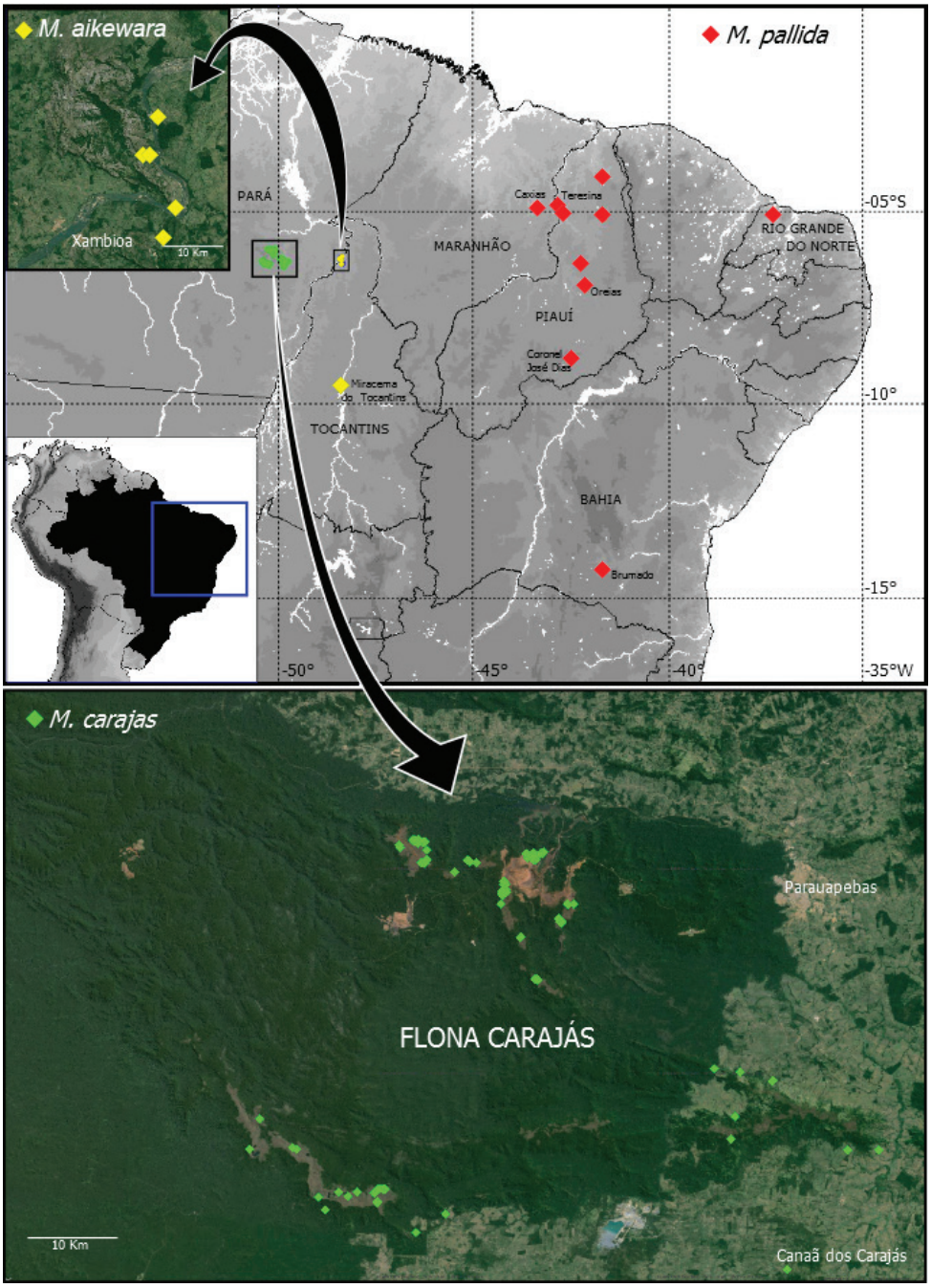


Figure 14. Map showing records of the three new *Misionella* species in Brazil. Green diamond = *M. carajas* sp. n., yellow diamond = *M. aikewara* sp. n., red diamond = *M. pallida* sp. n.

Discussion

Phylogenetic placement. The three new species herein described superficially resemble *Filistatoides* F.O. Pickard-Cambridge due to the elongate palps and bulbs, and by the female genitalia with a single pair of spermathecae (see Gray 1995; Ramírez and Grismado 1997). However, they share two derived states with the South American genera *Pikelinia* and *Misionella*: the cymbium fused to the tegulum (Fig. 6C, D) and the second metatarsus of males modified and bearing retrolateral macrosetae (Figs 1G–I, 2A, 4C–D, 5D–F, 7). Currently, *Misionella* is defined by the presence of a modified metatarsus II combined with the absence of a tibial apophysis in the male palp (Ramírez and Grismado 1997), both characters being present in the three new species. However, several undescribed filistatid species not treated here have intermediate morphologies between *Misionella* and *Pikelinia* (Magalhaes, unpublished data), blurring the limits between the two genera. The three species newly described apparently form a monophyletic group, supported by the unilobulate spermathecae, long bulbs, and the absence of pigment rings in the legs. Their placement in *Misionella* is not satisfactory, and a new genus could be proposed. However, as stressed above, the limits between South American filistatid genera are currently somewhat dubious, and several genus-defining characters are apparently homoplastic. A new phylogenetic analysis of the Filistatidae is in progress by the second author, and we think it is more prudent not to erect a new genus at the moment.

Biogeography. Filistatids are known to occur mainly in arid and semi-arid environments. To date, *Misionella* have been an exception as they seem to prefer more humid habits: *M. mendensis* occurs in the Cerrado (a savannah) and the Atlantic Forest, and *M. jaminawa* is Amazonian (Ramírez and Grismado 1997; Grismado and Ramírez 2000). On the other hand, *Misionella pallida* sp. n. seems to be restricted to the western border of the Caatinga, a seasonally dry tropical forest with a semi-arid climate (Pennington et al. 2000). *Misionella carajas* sp. n. and *M. aikewara* sp. n. occur in the humid Amazon, but they are restricted to caves. Caves often have different microclimatic conditions than the surroundings, and sometimes harbor relict species (*e.g.* Malek-Hosseini et al. 2015). It has been hypothesized that the limits of the seasonally dry tropical forests of Brazil have changed in response to recent climatic fluctuations (Pennington et al. 2000; Magalhaes et al. 2014). Thus, it can be hypothesized that the ancestors of *M. carajas* sp. n. and *M. aikewara* sp. n. also lived in dry conditions, and that they reached their current distribution during an event of expansion of the dry forests, being subsequently 'trapped' in the caves as the dry forests receded. A dated phylogeny of South American prithine would help shed some light on these questions.

Acknowledgments

We thank the curators of the collections cited above. We are grateful to Beatriz Mauricio, the operator of the Centro de Microscopia Eletrônica at the Instituto Butantan,

and F. Tricárico, at the MACN Microscopy Laboratory of Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", for SEM images. Thanks to Sergei Zonstein, an anonymous reviewer and Cor Vink for their comments on the manuscript. Robson Zampaulo provided information on caves and biology, and L.S. Carvalho provided specimens and natural history data of *M. pallida*. This study was financially supported by Vale S.A., OAPBio, CNPq 443270/2015-5 grant to IC, CNPq 301776/2004-0 and FAPESP 2011/50689-0 grants to ADB, a CONICET doctoral fellowship to ILFM and grants PIP 2012-0943 from CONICET and PICT 2011-1007 from FONCyT.

References

Carmo FF, Jacobi CM (2013) A vegetação de canga no Quadrilátero Ferrífero, Minas Gerais: caracterização e contexto fitogeográfico. Rodriguésia 64: 527–541. doi: 10.1590/S2175-78602013000300005

Grismado CJ, Ramírez MJ (2000) Notes on the spider genus *Misionella* with a description of a new species from Brazil (Araneae: Filistatidae). Studies on Neotropical Fauna and Environment 35: 161–163. doi: 10.1076/0165-0521(200008)35:2;1-9;FT161

Gray MR (1995) Morphology and relationships within the spider family Filistatidae (Araneae: Araneomorphae). Records of the Western Australian Museum 52: 79–89.

Mello-Leitão CF (1920) Algumas aranhas novas. Revista da Sociedade Brasileira de Sciências 3: 169–176.

Malek-Hosseini MJ, Grismado CJ, Sadeghi S, Bakhshi Y (2015) Description of the first cave dwelling species of the spider genus *Trilacuna* Tong & Li from Iran (Araneae: Oonopidae). Zootaxa 3972: 549–561. doi: 10.11646/zootaxa.3972.4.6

Magalhaes ILF, Oliveira U, Santos FR, Vidigal THDA, Brescovit AD, Santos AJ (2014) Strong spatial structure, Pliocene diversification and cryptic diversity in the Neotropical dry forest spider *Sicarius cariri*. Molecular Ecology 23: 5323–5336. doi: 10.1111/mec.12937

Pennington RT, Prado DE, Pendry CA (2000) Neotropical seasonally dry forests and Quaternary vegetation changes. Journal of Biogeography 27: 261–273. doi: 10.1046/j.1365-2699.2000.00397.x

Pinto-da-Rocha R, Andrade R (2012) A new species of *Cryptocellus* (Arachnida: Ricinulei) from Eastern Amazonia. Zoologia 29: 474–478. doi: 10.1590/S1984-46702012000500012

Ramírez MJ, Grismado CJ (1997) A review of the spider family Filistatidae in Argentina (Arachnida, Araneae), with a cladistic reanalysis of filistatid genera. Entomologica Scandinavica 28: 319–349.

ZooKeys 589: 97–106 (2016)
doi: 10.3897/zookeys.589.7739
http://zookeys.pensoft.net

SHORT COMMUNICATION

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Taxonomic recovery of the ant cricket *Myrmecophilus albicinctus* from *M. americanus* (Orthoptera, Myrmecophilidae)

Takashi Komatsu[1], Munetoshi Maruyama[1]

1 *Kyushu University, Hakozaki 6-10-1, Higashi-ku, Fukuoka 812-8581 Fukuoka, Japan*

Corresponding author: *Takashi Komatsu* (corocoro1232000@yahoo.co.jp)

Academic editor: *F. Montealegre-Z* | Received 8 January 2016 | Accepted 12 April 2016 | Published 16 May 2016

http://zoobank.org/9956EB10-A4CE-4933-A236-A34D809645E8

Citation: Komatsu T, Maruyama M (2016) Taxonomic recovery of the ant cricket *Myrmecophilus albicinctus* from *M. americanus* (Orthoptera: Myrmecophilidae). ZooKeys 589: 97–106. doi: 10.3897/zookeys.589.7739

Abstract

Myrmecophilus americanus and *M. albicinctus* are typical myrmecophilous insects living inside ant nests. These species are ecologically important due to the obligate association with tramp ant species, including harmful invasive ant species. However, the taxonomy of these "white-banded ant crickets" is quite confused owing to a scarcity of useful external morphological characteristics. Recently, *M. albicinctus* was synonymized with *M. americanus* regardless of the apparent host use difference. To clarify taxonomical relationship between *M. albicinctus* and *M. albicinctus*, we reexamined morphological characteristics of both species mainly in the viewpoint of anatomy. Observation of genitalia parts, together with a few external body parts, revealed that *M. albicinctus* showed different tendency from them of *M. americanus*. Therefore, we recover *M. albicinctus* as a distinct species on the basis of the morphology.

Keywords

Formicidae, host specificity, myrmecophily, symbiont, synonymy

Introduction

Myrmecophilus (*Myrmophilina*) *americanus* Saussure, 1877 (Orthoptera: Myrmecophilidae) (Figs 1a, 2a) is a typical example of an ant guest that lives inside ant nests. This species, similar to its congeners, eats food found inside the ant nest, either by itself or via mouth-to-mouth feeding by the ants (Wetterer and Hugel 2008). Its body color is totally black except for a single white band on the mesonotum. *Myrmecophilus americanus* was first described on the basis of a single female specimen collected in Colombia (Saussure 1877). The species is currently known to be distributed across tropical Asia, including on small islands, northern Africa, and the Neotropics (Wetterer and Hugel 2008). Its host-ant-species specificity is quite high; it has been collected exclusively (Wetterer and Hugel 2008) from nests of the longhorn crazy ant, *Paratrechina longicornis* (Latreille 1802). Because of its broad distribution, however, specimens of *M. americanus* from several localities have been given different species names. For example, Wasmann (1905) described *M. prenolepidis* from India, and Gorochov (1994) described *M.* (*Eumyrmecophilus*) *microscopicus* from Seychelles, but both these species have since been synonymized with *M. americanus*, the former by Schimmer (1909) and the latter by Hugel (2006). On the other hands, Ebner (1956) described *M. robustus* from Egypt though it has been also synonymized with *M. americanus* by Chopard (1968).

Myrmecophilus albicinctus (Chopard 1924) (Figs 1b, 2b) was first described on the basis of four females (the holotype and three paratypes) collected from India, but Ingrisch (2010) considered this species to be indistinguishable from *M. americanus* due

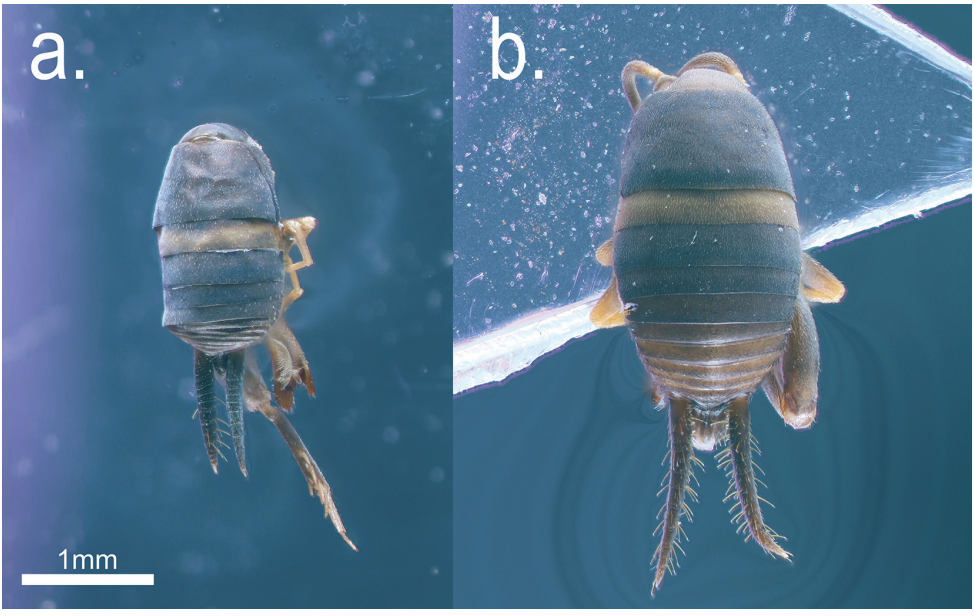


Figure 1. Specimens of the two *Myrmecophilus* species in dorsal view: *M. americanus* (**a**) and *M. albicinctus* (**b**).

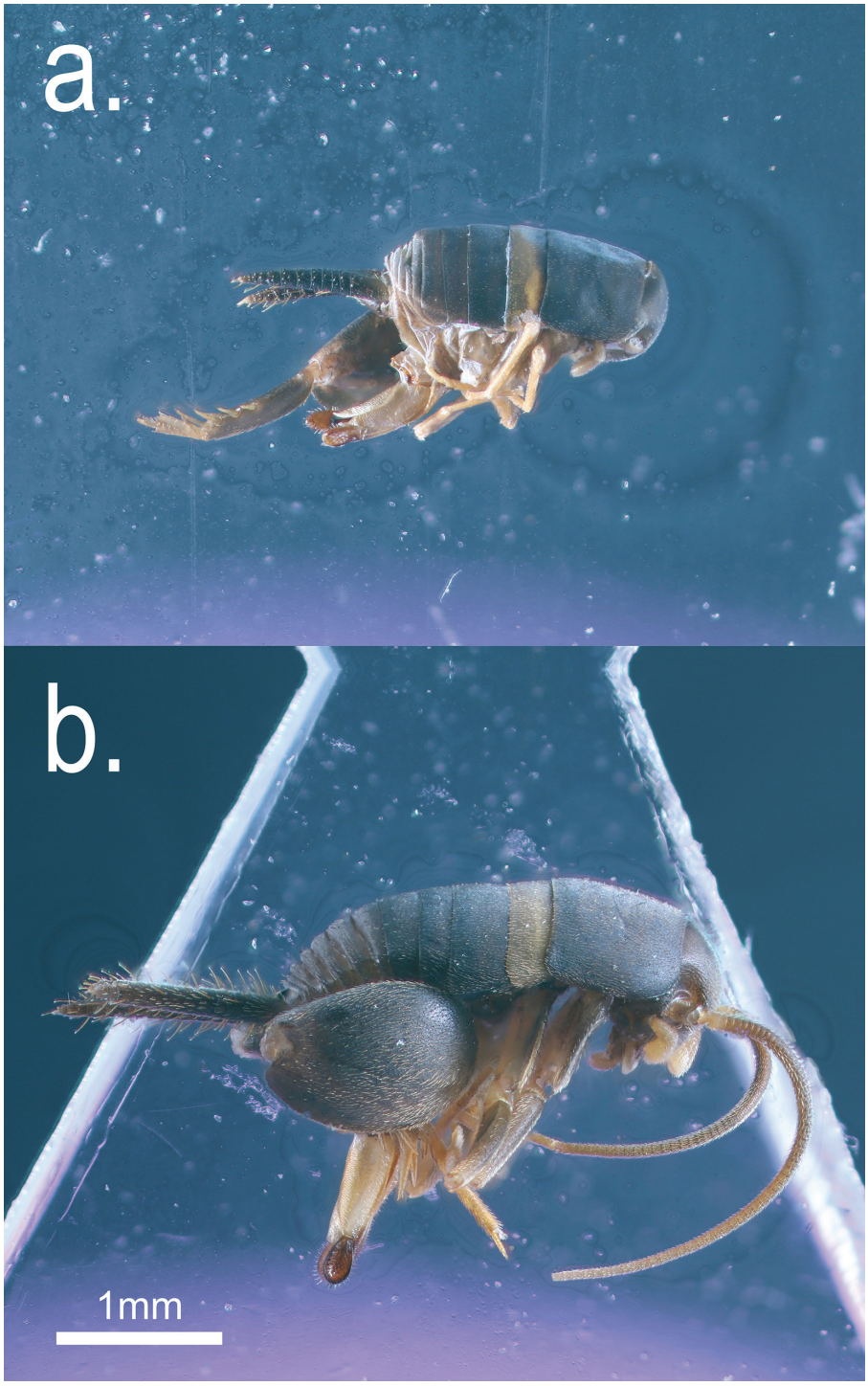


Figure 2. Specimens (same as those shown in Fig. 1) of the two *Myrmecophilus* species in lateral view: *M. americanus* (**a**) and *M. albicinctus* (**b**).

Table 1. Past literatures including host ant record of *Myrmecophilus americanus* and *M. albicinctus*. For records of *M. americanus*, Wetterer and Hugel (2008) have written up in detail.

Recorded species	Host ant species	Author
Myrmecophilus prenolepidis	*Prenolepis* (= *Paratrechina*) *longicornis*	Wasmann (1905)
Myrmecophilus albicincta (=*albicinctus*)	*Camponotus mitis*	Chopard (1924)
Myrmecophilus robustus	*Camponotus* sp.	Ebner (1956)
Myrmecophilus albicinctus	*Anoplolepis gracilipes*	Ichikawa et al. 2000
Myrmecophilus microscopicus	*Paratrechina longicornis*	Hugel and Blard (2005)
Myrmecophilus albicinctus	*Anoplolepis gracilipes*, *Pheidole* spp. (the latter is quite rare case)	Maruyama (2006)
Myrmecophilus americanus	*Paratrechina longicornis*, *Camponotus* sp. (the latter is only single record)	Wetterer and Hugel (2008)
Myrmecophilus albicinctus	*Anoplolepis gracilipes*	Komatsu et al. (2009)
Myrmecophilus albicinctus	*Anoplolepis gracilipes*	Murai and Ito (2011)
Myrmecophilus americanus	*Paratrechina longicornis*	Wetterer and Hugel 2014

to the similarity of their morphological characteristics. In contrast to *M. americanus*, however, *M. albicinctus* is known from only tropical Asia, including small islands (Maruyama 2006, Murai and Ito 2011). Moreover, recent studies have indicated that it is exclusively found in nests of the yellow crazy ant, *Anoplolepis gracilipes* (Maruyama 2006, Komatsu et al. 2009, Murai and Ito 2011), although the holotype specimen was collected from a *Camponotus mitis* nest (Chopard 1924). Laboratory experiments have shown that, from the perspective of behavioral ecology, *M. albicinctus* is closely dependent on *A. gracilipes* (Komatsu et al. 2009). With regard to the taxonomy of this species, Ichikawa (2001), independently of Hugel and Blard (2005) and Hugel (2006), synonymized *M. microscopicus* with *M. albicinctus*. Thus, the taxonomic status of *M. americanus* and *M. albicinctus* is quite confused (Ingrisch 2010). The host ant species of both *M. americanus* and *M. albicinctus* are well-known tramp ants, and *A. gracilipes* in particular is known to be a highly destructive invasive species. From the viewpoint of pest control, therefore, the taxonomy of parasites and myrmecophilous insects associated with this invasive ant species is of fundamental interest.

Recently, Ingrisch (2010) synonymized *M. albicinctus* with *M. americanus* because no morphological characteristic except body size was found to clearly distinguish them. However, we have previously suggested, following Wetterer and Hugel (2008), that each of these two species depends strictly on a different host ant species, and, moreover, we showed by a preliminary molecular phylogenetic analysis that they can be genetically differentiated (Komatsu et al. 2009, Komatsu et al. unpublished). In addition, we have found clear morphological differences between these two *Myrmecophilus* species.

For recovery of "*Myrmecophilus albicinctus*", there is problem of validity to use the name toward the species. As above mentioned, *M. albicinctus* have once synonymized with *M. americanus.* In addition, there is an older synonym of *M. americanus*; that is, *M. prenolepidis*. Under normal circumstances, it should be used the name of *M. prenolepidis* toward the recovered species. However, *M. prenolepidis* was described on the basis of specimens collected from nest of *Prenolepis longicornis* (Roger, 1863) that is synonymized

as *Paratrechina longicornis* (Emery 1925). The host specificity of *M. albicinctus* toward *A. gracilipes* is strong in principle so it is unlikely that it is collected from nests of *P. longicornis.* Given this, the specimens that formerly regards as *M. prenolepidis* can be regarded as not *M. albicinctus* but *M. americanus* which we call in present paper. Therefore, we apply the name of *M. albicinctus* for the recovered species. A series of old and recent host ant species records for *M. americanus* and *M. albicinctus* are listed in Table 1.

Methods

Sampling

Field sampling of *Myrmecophilus americanus* and *M. albicinctus* in the Ryukyu Islands and in southeast Asia was conducted from 2005 to 2015. Ant crickets were collected from nests of *Anoplolepis gracilipes* and *Paratrechina longicornis* by locating nest entrances, turning over stones, or breaking up decayed logs and stumps. Whenever ant crickets were found, as many as possible were collected and preserved in absolute alcohol.

Examination of samples

One of us (TK) examined specimens that he collected or were collected by colleagues. In addition, he visited the Muséum national d'Histoire naturelle (MNHN) to examine both type specimens (*M. americanus* and *M. albicinctus*).

The collected ethanol-preserved specimens were used for morphological observation. Specimens were dissected to observe their genitalia (abdominal terminalia). Each specimen was softened before dissection by warming (60 °C for 30–60 min) it in a small ceramic bowl (2.5 cm in diameter) with a small amount of water. Then, the specimen was dissected in water at high magnification under a stereomicroscope (Olympus SZ-40, ×6.7–80). The abdominal apex was removed from each specimen and dissected. Body parts were soaked in a warm 5–8% solution of potassium chloride (60 °C, 20–60 min), cleaned in 30% ethanol (5 min), and dehydrated in 99% ethanol (5 min). The dehydrated materials were mounted in Euparal (Chroma-Gesellschaft) on glass slides for detailed observation.

Results

Taxonomy

Myrmecophilus (*Myrmophilina*) *americanus* Saussure, 1877

Material examined. 3♂ and 1♀, collected from 50 Ngamwongwan Rd. ChatuChak Bangkok, Thailand, 6-X-2007, Komatsu T.; 1♀, Plot 256, Tingkat Perusahaan 5, Ka-

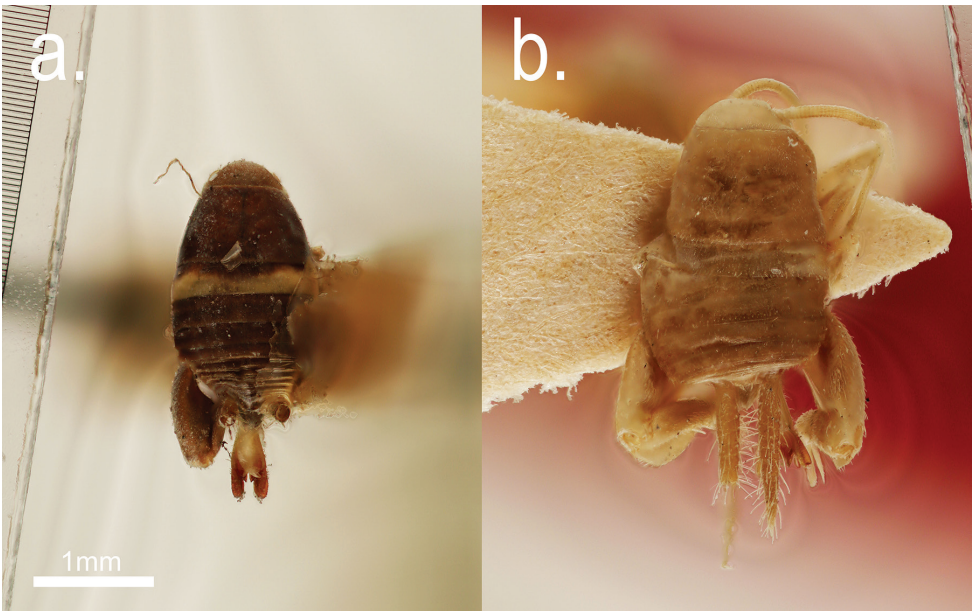


Figure 3. Type specimens: *M. americanus* (**a**) and *M. albicinctus* (**b**).

wasan Perindustrian Perai 2, Perai, Penang, Malaysia, 28-I-2011, Sumino T.; 1♀, Andalas University, Jl. Limau Manis, Kecamatan Pauh, Padang, Sumatera Barat 25163, Indonesia, 27-XI-2013, Komatsu T.; 1♂, Lembaga Ilmu Pengetahuan Indonesia, JL. Raya Jakarta Bogor km 46, Cibinong 16911, Indonesia, 20 VI 2013, Komatsu T.

Type material. Syntype 1♀: Barkuda Id., Chilka Lake, Ganjam dist., Madras Pres. 4-19-1919. F. H. Gravery, Zool. Surv. Ind. (MNHN) (Fig. 3a)

Diagnosis. Hind tarsus is relatively short (less than 1 mm, Fig. 4a); male phallic complex with pseudepiphallic ancorae short and roughly rounded with no dorsal branch. Ventral appendage of pseudepiphallic ancora somewhat predominant with both ends roughly square (Fig. 4b); male tenth abdominal tergite bituberculate, with scarce hair but without long strong spines (Fig. 4c); female ovipositor notably short and spoon-shaped in lateral view. Apical valves on both dorsal and ventral margins rounded, more than in other *Myrmecophilus* species (both *M. americanus* and *M. albicinctus* have rounded valves, with those of latter being more rounded) (Fig. 4d, e).

Myrmecophilus albicinctus Chopard, 1924, sp. rev.

Material examined. 1♀, collected from Koshidake, Iheya-jima, Okinawa, Japan, 5-IV-1996, Inada S.; 2♂, Gusukube-sunagawa, Miyakojima-shi, Miyako-jima, Okinawa, Japan, 8-VI-1996, Inada S.; 1♂ and 1♀, collected from Urasoe, Okinawa-jima, Okinawa, Japan, 24-VII-2007, Komatsu T.; 3♂ and 1♀, Yona, Kunigami-son, Okinawa-jima, Okinawa, Japan, 6-VIII-2007, Komatsu T.; 2♀, Field Studies Centre of the University of

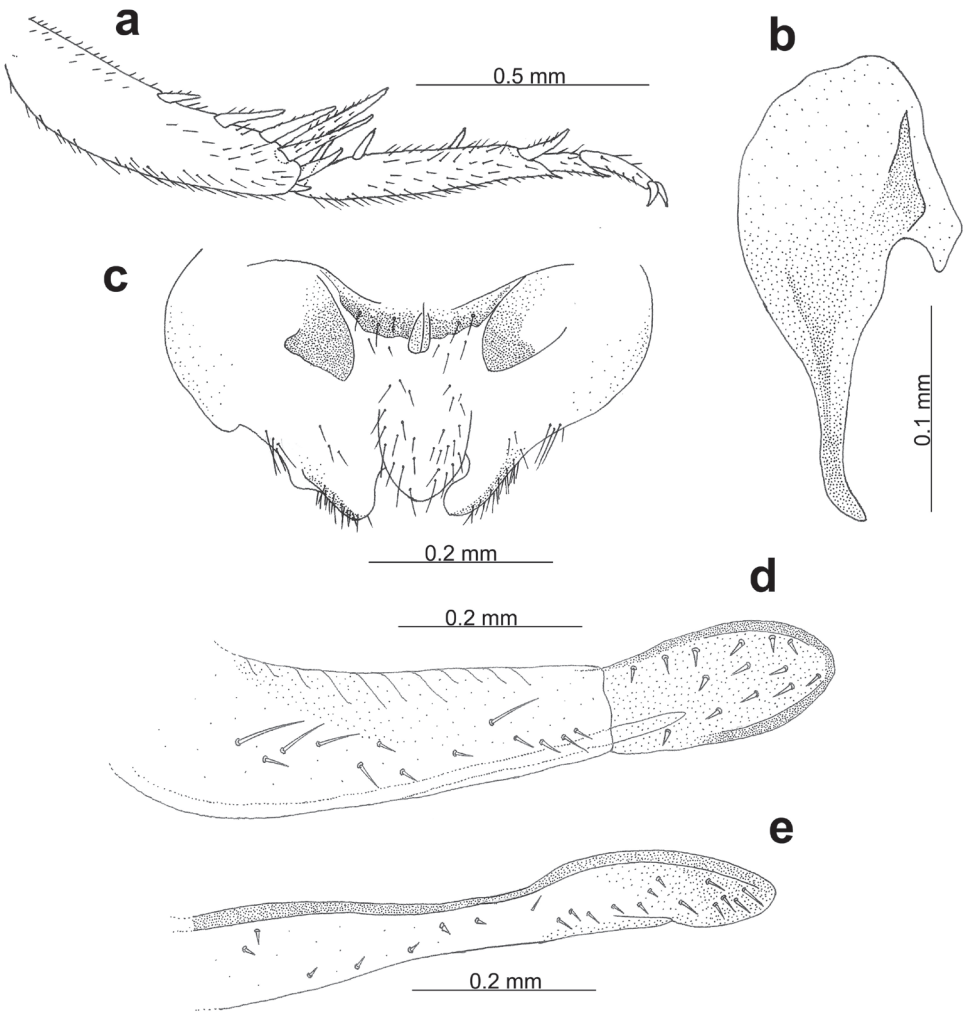


Figure 4. *Myrmecophilus americanus*. Hind tibia and tarsus, male inferior view (**a**); male pseudepiphallic ancora (**b**); male abdominal apex showing tubercles of the last abdominal tergite (**c**); female ovipositor, lateral view, dorsal margin (**d**); female ovipositor, lateral view, ventral margin (**e**).

Malaya, Ulu Gombak, Selangor, Malaysia, 25-X-2012, Komatsu T.; 3♀, Bogor Botanical Gardens, Jalan Ir. Haji Juanda No.13, 16122, Indonesia, 22-XI-2013, Komatsu M.; 1♂, Andalas University, Jl. Limau Manis, Kecamatan Pauh, Padang, Sumatera Barat 25163, Indonesia, 1-XII-2013, Komatsu M; 1♂, 16 km Point, Kaeng Krachan National Park, Phetchaburi, Thailand, 28-VI-2014, Komatsu T.; 4♂ and 2♀, Jalan Universiti, 50603 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur, Malaysia, 28-XI-2005, Komatsu T.; 2♂ and 1♀, Daruma-yama, Kume-jima, Okinawa, Japan, 9-XII-2014, Komatsu T.

Type material. Paratype 2♂2♀: Pattambi, Molabas Dist., F. H. Gravely V. 30 and *Anoplolepis longipes*. (Fig. 3b).

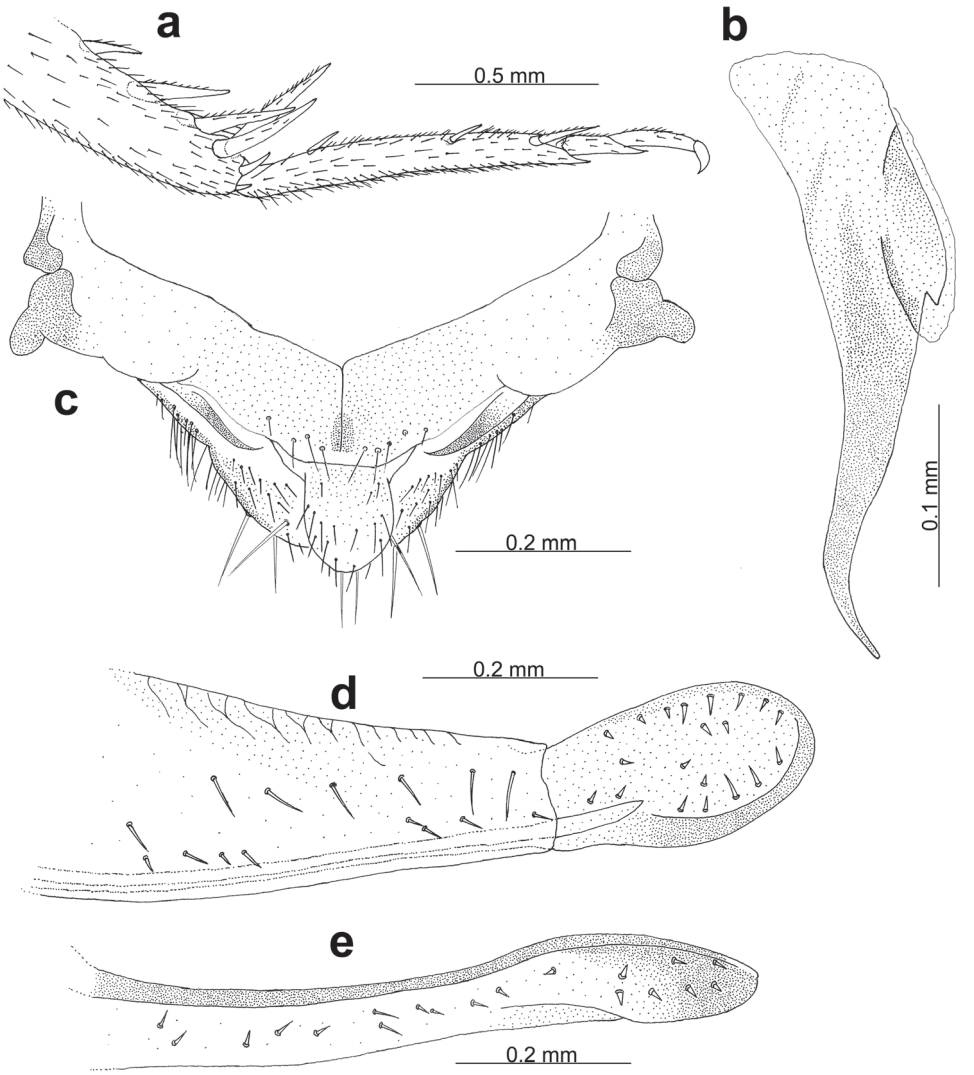


Figure 5. *M. albicinctus*. Hind tibia and tarsus, male inferior view (**a**); male pseudepiphallic ancora (**b**); male abdominal apex showing tubercles of the last abdominal tergite (**c**); female ovipositor, side view, dorsal margin (**d**); female ovipositor, side view, ventral margin (**e**).

Diagnosis. Hind tarsus is relatively long (more than 1 mm, Fig. 5a); male phallic complex with pseudepiphallic ancorae straightly elongate with no dorsal branch. Ventral appendage of pseudepiphallic ancora considerably reduced with both ends angular (Fig. 5b); male tenth abdominal tergite bituberculate, with rich hair and long strong spines (Fig. 5c); female ovipositor closely resembles that of *M. americanus*, except the apical valve on the dorsal margin is more rounded in lateral view (Fig. 5d, e).

Remark. This species can be clearly discriminated from *M. americanus* on the basis of the described diagnostic characteristics. Therefore, we recognize *M. albicinctus* as a distinct species.

Discussion

With regard to the taxonomy of *Myrmecophilus* ant crickets, Ingrisch (2010) has stated that better characteristics than host specificity are needed to differentiate species. In fact, some species of *Myrmecophilus* are host-generalists and do not show any apparent host specificity (Komatsu et al. 2009) whereas other *Myrmecophilus* species, including *M. americanus* and *M. albicinctus*, are characterized by strict host-species specificity (Wetterer and Hugel 2008; Komatsu et al. 2009). It has been suggested that host-species differentiation is one cause of speciation (Schönrogge et al. 2002, Ugelvig et al. 2011). Given the similarities of *M. americanus* and *M. albicinctus*, they may represent a transitional phase of speciation via host switching.

Acknowledgements

We thank Mr. Tomoki Sumino (Malaysia) and Satoshi Inada (Japan) for collecting material; Prof. Rosli Hasim (University of Malaya), Dr. Watana Sakchoowong (Department of National Parks, Wildlife and Plant Conservation), Dr. Wara Asfiya (Division of Zoology, Research Center for Biology, Indonesian Institute of Sciences), Dr. Sri Hartini (Zoology Division, Research Center for Biology, Indonesian Institute of Science–LIPI), and Dr. Henny Herwina (Universitas Andalas) for obtaining permission to conduct this research in their institutions; and Dr. Laure Desutter-Grandcolas (Muséum national d'Histoire naturelle) for permission to examine the type specimens. This work was supported by the Japan Society for the Promotion of Science KAKENHI Grant Number 26·931 to TK.

References

Chopard L (1924) The fauna of an island in the Chilka Lake. The Dermaptera and Orthoptera of Barkuda Island. Records of the Indian Museum Calcutta 26: 165–191.

Chopard L (1968) Fam. Gryllidae: Subfam. Mogoplistinae, Myrmecophilinae, Scleropterinae, Cachoplistinae, Pteroplistinae, Pentacentrinae, Phalangopsinae, Trigonidiinae, Eneopterinae; Fam. Oecanthidae, Gryllotalpidae. In: Beier M (Ed.) Orthopterorum Catalogus 12: 213–500.

Ebner R (1956) Ueber einige fuer Aegypten neue oder seltene Orthopteren. Bulletin de la Societe Entomologique d'Egypte 40: 11–20.

Emery C (1925) Hymenoptera, Formicidae, Formicinae. In: Wytsman MP (Ed.) Genera Insectrum fasc 183. Nabu Press, Charleston, 302 pp.

Gorochov AV (1994) Contribution to the knowledge of Grylloidea (Orthoptera) of the Seychelles. Entomologicheskoe Obozrenie 73: 95–102. [Russian; English translation in Entomological Review 73: 103–110]

Hugel S (2006) Note synonymique sur *Myrmecophilus americanus* Saussure, 1877, et presence de *M. quadrispina* Perkins, 1899, à l'île Maurice (Orth., Myrmecophilinae). Bulletin de la Société Entomologique de France 111(4): 448.

Hugel S, Blard F (2005) Présence de Grillons du genre *Myrmecophilus* à l'île de la Réunion (Orthoptera, Myrmecophilinae). Bulletin de la Société Entomologique de France 110: 387–389.

Ichikawa A (2001) New species of Japanese crickets (Orthoptera, Grylloidea) with notes on certain taxa. Tettigonia, Memoirs of the Orthopterological Society of Japan 3: 45–58.

Ichikawa A, Murai T, Honda E (2000) Monograph of Japanese crickets (Orthoptera; Grylloidea). Bulletin of the Hoshizaki Green Foundation 4: 257–332.

Ingrisch S (2010) Review of the ant loving crickets from South-East Asia (Orthoptera: Gryllidae, Myrmecophilinae). Entomologische Zeitschrift 120(1): 3–12.

Komatsu T, Maruyama M, Itino T (2009) Behavioral differences between two ant cricket species in Nansei Islands: host-specialist versus host-generalist. Insectes Sociaux 56(4): 389–396. doi: 10.1007/s00040-009-0036-y

Latreille PA (1802) Histoire Naturelle des Fourmis, et recueil de mémoires et d'observations sur les abeilles, les faucheurs, et autres insects. Pierre-Théophile Barrois, Paris, 445 pp.

Maruyama M (2006) Family Myrmecophilidae Saussure, 1870. In: Orthopterological Society of Japan (Ed.) Orthoptera of the Japanese Archipelago in Color. Hokkaido University Press, Sapporo, 490–492.

Murai T, Ito F (2011) Myrmecophilidae. In: Orthopterological Society of Japan (Ed.) A Field Guide to the Orthoptera of Japan. Hokkaido University Press, Sapporo, 307–310.

Saussure HD (1877) Mélanges Orthoptérologiques, V^{eme} fascicule. Gryllides. Mémoires de la Société de Physique et d'Histoire Naturelle de Genève 25(1): 1–352.

Schimmer F (1909) Beitrag zu einer Monographie der Gryllodeengattung *Myrmecophila* Latr. Zeitschrift für Wissenschaftliche Zoologie 93: 409–534. [pls 22–24]

Ugelvig LV, Vila R, Pierce NE, Nash DR (2011) A phylogenetic revision of the *Glaucopsyche* section (Lepidoptera: Lycaenidae), with special focus on the *Phengaris–Maculinea* clade. Molecular phylogenetics and evolution 61(1): 237–243. doi: 10.1016/j.ympev.2011.05.016

Wasmann E (1905) Zur Lebensweise einiger in- und ausländischer Ameisengäste (148. Beitrag zur Kenntnis der Myrmecophilen und Termitophilen). Zeitschrift für Wissenschaftliche Insektenbiologie 9(1): 329–336, 384–390, 418–428.

Wetterer JK, Hugel S (2008) Worldwide spread of the ant cricket *Myrmecophilus americanus*, a symbiont of the longhorn crazy ant *Paratrechina longicornis*. Sociobiology 52: 157–165.

Wetterer JK, Hugel S (2014) First North American records of the old world ant cricket *Myrmecophilus americanus* (Orthoptera, Myrmecophilidae). Florida Entomologist 97(1): 126–129.

ZooKeys 589: 107–121 (2016)
doi: 10.3897/zookeys.589.7320
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Three new species of *Osmylus* Latreille from China (Neuroptera, Osmylidae)

Han Xu[1], Yongjie Wang[2], Zhiqi Liu[1]

1 *Department of Entomology, China Agricultural University, Beijing 100094, China* **2** *College of Life Sciences, Capital Normal University, Beijing 100048, China*

Corresponding authors: *Yongjie Wang* (wangyjosmy@foxmail.com); *Zhiqi Liu* (zhiqiliu@cau.edu.cn)

Academic editor: *S. Winterton* | Received 26 November 2015 | Accepted 28 March 2016 | Published 16 May 2016

http://zoobank.org/E7489ECB-E933-445C-9CE4-EFEC8D7393F9

Citation: Xu H, Wang Y, Liu Z (2016) Three new species of *Osmylus* Latreille from China (Neuroptera, Osmylidae). ZooKeys 589: 107–121. doi: 10.3897/zookeys.589.7320

Abstract

Three new species of *Osmylus* Latreille are described from China: *Osmylus maoershanicola* **sp. n.**, *Osmylus shaanxiensis* **sp. n.** and *Osmylus angustimarginatus* **sp. n.** These new species are distinguishable from other related species by the shape of the 9th tergite of both sexes, as well as the shape of gonarcus, mediuncus and spermatheca. A key is given to differentiate Palaearctic and Oriental species of *Osmylus*.

Keywords

New species, Oriental region, Osmylidae, *Osmylus*, Palaearctic region

Introduction

The genus *Osmylus* Latreille (Osmylidae: Osmylinae) contains 21 species distributed in the Palaearctic and Oriental regions, 20 species of which are distributed in Asia and only one, *O. fulvicephalus*, which is widespread in Europe (Banks 1947, Canbulat 2013, Iwata 1928, Kozhanchikov 1951, Krüger 1912, 1913, Makarkin 1985, McLachlan 1870, 1875, Nakahara 1914, New 1991, Yang 1987, 1988, 1997, 1999). The first *Osmylus* species of the Chinese fauna, *Osmylus* (*Lysmus*) *oberthurinus*, was described

Figure 1. Distribution of *Osmylus* in China. ♦ = *O. angustimarginatus* sp. n.; △ = *O. biangulus*; ▼ = *O. bipapillatus*; ☆ = *O. conanus*; ★ = *O. fuberosus*; ⬣ = *O. lucalatus*; ▲ = *O. maoershanicola* sp. n.; ◇ = *O. megistus*; □ = *O. minisculus*; ○ = *O. shaanxiensis* sp. n.; ⬡ = *O. taiwanensis*; ■ = *O. wuyishanus*; ➢ = *O. xizangensis*.

by Navás (1910) and then 12 species described successively by Banks (1947), Yang (1987, 1988, 1997, 1999) and Wang and Liu (2010), with higher diversity in Tibet (four species) and Shaanxi Province (four species) (Fig. 1).

The biology of osmylids is still poorly known. *Osmylus* is known to be univoltine and adults feed as generalists on fungal spores, pollen, algae, mites and insects; they sit temporarily on foliage of plants along streams or river banks in daytime (Devetak 2007, Gepp 1976, Withycombe 1923). The biology of larvae remains controversial. Latreille (1805) and Stein (1838) deduced that the larvae of *Osmylus* are aquatic. However, Stitz (1936) and Eisner (1989) reported correctly that the larvae simply live in the water margin (the riparian interface) but cannot survive submersion. Accordingly, the larvae should definitely be regarded as terrestrial.

Materials and methods

The specimens in this study were examined under an Optec SZ760 stereomicroscope with direct light. The terminal of abdomens were removed and soaked in the 10% NaOH for boiling water bath and stored in a glycerin-filled micro-vial mounted on

the pin beneath specimen. The terminology for wing venation and genitalia follows New (1983), Adams (1969) and New (1983). All type specimens are deposited in the Entomological Museum of China Agricultural University (CAU), Beijing.

Taxonomy

Genus *Osmylus* Latreille

Osmylus Latreille, 1802: 289. Type species: *Hemerobius fulvicephalus* Scopoli, 1763: 270.
Dictyosmylus Navás, 1910: 189. Type species: *Dictyosmylus lunatus* Navás, 1910: 189, by monotypy.

Diagnosis. Moderate to large body size (body length 15–20 mm); forewing generally large and broad (length 20–30 mm), with numerous fragmentary marks; two nygmata present at the center and the proximal base of wing between MP and Rs; veins dark brown; costal cross-veins generally bifurcate distally, without interlinking veinlets; cross-veins among branches of Rs forming at least two series of gradates; MP forked close to the base, MP_2 with many branches. The hindwing resembles the forewing in shape, but with fewer spots. The 9th tergite has variably-shaped dorsal process. Genitalia are composed of a gonarcus and a mediuncus; the gonarcus is variable in shape, consisting of a sclerotized and pilose external section posteriorly with a lightly sclerotized anterior-lateral section, the latter laterally with an anterior rod shaped process (i.e., baculum of some authors) which is sometimes articulated. The mediuncus (i.e., parameres of some authors) is curved with a fused base (although the shape is variable in *O. pachycaudatus*). The mediuncus is subtended laterally by the rod-shaped, paired parameres (i.e., subarcus of other authors) that are not fused anteriorly. The female 9th tergite occasionally has a ventral process, the gonapophysis lateralis is generally finger-like and articulated with stylus distally, and the spermatheca is either oval or cylindrical in shape.

Included species. *Osmylus angustimarginatus* sp. n., *O. biangulus* Wang & Liu, *O. bipapillatus* Wang & Liu, *O. cilicicus* Krüger, *O. conanus* Yang, *O. decoratus* Nakahara, *O. fuberosus* Yang, *O. fulvicephalus* (Scopoli), *O. gussakovskii* Kozhanchikov, *O. hyalinatus* McLachlan, *O. kisoensis* Iwata, *O. lucalatus* Wang, *O. maoershanicola* sp. n., *O. megistus* Yang, *O. minisculus* Yang, *O. multiguttatus* McLachlan, *O. pachycaudatus* Wang, *O. posticatus* Banks, *O. pryeri* McLachlan, *O. shaanxiensis* sp. n., *O. taiwanensis* New, *O. tessellatus* McLachlan, *O. wuyishanus* Yang, *O. xizangensis* Yang.

Comments. *Osmylus* has been often confused with three other genera, *Grandosmylus* Makarkin, 1985, *Parosmylus* Needham, 1909 and *Plethosmylus* Krüger, 1913. Banks (1913) advanced that *Parosmylus* should be a junior synonym of *Osmylus* because the spur on the coxa in *Parosmylus* is also present in some species of *Osmylus*. Krüger (1913) erected the genus *Plethosmylus* based on venation characters (presence of interlink veinlets between costal cross-veins). Nakahara (1914) considered the opinion of Krüger subjective and synonymized the latter genus. Kuwayama (1953, 1962)

again separated *Plethosmylus,* differentiating it from *Osmylus* by the presence of interlinking veinlets among the costal and two basal Rs-Mp cross-veins before the proximal nygma. However, Makarkin (1985) revised the status of *Plethosmylus*, synonymizing it with *Osmylus* and establishing a new subgenus *Plesiosmylus* within *Osmylus*. He also established a new genus *Grandosmylus*, separated from *Osmylus* by the irregular gradate cross-veins and the shape of 9th sternite in males and 8th sternite in females; this opinion was accepted by Sekimoto (2011) in his revision of Japanese *Osmylus.* The relationship among *Grandosmylus*, *Parosmylus* and *Plethosmylus* remains unclear. Wang and Liu (2009) clarified the generic status of *Parosmylus*, after reviewing specimens from mainland China, and they concluded that both genera could be valid due to differences in the number of gradate series, the configuration of gonarcus and the shape of spermatheca (Wang and Liu 2009). Furthermore, after re-examining the specimens of *Plethosmylus* from mainland China, we observed that *Osmylus* and *Plethosmylus* possessed significant differences in male genitalia (the configuration of gonarcus) and in female genitalia. Moreover, the interlink veinlets among costal cross-veins could conveniently divide them. Considering the vague relationships among these genera, we consider is suitable to maintain them as separate genera until a robust phylogenetic work can be conducted in the future. In this paper, three new species of *Osmylus* are described from China: *O. maoershanicola* sp. n. *O. shaanxiensis* sp. n. and *O. angustimarginatus* sp. n., primarily based on genital characters.

Key to *Osmylus* species in the Palaearctic and Oriental regions

(Note: *Osmylus kisoensis* is not included as it is only known from the larval stage, while *O. cilicicius* and *O. posticatus* are poorly known and could not be included in the key.)

1 The structure of spermatheca complicated (Fig. 2a) ..... ***O. megistus***
– The structure of spermatheca simple (Fig. 2b–h)..... **2**
2 The 7th sternite in female with a median preapical protuberance **3**
– The 7th sternite in female without any protuberance..... **4**
3 Spermatheca cylindrical and bent; anterior third of pronotum with median stripe..... ***O. taiwanensis***
– Spermatheca oval; frons with dark brown X-shaped marking; pronotum with yellow and median stripe ..... ***O. decoratus***
4 The gonapophyses lateralis cone-shaped, spermatheca pyriform ... ***O. minisculus***
– The gonapophyses lateralis finger-like or fusiform..... **5**
5 9th tergite in male with a distinct dorsal process (Figs 3a–d, 5a, 9a)..... **6**
– 9th tergite in male without distinct dorsal processes (Figs 3e, 7a)..... **14**
6 Gonarcus with a sharpened process along dorsal margin in lateral view..... ..... ***O. pryeri***
– Gonarcus without processes along dorsal margin in lateral view..... **7**
7 Forewing relatively narrow, membrane hyaline with slight metallic luster..... ..... ***O. lucalatus***

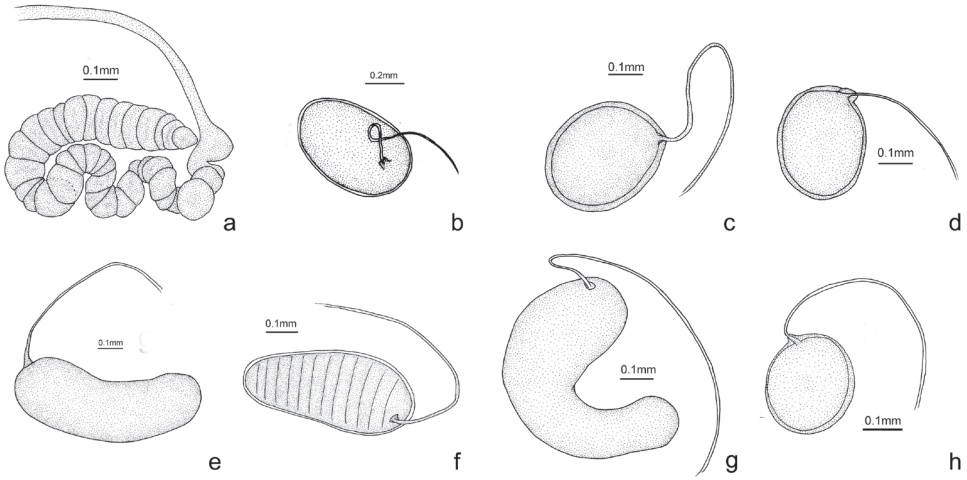


Figure 2. Spermathecae. **a** *O. megistus* **b** *O. lucalatus* **c** *O. angustimarginatus* sp. n. **d** *O. maoershanicola* sp. n. **e** *O. biangulus* **f** *O. fuberosus* **g** *O. shaanxiensis* sp. n. **h** *O. wuyishanus*.

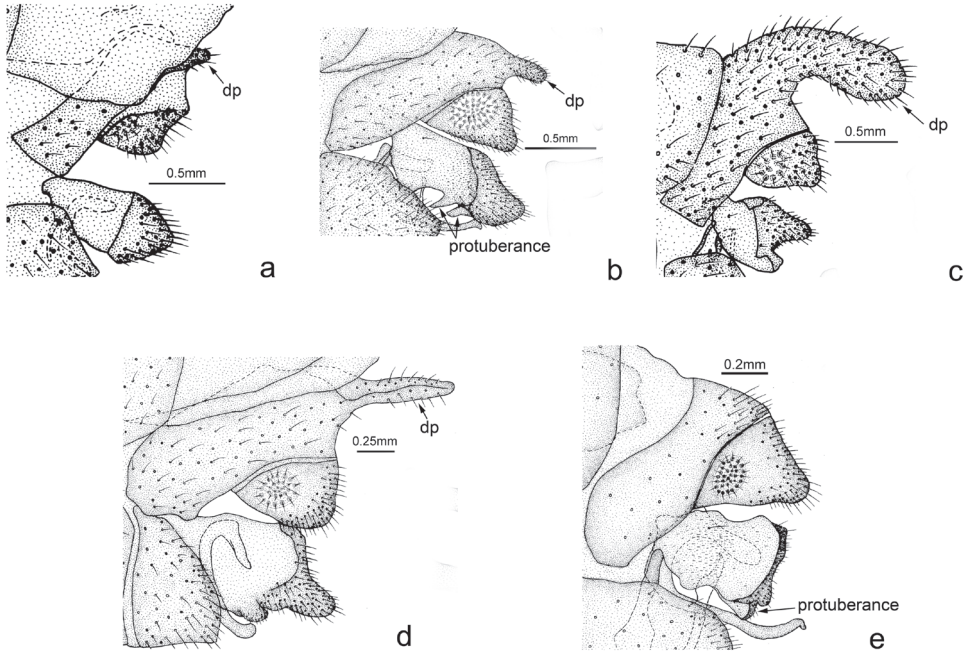


Figure 3. Male terminalia, lateral view. **a** *O. lucalatus* **b** *O. biangulus* **c** *O. pachycaudatus* **d** *O. bipapillatus* **e** *O. fuberosus*. Abbreviation: dp, dorsal process.

– Forewing broad, membrane dull hyaline.....**8**

8 The length of process of 9th tergite in male slightly longer than width (Figs 3b, 5a, 9a).....**9**

–	The length of process of 9th tergite in male significantly longer than width (Fig. 3c–d) **13**
9	The process of 9th tergite in male cone-shaped..... ***O. hyalinatus***
–	The process of 9th tergite in male bar-shaped..... **10**
10	Gonarcus with one or two lateral posteroventral protuberances **11**
–	Gonarcus without any lateral posteroventral protuberance..... **12**
11	Gonarcus with only one lateral posteroventral protuberance in lateral view (Fig. 5b)..... ***O. maoershanicola* sp. n.**
–	Gonarcus with two lateral posteroventral protuberances in lateral view (Fig. 3b)..... ***O. biangulus***
12	Mediuncus apically hook-shaped ..... ***O. fulvicephalus***
–	Mediuncus apically slender and straight (Fig. 5d)... ***O. angustimarginatus* sp. n.**
13	The process of 9th tergite in male cylindrical (Fig. 3c), mediuncus arch-like in lateral view, anterior arm of gonarcus with a distal right-angle bend .... ***O. pachycaudatus***
–	The process of 9th tergite in male subulate (Fig. 3d), mediuncus C-shaped in lateral view..... ***O. bipapillatus***
14	Distal part of gonarcus flat and quadrate, with a posteroventral protuberance (Fig. 3e) ..... ***O. fuberosus***
–	Distal part of gonarcus approximately triangular..... **15**
15	Distal part of gonarcus with one or more conspicuous protuberances **16**
–	Distal part of gonarcus with an inconspicuous protuberance..... **20**
16	8th sternite in female with two ventral protuberances; distal part of gonarcus with a ventral and rod-like protuberance..... ***O. gussakovskii***
–	8th sternite in female without any protuberance **17**
17	Distal part of gonarcus with two protuberances in later view; pronotum with slender yellow marking on anterior half and yellow spot over posterior margin ..... ***O. tessellatus***
–	Distal part of gonarcus with only one protuberance..... **18**
18	Cross-veins among branches of Rs forming 3 series of gradates; forewing and hindwing with approximately rounded spots ..... ***O. multiguttatus***
–	Cross-veins among branches of Rs forming 2 series of gradates..... **19**
19	9th tergite in female with a median narrowing in lateral view (Fig. 7e); meso- and metanotum dark brown, some sclerites brown .... ***O. shaanxiensis* sp. n.**
–	9th tergite in female slightly tapered medially in lateral view; meso- and metanotum yellowish brown with black streaks ..... ***O. xizangensis***
20	Distal part of gonarcus forming a large triangular sclerite; outer gradates of forewing with brown marks..... ***O. wuyishanus***
–	Distal part of gonarcus forming a narrow and small sclerite; inner gradates of forewing with brown marks..... ***O. conacus***

***Osmylus maoershanicola* sp. n.**
http://zoobank.org/515687EC-B4FF-489C-A12D-4B41DEC81A34
Figs 4, 5

Material examined. Holotype Male, CHINA: Guangxi (Province): Maoershan (Nature Reserve), [25°48'N, 110°24'E], 9.viii.2005, leg. Ping Zhao. Verbatim label data (translated from Chinese): CHINA: Guangxi Prov., Maoershan/ 9.viii.2005/ Ping Zhao/ CAU. Condition: Antennal flagellum missing. Abdomen terminalia cleared in KOH, and stored in the micro-vial pinned below the specimen. Paratype. 1 female (left antenna damaged), same data as holotype (CAU).

Diagnosis. Male: 9th tergite with a short finger-like dorsal process; ectoproct cone-shaped. Gonarcus distally triangular with a ventral, triangular, membranous protuberance in lateral view. Female: gonapophysis lateralis approximately fusiform; spermatheca oval.

Description. *Head*. Vertex yellowish-brown with brown setae; eye dark gray, ocelli yellow, area within ocelli black. Antennal flagellum missing, scape and pedicel dark brown; frons yellow. *Thorax*. Pronotum dark brown, posterior margin slightly wider,

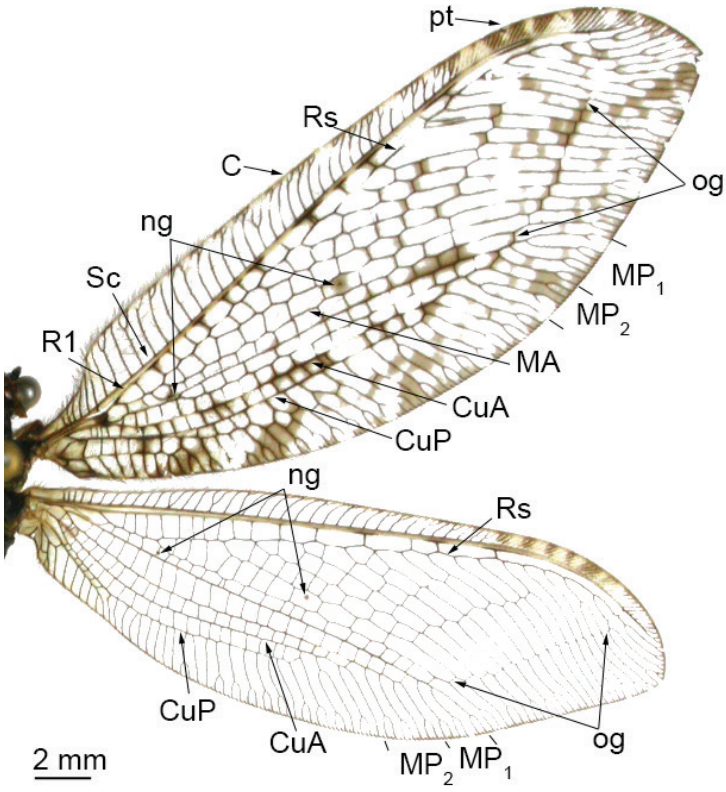


Figure 4. Wings of *Osmylus maoershanicola* sp. n., forewing (upper) and hindwing (lower). Abbreviations: ng, nygmata; pt, pterostigma; og, outer gradates.

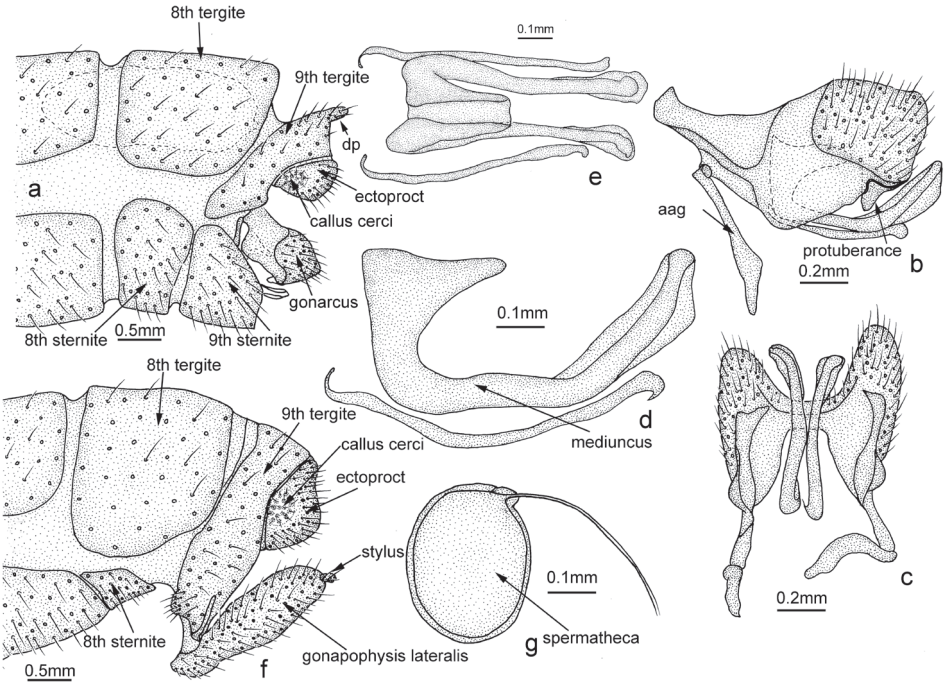


Figure 5. *Osmylus maoershanicola* sp. n. **a–e** Male: **a** apex of the abdomen and genitalia, lateral view **b** genitalia, lateral view **c** genitalia, ventral view **d** mediuncus, lateral view **e** mediuncus, dorsal view **f–g** Female: **f** apex of the abdomen and genitalia **g** spermatheca, lateral view (spiracula omitted). Abbreviation: dp, dorsal process; aag, anterior arm of gonarcus.

with black brown setae; meso- and metanotum black with brown setae. Legs yellow with brown setae; pretarsal claws dark brown.

Wing (Fig. 4). Forewing length 27–28 mm, width 9–10 mm. Membrane hyaline, with many sparse, fuscous spots; pterostigma brown; nygmata light brown; veins dark brown; Rs with 13–14 branches, outer gradate cross-veins edged with fuscous stains; R1-Rs cross-veins edged with brown marks; short cross-veins are present among the branches of CuP. Hindwing length 23–24 mm, width 7–8 mm. Membrane hyaline; pterostigma light yellow.

Male terminalia (Fig. 5a–e). Scent glands slender. 9th tergite long and narrow with a short, dorsal finger-like process (Fig. 5a), ventral margin slightly tapered. 9th sternite trapezoidal in lateral view. Ectoproct triangular in lateral view, callus cerci round. Distal part of gonarcus well sclerotized and approximately triangular, ventral part membranous with a triangular protuberance in lateral view (Fig. 5b); anterior arm of gonarcus slender; mediuncus dilated basally with a sharp backward end, slender apically and coated by a membrane in lateral view; rod-shaped paramere beneath the mediuncus slightly bent in lateral view, posterior end sharp.

Female terminalia (Fig. 5f–g). 8th sternite approximately trapezoidal; 9th tergite long and narrow with a ventral hemispherical tubercle in lateral view; ectoproct triangular in lateral view, callus cerci round, presenting in middle; gonapophysis lateralis approximately fusiform, stylus cylindrical; spermatheca simple, approximately spherical.

Distribution. Presently known only from Guangxi Province, China.

Etymology. The specific name '*maoershanicola*' refers to 'Maoershan Mountain', the type locality.

Remarks. The dorsal finger-like process of 9th tergite of *Osmylus maoershanicola* sp. n. is similar to *O. pryeri* and *O. biangulus*, but this new species can be identified by the distinctive shape of the gonarcus. There are two prominent ventral protuberances in the distal part of gonarcus of *O. pryeri* and *O. biangulus* (Fig. 3b) but only one in *O. maoershanicola* sp. n. (Fig. 5b). Furthermore, the distal gonarcus is cone-shaped in *O. biangulus* but triangular in *O. maoershanicola* and the spermatheca is short and bent rod-like in *O. biangulus* (Fig. 2e) but approximately spherical in *O. maoershanicola* (Fig. 5g).

***Osmylus shaanxiensis* sp. n.**

http://zoobank.org/0815CDFE-15C7-4C26-AC13-46F03D535A44

Figs 6, 7

Material examined. Holotype Male. CHINA: Shaanxi (Province): Houzhenzi (town), [33°51'N, 107°50'E] 12.viii.2007, leg. Yang Shi. Verbatim label data (translated from Chinese): CHINA: Shaanxi, Houzhenzi/ 12.viii.2007/ Yang Shi/ CAU. Condition: Antennal flagellum missing. Terminalia cleared in KOH, and stored in the microvial pinned below the specimen. Paratype. 1 female, same data as holotype (CAU). 1 female, CHINA: Gansu (Province): Diebu (county), Lazikou. 1700m, [34°03'N, 103°54'E] 12.viii.1980, Chikun Yang (CAU).

Diagnosis. Wing broad, with numerous dark brown spots on the margin. Male: 9th tergite with a median narrowing, with a small tuberous dorsal process in lateral view; protuberance of posteroventral gonarcus papillary. Base of mediuncus knife-shaped in lateral view. Female: gonapophysis lateralis basally fused with a triangular sclerite, spermatheca bent, cylindrical.

Description. *Head*. Vertex dark brown. Ocelli yellow, area comprised among ocelli dark brown, eye dark brown; frons brown. *Thorax*. Pronotum dark brown with yellow long setae; meso- and metanotum dark brown. Legs yellow with dark yellow setae, pretarsal claws dark brown.

Wings (Fig. 6). Forewing length 22–25 mm, width 8–9 mm. Membrane hyaline, with numerous dark brown spots on the margin; pterostigma and nygmata brown; veins dark brown, some edged with dark brown spots; Rs with 12–13 branches, gradates cross-veins with brown marks. Hindwing length 20–22 mm, width 7–8 mm. Membrane hyaline; pterostigma light brown.

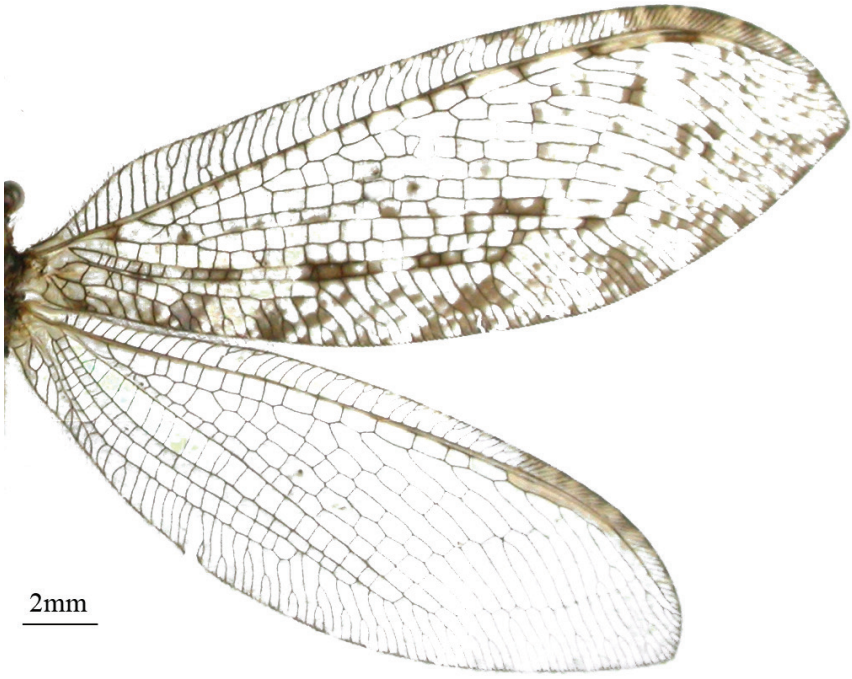


Figure 6. Wings of *Osmylus shaanxiensis* sp. n., forewing (upper) and hindwing (lower).

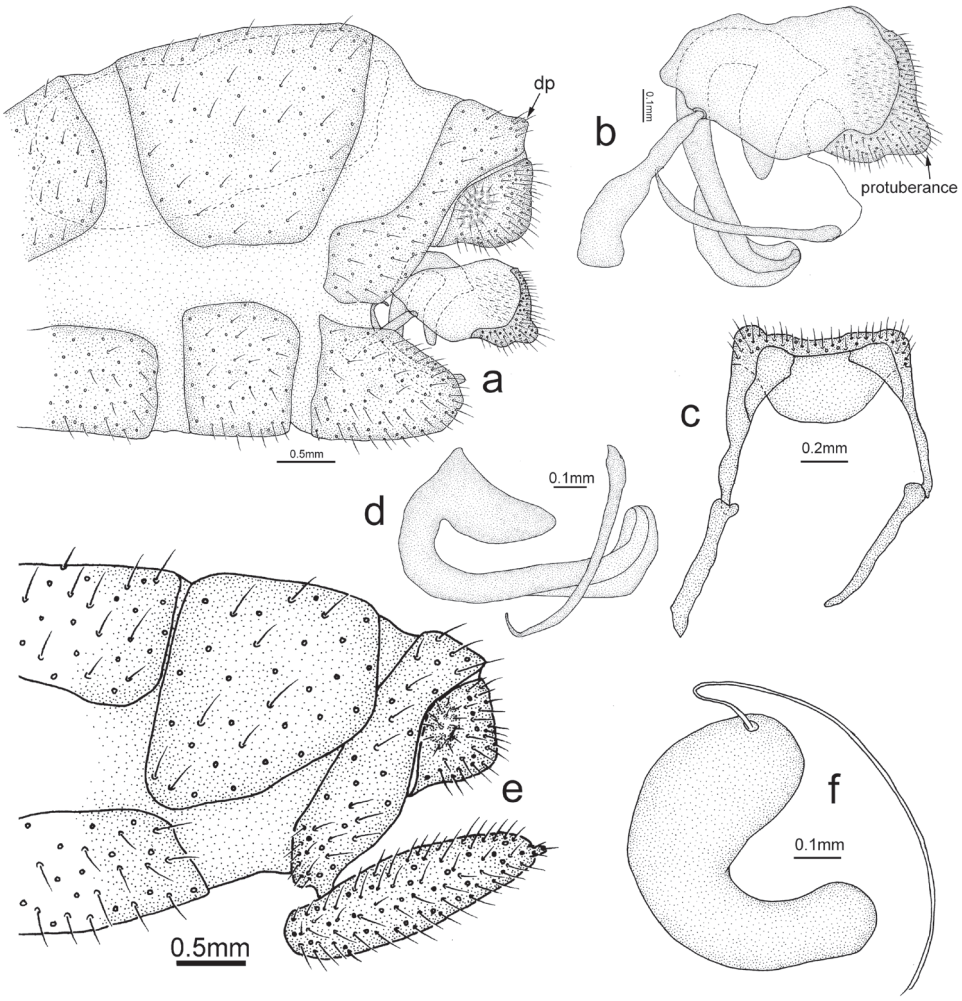
Male Terminalia (Fig. 7a–d). Scent glands stout; 9th tergite with a median narrowing in lateral view, with a small hemispheric dorsal process; ectoproct triangular in lateral view, callus cerci oval; gonarcus sclerotized distally and posteroventrally ending into a papilla in lateral view; anterior arm of gonarcus slender, basally dilated; mediuncus basally dilated, knife-shaped, more slender apically in lateral view; rod-shaped paramere slender and bent in lateral view, dilating from base to end.

Female Terminalia (Fig. 7e–f). 8th sternite reduced; 9th tergite narrow; ectoproct approximately conical, callus cerci round; gonapophysis lateralis fusiform, apex with a long finger-like stylus; spermatheca cylindrical, bent and slightly dilated basally.

Distribution. China (Shaanxi, Gansu).

Etymology. The specific name '*shaanxiensis*' refers to 'Shaanxi Province', the type locality.

Remarks. The new species can be distinguished from other species by the small hemispheric dorsal process of the 9th tergite in male (Fig. 7a). Although *O. shaanxiensis* sp. n. is similar to *O. conanus*, they can be easily separated by the differences of gonarcus and gonapophysis lateralis. The distal part of gonarcus in *O. conanus* protrudes slightly but the same part in *O. shaanxiensis* sp. n. protrudes significantly in lateral view (Fig. 7b). Also compared with *O. conanus*, the spermatheca in *O. shaanxiensis* sp. n. is longer and more bent (Fig. 7f).



Figures 7. *Osmylus shaanxiensis* sp. n. **a–d** Male: **a** apex of the abdomen and genitalia **b** genitalia, lateral view **c** genitalia, ventral view **d** mediuncus, lateral view **e–f** Female: **e** apex of the abdomen and genitalia **f** spermatheca, lateral view. Abbreviation: dp, dorsal process.

***Osmylus angustimarginatus* sp. n.**
http://zoobank.org/8018E2FE-CCBB-4BD5-8D9D-B567A84A097A
Figs 8, 9

Material examined. Holotype Male. CHINA: Chongqing: Jiangjin (District): Simianshan (mountain), [28°38'N, 106°24'E] 17.vi.2006, leg. Weiwei Zhang. Verbatim label data (translated from Chinese): CHINA: Chongqing, Jiangjin, Simianshan/ 17.vi.2006/ Weiwei Zhang/ PC. Terminalia cleared in KOH, and stored in a microvial pinned below the specimen. Paratype. 1 female, same data as holotype; 1 male, 1 female, same locality as holotype. 21-23.ix.2007, leg. Weiwei Zhang.

Figure 8. Wings of *Osmylus angustimarginatus* sp. n., forewing (upper) and hindwing (lower).

Diagnosis. Male: 9th with a finger-like dorsal process. Gonarcus distally triangular in lateral view, ventral margin well sclerotized; base of mediuncus slightly protuberant distally in lateral view. Female: gonapophysis lateralis finger-like; spermatheca approximately spherical.

Description. *Head*. Vertex yellow brown, with dark brown setae; ocelli light yellow, area comprised among ocelli dark brown; eyes gray with metallic reflection; frons black. *Thorax.* Pronotum dark brown, with yellow setae; meso- and metanotum fuscous, with black stripes. Legs yellow, with short setae, pretarsal claws dark brown.

Wings (Fig. 8). Forewing length 27–29 mm, width 8–9 mm. Wings elongated; membrane hyaline, with numerous brown spots; pterostigma brown, nygmata light brown; veins dark brown, some edged with dark brown spots; Rs with 13–14 branches; cross-veins are present among branches of CuP. Hindwing length 25–26 mm, width 7–8 mm; membrane hyaline; pterostigma light brown.

Male Terminalia (Fig. 9a–e). Scent glands stout. 9th tergite wide, with a finger-like process; 9th sternite approximately rectangular in lateral view; ectoproct small, callus cerci round; gonarcus distally well sclerotized and triangular in lateral view, ventral margin well sclerotized; anterior arm of gonarcus slender and basally dilated; mediuncus slightly finger-like at base, more slender apically in lateral view; rod-shaped paramere beneath the mediuncus slightly bent in lateral view.

Female Terminalia (Fig. 9f–h). 8th sternite approximately square in lateral view. 9th tergite narrow; ectoproct conical, callus cerci round; gonapophysis lateralis long and finger-like, with a long conical stylus; spermatheca approximately spherical.

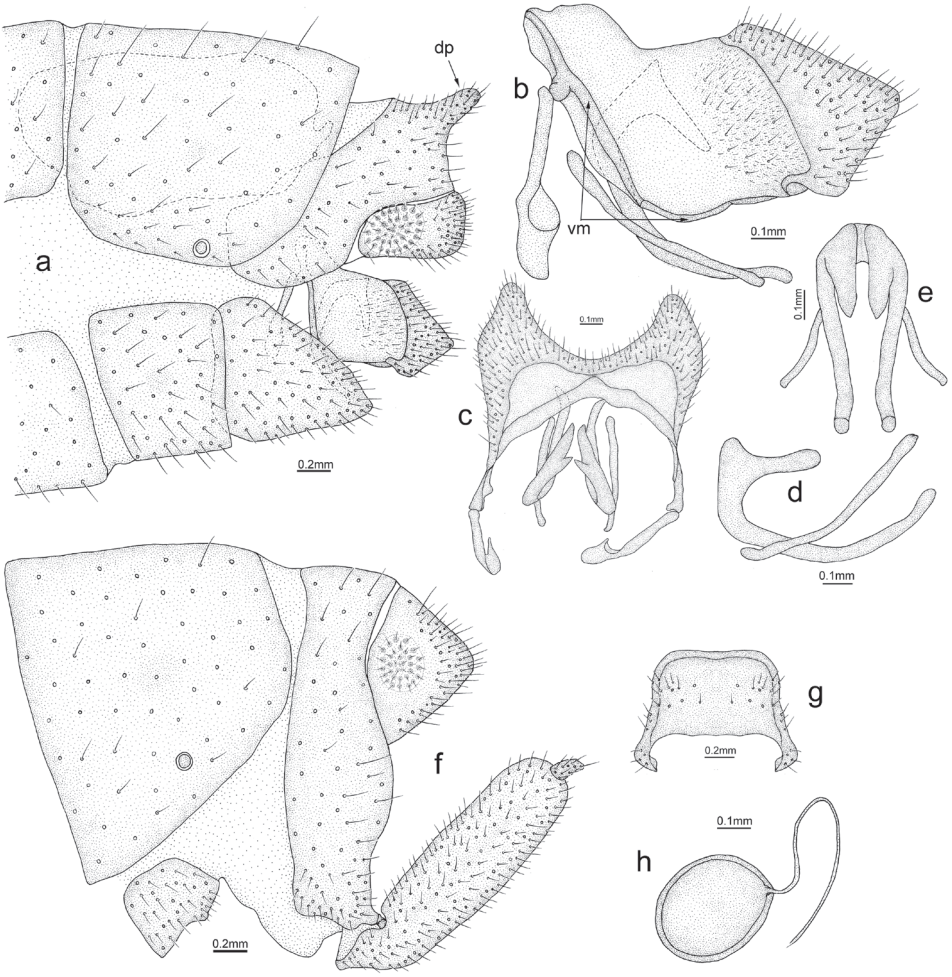


Figure 9. *Osmylus angustimarginatus* sp. n. **a–e** Male: **a** apex of the abdomen and genitalia **b** genitalia, lateral view **c** genitalia, dorsal view **d** mediuncus, lateral view **e** mediuncus, dorsal view **f–h** Female: **f** apex of the abdomen and genitalia **g** subgenital plate, ventral view **h** spermatheca, lateral view. Abbreviations: dp, dorsal process; vm, ventral margin.

Distribution. Known only from Chongqing, China.

Etymology. The specific name '*angustimarginatus*' the compound of Latin deribation, from *angusti-* (narrow) and *marginatus-* (margin), refers to the well sclerotized ventral margin of the gonarcus in lateral view.

Remarks. The dorsal process of 9th tergite in the male of *O. angustimarginatus* sp. n. is finger-like (Fig. 9a), closely resembling the condition observed in *O. maoershanicola* sp. n. However, the ventral margin of the gonarcus of *O. angustimarginatus* sp. n. is well sclerotized (Fig. 9b), clearly differentiating it from *O. maoershanicola* sp. n. Moreover, female gonapophysis lateralis of *O. angustimarginatus* sp. n. is more slender in comparison with the fusiform gonapophysis lateralis of *O. maoershanicola* sp. n. (Fig. 5f).

Acknowledgements

We sincerely thank Zhang Weiwei (Beijing) for collecting the specimens. This research is supported by the National Natural Science Foundation of China (grants 31272352, 31301905 and 41372013), the China Postdoctoral Science Foundation (grant 2012T50113), the Beijing Natural Science Foundation (grant 5132008), and Ph.D. Programs Foundation of Ministry of Education of China (grant 20131108120005).

References

Adams PA (1969) A New Genus and Species of Osmylidae [neuroptera] from Chile and Argentina, with a Discussion of Planipennian Genitalic Homologies. Postilla 141: 1–11. doi: 10.5962/bhl.part.24591

Banks N (1913) Synopses and descriptions of exotic Neuroptera. Transaction of the American Entomological Society 39: 201–242. http://www.jstor.org/stable/25076911

Banks N (1947) Some neuropterous insects from Szechwan, China. Fieldiana: Zoology, Chicago Natural History Museum 31: 99. doi: 10.5962/bhl.title.2821

Canbulat S (2013) Redescription of *Osmylus multiguttatus* McLachlan, 1870 (Neuroptera: Osmylidae) with distributional remarks. Zootaxa 3741(3): 385–390. doi: 10.11646/zootaxa.3741.3.7

Devetak D, Duelli P (2007) Intestinal contents of adult *Osmylus fulvicephalus* (Scop.) (Neuroptera, Osmylidae). Annales for Istrian and Mediterranean Studies, Series Historia Naturalis 17: 93–98. http://www.dlib.si/stream/URN:NBN:SI:DOC-5XYMHHIP/f7db0a9c-547e-4e38-8bf6-2bc28353e082/PDF

Eisner M (1989) Biologie und Larvalmorphologie der wasserlebenden Neuropteren Mitteleuropas (Neuropteroidea; Megaloptera, Planipennia). PhD Thesis, University Graz, Graz.

Iwata M (1928) A new hemerobiid larva (Neuroptera), *Osmylus kisoensis* n. sp. Kontyû 2: 215–220. http://lacewing.tamu.edu/neuropterida/neur_bibliography/edoc12/iwata1928ref3111-340.pdf

Kozhanchikov IV (1951) New Asiatic species of Osmylidae (Neuroptera). Entomologicheskoe Obozrenie 31: 523–528.

Krüger L (1912) Osmylidae. Beiträge zu einer Monographie der Neuropteren-Familie der Osmyliden. I. *Osmylus chrysops* L. Stettiner Entomologische Zeitung 73: 319–373. http://lacewing.tamu.edu/neuropterida/neur_bibliography/edoc12/kruger1912ref3464s-2148.pdf

Krüger L (1913) Osmylidae. Beiträge zu einer Monographie der Neuropteren-Familie der Osmylidae. Stettiner Entomologische Zeitung 74: 3–123. http://lacewing.tamu.edu/neuropterida/neur_bibliography/edoc12/kruger1913ref3465s-2253.pdf

Kuwayama S (1953) The insect fauna of Mt. Ishizuchi and Omogo Valley, Iyo, Japan: Neuroptera. Transaction of Shikoku Entomological Society 3: 177–180.

Kuwayama S (1962) A revisional synopsis of the Neuroptera in Japan. Pacific Insects 4: 325–412.

Latreille PA (1805) Histoire naturelle, générale et particulière des crustacés et des insectes (Vol. 13). F. Dufart, Paris, 432 pp. http://lacewing.tamu.edu/neuropterida/neur_bibliography/edoc12/latreille1805ref3639-5194.pdf

Makarkin VN (1985) Review of the family Osmylidae (Neuroptera) of the fauna of the USSR. In: Lehr PA, Storozhenko SYu (Eds) Taxonomy and ecology of arthropods from the Far East. Far Eastern Scientific Centre, Vladivostok, 35–47.

McLachlan R (1870) New species etc. of Hemerobiina-second series (*Osmylus*). Entomologist's Monthly Magazine 6: 195–201. http://lacewing.tamu.edu/neuropterida/neur_bibliography/edoc12/mclachlan1870ref350-345.pdf

McLachlan R (1875) A sketch of our present knowledge of the neuropterous fauna of Japan (excluding Odonata and Trichoptera). Entomological Society of London 23: 179–181. doi: 10.1111/j.1365-2311.1875.tb01906.x

Nakahara W (1914) On the Osmylinae of Japan. Annotationes Zoologicae Japonenses 8: 489–518.

Navás L (1910) *Osmylides exotiques* (insectes névroptères) nouveaux. Annales de la Société Scientifique de Bruxelles 34: 188–195. http://lacewing.tamu.edu/neuropterida/neur_bibliography/edoc12/navas1910ref517-347.pdf

New TR (1983) A revision of the Australian Osmylidae: Kempyninae (Insecta: Neuroptera). Australian Journal of Zoology 31: 393–420. doi: 10.1071/ZO9830393

New TR (1991) Osmylidae (Insecta: Neuroptera) from the Oriental Region. Invertebrate Taxonomy 5: 1–31. doi: 10.1071/IT9910001

Sekimoto S (2011) Revision of the genus *Osmylus* (Neuroptera: Osmylidae: Osmylinae) of Japan. Insecta matsumurana. Series entomology. New series 67: 1–22. http://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/47450/1/01%20Sekimoto.pdf.pdf

Stein F (1838) Entwicklungs-Geschichte mehrere Insektengattungen aus der Ordnung der Neuropteren. Archiv für Naturgeschichte 4: 315–333.

Von Johann GEPP (1976) Die Neuropteren von Graz: Ein Beitrag zur Kenntnis der mitteleuropäischen Großstadtfauna.-Mitt. Naturwiss. Ver. Steiermark 105: 265–278. http://www.zobodat.at/pdf/MittNatVerSt_105_0265-0278.pdf

Wang YJ, Liu ZQ (2009) Two new species of *Parosmylus* Needham (Neuroptera, Osmylidae) from China, with a key to Chinese species. Zootaxa 1985: 57–62. http://mapress.com/zootaxa/2009/f/z01985p062f.pdf

Wang YJ, Liu ZQ (2010) New species of *Osmylus* Latreille from Henan, China (Neuroptera: Osmylidae). Zootaxa 2363: 60–68.

Withycombe CL (1923) XX – Notes on the Biology of some British Neuroptera (Planipennia). Transactions of the Royal entomological Society of London 70(3–4): 501–594. doi: 10.1111/j.1365-2311.1923.tb02844.x

Yang CK (1987) Neuroptera. In: Zhang S (Ed.) Agricultural insects, spiders, plant diseases and weeds of Xizang. Vol. 1. Xizang Renmin Press House, Tibet, China, 191–194.

Yang CK (1988) Neuroptera: Osmylidae, Dilaridae, Hemerobiidae, Chrysopidae, Mantispidae, Myrmeleontidae, Ascalaphidae, Corydalidae. In: Huang FS (Ed.) Insects of Mt. Namjagbarwa region of Xizang. Science Press, Beijing, China, 195–196.

Yang CK (1997) Neuroptera: Osmylidae. In: Yang XK (Ed.) Insects of the Three Gorge Reservoir Area of Yangtze River. Chongqing Press, Chongqing, China, 580–581.

Yang CK (1999) Osmylidae. In: Huang BK (Ed.) Fauna of Insects Fujian Province of China Vol. 3. Fujian Science and Technology Press, Fuzhou, China, 96–98.

ZooKeys 589: 123–142 (2016)
doi: 10.3897/zookeys.589.7847
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

The morphology of the immature stages of *Metadonus vuillefroyanus* (Capiomont, 1868) (Coleoptera, Curculionidae, Hyperini) and notes on its biology

Jiří Skuhrovec[1], Petr Bogusch[2]

1 *Group Function of Invertebrate and Plant Biodiversity in Agrosystems, Crop Research Institute, Prague 6–Ruzyně, Czech Republic* **2** *Department of Biology, Faculty of Science, University of Hradec Králové, Rokitanského 62, CZ-500 03 Hradec Králové, Czech Republic*

Corresponding author: *Jiří Skuhrovec* (jirislavskuhrovec@gmail.com)

Academic editor: *M. Alonso-Zarazaga* | Received 21 January 2016 | Accepted 16 April 2016 | Published 16 May 2016

http://zoobank.org/8C00DA62-61C1-4387-B0B9-0A617D670D75

Citation: Skuhrovec J, Bogusch P (2016) The morphology of the immature stages of *Metadonus vuillefroyanus* (Capiomont, 1868) (Coleoptera, Curculionidae, Hyperini) and notes on its biology. ZooKeys 589: 123–142. doi: 10.3897/zookeys.589.7847

Abstract

Last instar larva and pupa of *Metadonus vuillefroyanus* (Capiomont, 1868) (Curculionidae: Hyperini) are described and compared with known larvae of the other 43 hyperine taxa. The thorn-like setae located on distinct black protuberances on the larval body are characteristic features of the genus *Metadonus* and the subgenus *Eririnomorphus* of the genus *Hypera*. The biological singularity of this species was studied and described. The variable colouration of larvae has been confirmed in association with the variability of the host plant's colouration at the studied localities. This species' reported inability to spin cocoons has been disproven. A different type of cocoon with two layers, where the inner layer consists of proteins from Malpighian tubules while the outer layer contains soil particles, is described. This type of cocoon is unique compared with those known from other hyperines, which usually pupate on or above the ground and do not use substrate particles in building their cocoons.

Keywords

Weevil, mature larva, pupa, larval development, life cycle, host plant, *Suaeda vera*, Amaranthaceae, Spain, Palaearctic region

Introduction

The phylogeny and taxonomy of hyperines is still unresolved. Recently, hyperines together with Bagoini and Gonipterini have been considered unclassifiable tribes in Curculionidae (Oberprieler et al. 2014). However, the hyperines have been also treated as other groups; e.g. a subfamily Hyperinae (e.g., Thompson 1992; Zimmerman 1992; Alonso-Zarazaga and Lyal 1999; Anderson 2002; Marvaldi et al. 2002; Marvaldi and Lanteri 2005; Bouchard et al. 2011); within a subfamily Brachycerinae (Kuschel 1995); as a tribe of Curculioninae (Oberprieler et al. 2007; McKenna et al. 2009); as a tribe of Entiminae (Legalov 2011a, b); and finally, in a clade that also included Entiminae, Cyclominae and Gonipterini (Hunt et al. 2007; Hundsdoerfer et al. 2009; McKenna et al. 2009; Gunter et al. 2015).

Hyperines have characteristic shapes, but the genera recently included in the tribe have so far shown no distinct diagnostic or synapomorphic characters that would permit a satisfactory concept of Hyperini (Oberprieler et al. 2014) to be drawn up. Their only unique feature appears to be the peculiar meshed cocoon spun by the larvae from strands of protein secreted by the Malpighian tubules (Scherf 1964; Kenchington 1983). The latest attempt to define this group was conducted by Petri (1901), who defined them using ten features. However, of these, only the characters of the trochanters, claws and pygidium hold true, and none of these are unique to Hyperini (Oberprieler et al. 2014). Petri (1901) divided the Hyperini into two subtribes, Hyperina and Cepurina, based on the shape of the mesepimera and the length of the metanepisterna and the relative width and angle of their junction with the mesepimera. Legalov (2007, 2010, 2011) classified this tribe into five subtribes: Cepurina, Hyperina, Coniatina, Macrotarrhusina and Phaeopholina, based on several morphological characters, but such a distinction requires a more comprehensive study of the whole tribe and is equally unlikely to yield meaningful synapomorphies to identify family group taxa within the group (Oberprieler et al. 2014). Oberprieler et al. (2014) and Skuhrovec (unpublished data) recently divided this tribe into three "operating" groups with different distributions: (1) the Palaearctic region (Hyperina), (2) the circumtropical region (Cepurina), and (3) the Australian/Pacific region (Australian Hyperini and *Phaeopholus* Roelofs, 1873).

Several recent taxonomic studies deal with the Palaearctic fauna of Hyperini. Skuhrovec (2005a, 2005b, 2006a, 2006b, 2007) studied the larvae of *Donus* Jekel, 1865 and *Hypera* Germar, 1817, he clarified (2008) the complex nomenclature of the large and important genera *Brachypera* Capiomont, 1868, *Donus* and *Hypera*, and revised (2012) the genus *Metadonus* Capiomont, 1868. Alonso-Zarazaga and Lyal (2002) transferred the monotypic genus *Herpes* Bedel, 1874, previously classified in Brachycerinae or Rhythirrinini but in Thecesternini by Alonso-Zarazaga and Lyal (1999), to Hyperini. Legalov (2011a) recently resurrected a number of subgenera of *Coniatus* Germar, 1817, *Hypera* and *Macrotarrhus* Bedel, 1906 to generic status, but these taxonomic acts were published without detailed justification, and this is the main reason why Skuhrovec (2013a) and Oberprieler et al. (2014) did not accept these

taxonomic changes. A detailed comparative study of hyperine immature stages is also necessary in this context because most larval and pupal characters are only known from the relatively well-studied genera *Brachypera*, *Donus*, *Hypera* and *Phelypera* Jekel, 1865 (Oberprieler et al. 2014), and the larvae of only a few other genera have been described (e.g., *Fronto* Petri, 1901, *Metadonus* and *Macrotarrhus*) (Zaslavskij 1959).

The genus *Metadonus* has been revised recently (Skuhrovec 2012) and now includes 10 species, all of which are known to be native to the Palaearctic region. They occur primarily in Asia, but exceptions include *Metadonus vuillefroyanus*, which is found in Spain, Morocco and Algeria, and *Metadonus anceps* (Boheman, 1842) and *Metadonus distinguendus* (Boheman, 1842), which occur in Ukraine, Moldavia, Romania, Turkey and Russia (Skuhrovec 2012, 2013b). Species of this genus live in extreme conditions (such as cold steppes, salinas and semi-deserts) (Skuhrovec 2012). Biological notes about host plants are known only for *Metadonus vuillefroyanus*, *M. distinguendus* and *M. anceps* (Skuhrovec 2008, 2012; Korotyaev et al. 2016). Velázquez de Castro et al. (2000) listed the first biological data about *Metadonus vuillefroyanus*, which occurs in salt wetlands; its host plant is *Suaeda vera* (synonym *S. fruticosa*) (Amaranthaceae) (Skuhrovec 2012).

The immature stages of *Metadonus vuillefroyanus* are here described for the first time. Knowledge of the immature stages and the life history of a species are important for both taxonomic and applied use and can help protect this species more effectively. Taking into account the information gathered by the first author about the biology of immature stages and adults of *Metadonus vuillefroyanus*, the second author undertook a study trip to Spain. In the present paper we provide biological data based on Bogusch's observations obtained during his field work in Spain, and we describe the immature stages of this species.

Materials and methods

The material used to describe the immature stages was collected, and field observations were conducted in the following localities: **SPAIN: Almería:** Cabo de Gata National Park, Cabo de Gata, Salinas, surroundings of salt marshes (36°46'48"N, 2°13'44"W, 2 m), 29-III-2014, 1 ♂ and 15 larvae swept from *Suaeda vera*; 31-III-2014, 5 larvae swept from *Suaeda vera*; Tabernas env., river valley (37°02'57"N, 2°24'28"W, 339 m), 1-IV-2014, 2 mature larvae swept from *Suaeda vera*, all P. Bogusch and A. Astapenková leg., P. Bogusch det., revised by J. Skuhrovec, in the collections of P. Bogusch and J. Skuhrovec. Descriptions of immature stages were done on four larvae and two pupae.

Part of the larval and pupal material was preserved in Pampel fixation liquid (4 parts glacial acetic acid, 6 parts 4% formaldehyde, 15 parts 95% ethyl alcohol and 30 parts distilled water) and used for the morphological descriptions. These specimens are now deposited in the Group Function of Invertebrate and Plant Biodiversity in Agrosystems of the Crop Research Institute (Prague, Czech Republic). Plants were identified by the collectors. To prepare the slides we followed May (1994). The head

of the larva was separated and cleared in a 10% potassium hydroxide (KOH) solution and then rinsed in distilled water. After clearing, the mouth parts were separated from the head capsule. The head capsule and all mouth parts were mounted on permanent microscope slides in Euparal. All other body parts were mounted on temporary microscope slides in 10% glycerine.

The observations and measurements were made using a light microscope with calibrated oculars (Olympus BX 40 and Nikon Eclipse 80i). The following measurements were taken for each larva: head width, length of the body (larvae fixed in a C-shape were measured along segments), width of the body in the widest place (metathorax or abdominal segments I–IV), and these for each pupa: length and width at the widest place. The thorax and abdomen were not sclerotised, and it is unlikely that the fixation process altered the weevils' proportions; measurements of these parts are given for comparison purposes only.

Drawings were made with a drawing tube on a light microscope and processed using a computer program (Adobe Photoshop, Corel Photo-Paint 11, GIMP 2). The thoracic spiracle is placed on the prothorax near the boundary of the prothorax and mesothorax, as shown in the drawing (see Fig. 8), but it is of mesothoracic origin (Marvaldi et al. 2002; Marvaldi 2003). The drawings show the thoracic and abdominal spiracles (see Figs 8–10). The numbers of setae of the bilateral structures are given for one side.

We used the terms and abbreviations for the setae of the mature larva and pupa studied following Scherf (1964), May (1977, 1994) and Marvaldi (1998a, 1999).

Results

Metadonus vuillefroyanus (Capiomont, 1868)

Phytonomus vuillefroyanus Capiomont, 1868: 135

Description of mature larva. *Measurements* (in mm). Body length: 10.0–14.0 (mean 12.0). The widest place in the body (abdominal segments II–VI) measures up to 2.5. Head width: 0.9–1.1 (mean 1.0).

Colouration. Dark brown to black head (Fig. 7). All thoracic and abdominal segments greenish with white longitudinal stripes on both sides of body, but this larva also has a thick longitudinal yellow stripe and parallel longitudinal pink to violet stripes in its dorsal part with small black short stripes inside; all setae are thorn-like, located on distinct black protuberances in very thin white transversal lines (Figs 7–10, 16).

Vestiture. Body elongated, slightly curved, rounded in cross section (Fig. 7). Setae on body thin, different in length (short to relatively long), black thorn-like, located on distinct black protuberances.

Head capsule (Fig. 1). Head suboval, flattened laterally, endocarinal line absent. Frontal sutures on head distinct, extended to antennae. Two stemmata (st), in the form

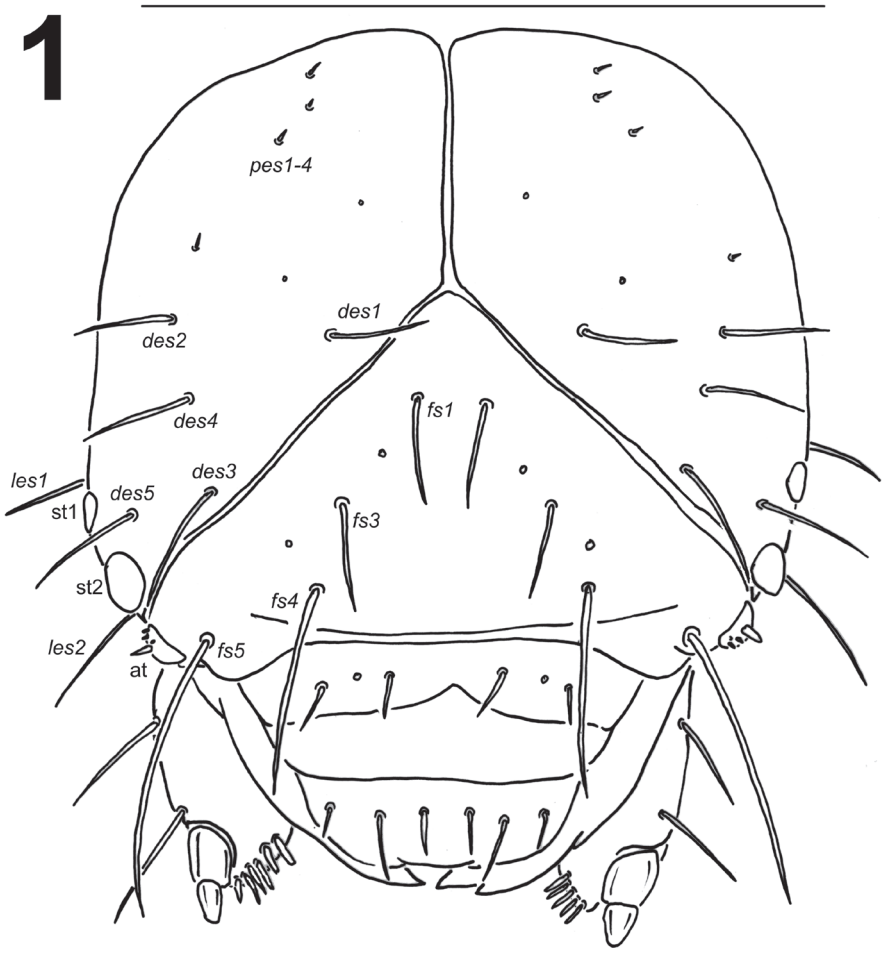
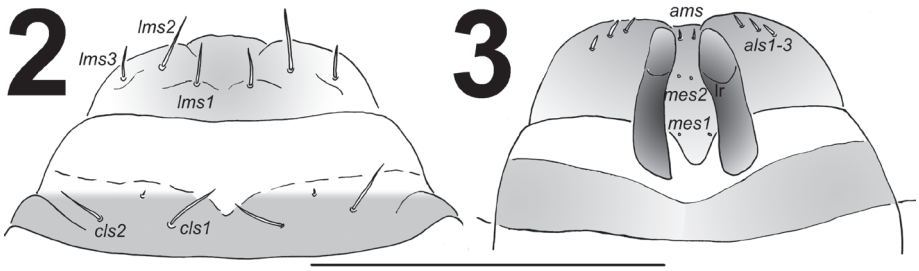


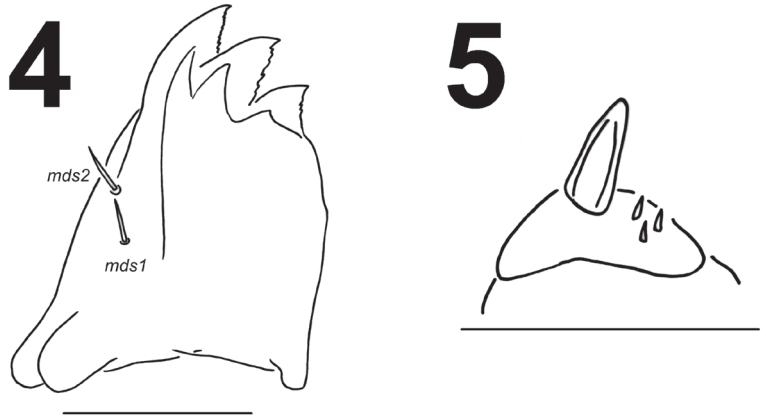
Figure 1. *Metadonus vuillefroyanus* mature larva head, dorsal view. Scale bar: 1 mm.

of a dark pigmented spot with convex cornea, both located on each side anterolaterally, close to each other. *Des1* and *des2* located in upper part of the central part of epicranium, *des1* near to the middle part of epicranium, and *des2* near to side of epicranium, *des3* located anteriorly on epicranium near to frontal suture, *des4* located in the central part of epicranium, *des5* located anterolaterally; all *des* very long, *des3* and *des5* slightly longer than remaining three setae (Fig. 1). *Fs1* and *fs3* placed medially, *fs2* absent, *fs4* located anterolaterally, and *fs5* located laterally, close to the epistoma; all setae long to very long, *fs5* distinctly longer than the remaining four setae (Fig. 1). *Les1–2* as long as *des1*; *ves1–2* short. Epicranial area with four postepicranial setae (*pes1–4*) and two sensilla.

Antennae located at the end of the frontal suture on each side, membranous and slightly convex basal article bearing one conical triangular sensorium, relatively long; basal membranous article with three sensilla different in both shape and length (Fig. 5).



Figures 2–3. *Metadonus vuillefroyanus* mature larva. **2** Labrum and clypeus **3** Epipharynx. Scale bar: 0.5 mm.



Figures 4–5. *Metadonus vuillefroyanus* mature larva head. **4** Right mandible **5** Antenna. Scales bars: 0.2 mm (**4**) and 0.1 mm (**5**).

Clypeus (Fig. 2) approx. 3 times as wide as long with two relatively long *cls*, almost equal in length, localized posterolaterally, and one sensillum; anterior margin rounded to the inside; median part covered by thorn-shaped cuticular processes.

Mouth parts. Labrum (Fig. 2) approximately 3.2 times as wide as long, with three pairs of piliform *lms*, of different lengths; *lms3* distinctly shorter than longer *lms1* and *lms2*; *lms1* placed close to the margin with clypeus, *lms2* located anteromedially and *lms3* located anterolaterally; anterior margin double sinuate. Epipharynx (Fig. 3) with three very short, piliform *als*, almost equal in length; one very short piliform *ams*; *mes* not distinct (but apparently there are two setal bases that may correspond to two small setae, not drawn); labral rods (lr) slightly elongated, sub-oval, apical part more sclerotised. Mandibles (Fig. 4) distinctly broad, trifid, tooth of unequal height; slightly truncate; both *mds* relatively long, piliform, located in distinct holes. Maxilla (Fig. 6) stipes with one *stps*, two *pfs* and one *mbs*, *stps* and *pfs1–2* very long, *pfs1* distinctly shorter than *pfs2*, *mbs* very short; mala with six bacilliform *dms*; five very short to minute

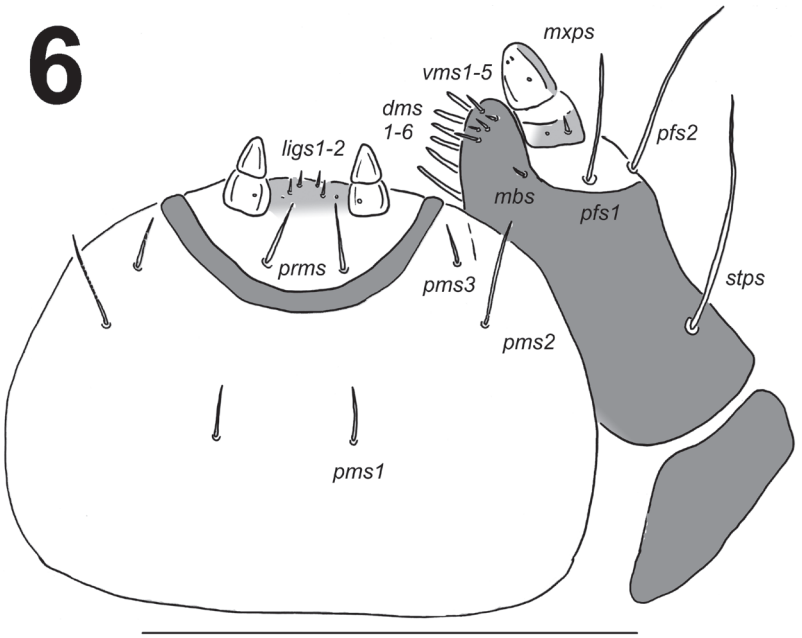


Figure 6. *Metadonus vuillefroyanus* mature larva head, maxillo-labial complex, ventral view. Scale bar: 0.5 mm.

vms, almost equal in length; *vms* distinctly shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with one very short *mxps* and two sensilla; length ratio of basal and distal palpomeres: 1:0.8; distal palpomere with one sensillum and a group of conical, cuticular apical processes. Praelabium (Fig. 6) oval-shaped and distinctly elongated, with one relatively long *prms*; ligula with sinuate margin and two piliform very short to minute *ligs*, unequal in length; premental sclerite well visible. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres: 1:0.9; distal palpomere with one sensillum and short, cuticular apical processes; basal palpomere with one dorsal sensillum. Postlabium (Fig. 6) with three *pms*, *pms1* located anteriorly, remaining two pairs laterally; *pms1* and *pms3* relatively long, *pms2* distinctly longer than others; surface of postlabium partly covered by cuticular processes.

Thorax. Prothorax distinctly smaller than meso- and metathorax. Spiracle bicameral, placed between the pro- and mesothorax (see Material and methods). Prothorax (Fig. 8) with 11 short to minute *prns* unequal in length, only 3 of them on small weakly pigmented dorsal sclerite, this sclerite subdivided in two triangular plates medially; two short *ps* and one short *eus*. Mesothorax (Fig. 8) with one short and one minute *prs*; four short *pds*; two short *as*; two very short *ss*; one short *eps*; one short and one minute *ps*; and one short and one minute *eus*. Chaetotaxy of metathorax (Fig. 8) almost identical to that of mesothorax. Each pedal area of thoracic segments well separated, with five short to minute *pda*, three of them on pigmented pedal area, unequal in length.

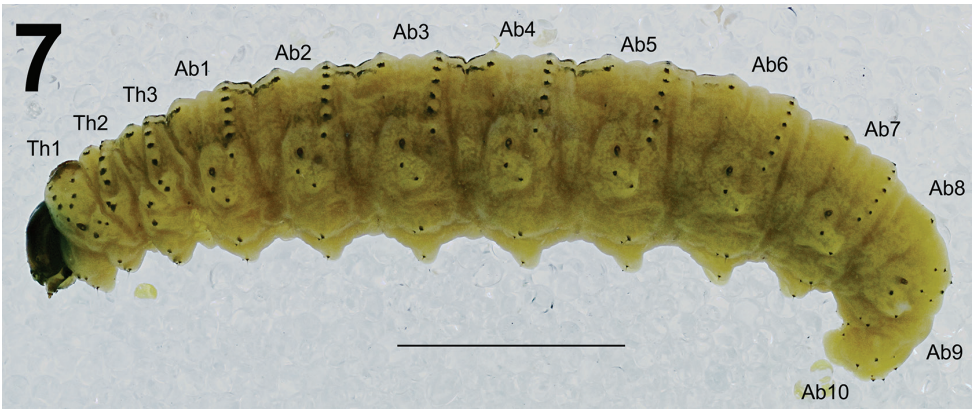


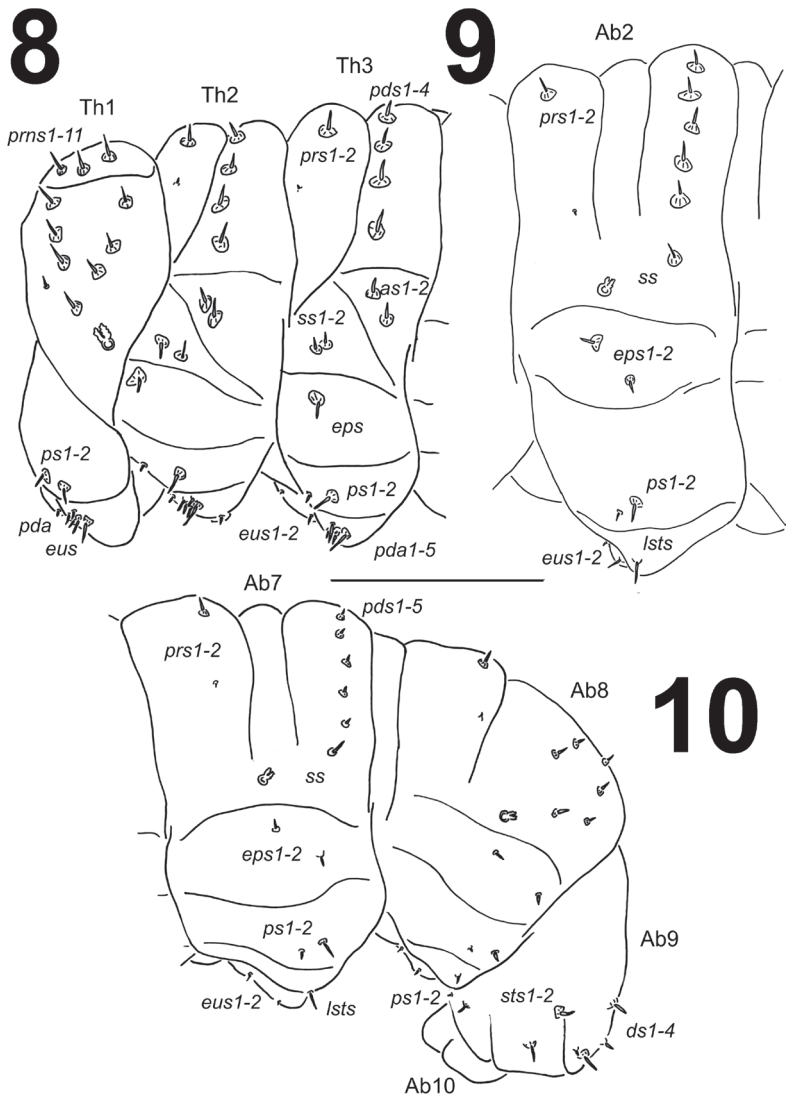
Figure 7. *Metadonus vuillefroyanus* mature larva habitus, lateral view. Scale bar: 3 mm.

Abdomen. Abdominal segments I–VI of almost equal length, next abdominal segments decreasing gradually to the terminal parts of the body. Abdominal segment X reduced to four anal lobes of unequal size, the dorsal being distinctly the largest, the lateral pair equal in size, and the ventral lobe very small. Anus located terminally; ambulatory ampullae bilobate to circular. Spiracles bicameral, the eight abdominal spiracles located laterally, close to the anterior margin of abdominal segments I–VIII. Abdominal segments I–VII (Figs 9–10) with one short and one minute *prs*; five short *pds*; one short *ss*; two short to very short *eps* of almost equal length; one short and one minute *ps*; one short *lsts*; one short and one minute *eus*. Abdominal segment VIII (Fig. 10) with one very short and one minute *prs*; five very short *pds*, *pds2* and *pds4* not in line; one very short *ss*; two very short *eps* of almost equal length; one very short and one minute *ps*; one very short *lsts*; and one very short and one minute *eus*. Abdominal segment IX (Fig. 10) with four *ds* (two *ds* very short, two *ds* minute); two very short *ps*; and one very short and one minute *sts*. Abdominal segment X (Fig. 10) without setae.

Description of pupa. *Measurements* (in mm). Body length: 7.0–8.0 (♂ 8.0; ♀ 7.0); at the widest region: 4.5–5.0. The widest place in the body is commonly between the apex of the meso- or metafemora. Unfortunately, both pupae were damaged and the measurements are not precise.

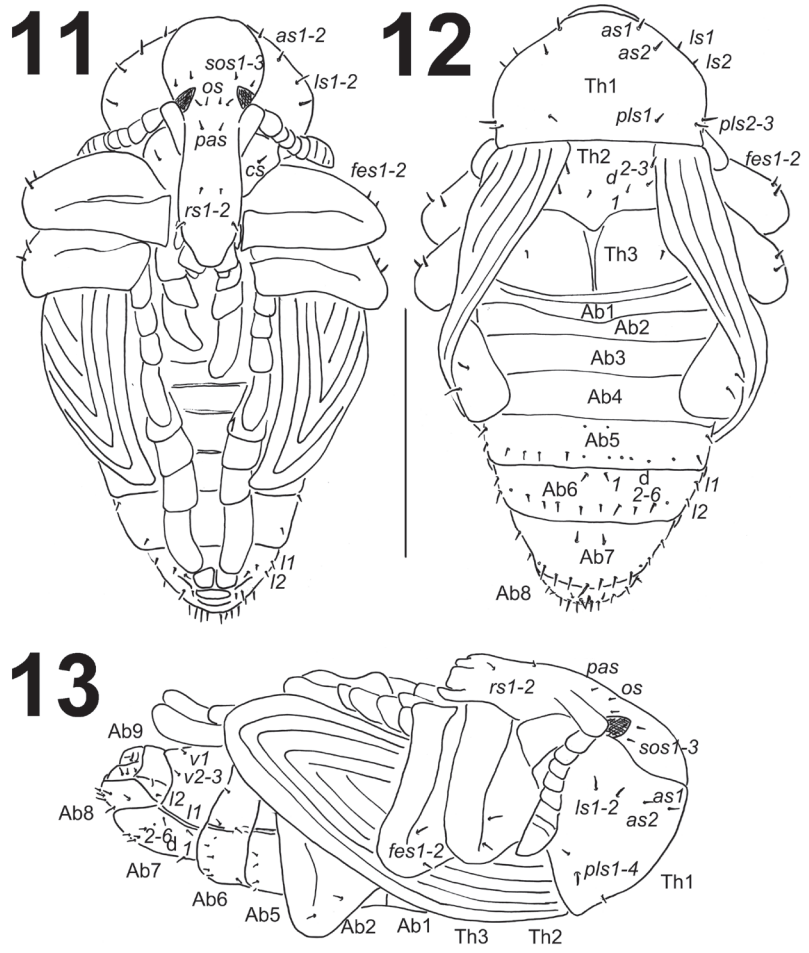
Colouration. Body yellowish with greenish abdomen (Figs 17, 19).

Morphology (Figs 11–13). Body stocky, cuticle smooth. Rostrum relatively long, approximately 2.5 times as long as wide, extended to metacoxae. Antennae relatively long and stout. Pronotum from 1.7 to 1.8 times as wide as long. Mesonotum and metanotum of almost equal length. Abdominal segments I–IV of almost equal length; abdominal segment V semi-circular, next abdominal segments diminish gradually to the end of the body. Abdominal segments VI–IX distinctly smaller than other abdominal segments. Gonotheca (abdominal segment IX) in females (one specimen) divided. Sexual dimorphism in weevil pupae is visible mainly in the length of rostrum and in the structure of abdominal segment IX: gonotheca of ♂ undivided and divided in ♀.



Figures 8–10. *Metadonus vuillefroyanus* mature larva habitus. **8** Lateral view of thoracic segments **9** Lateral view of abdominal segment II. **10** Lateral view of abdominal segments VII–X. Scale bar: 1 mm.

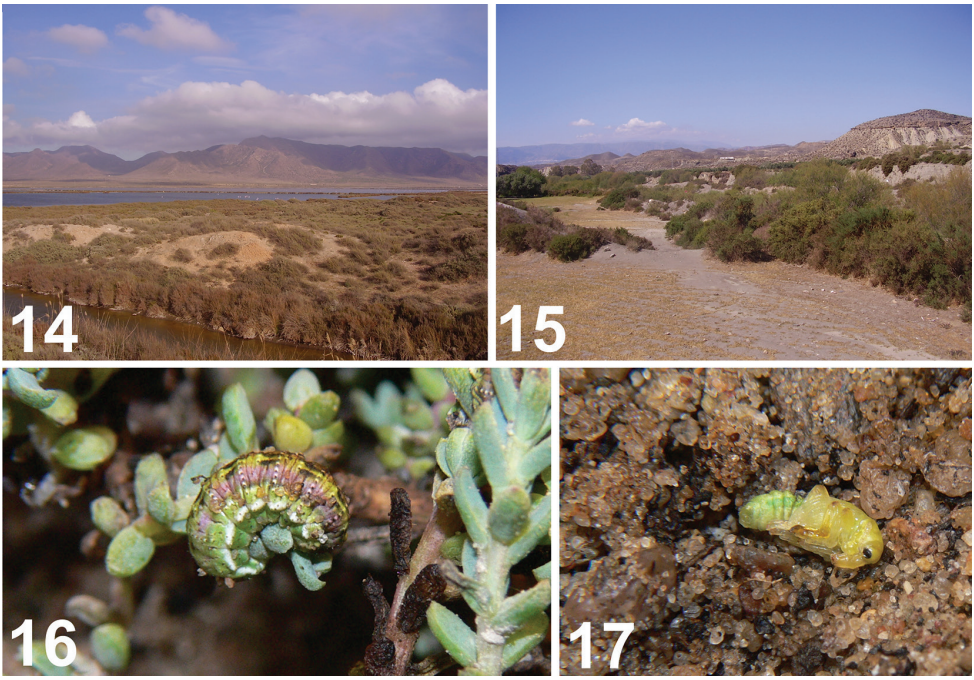
Chaetotaxy (Figs 11–13). Setae short to very short, unequal in length, light yellow, orange up to black. Setae well visible. Unfortunately, both pupae were damaged and it was not possible to observe chaetotaxy on some parts of body. Head capsule includes only three *sos*, one *os* and one *pas*. Rostrum with two *rs*. Setae on head capsule straight, as short as the remaining setae on thoracic and abdominal segments. Pronotum with two *as*, two *ls* and three *pls*, *ds* not observed. Dorsal parts of mesothorax with one seta located posteromedially, one seta posterolaterally and one seta located along its anterior



Figures 11–13. *Metadonus vuillefroyanus* pupa habitus. **11** Ventral view **12** Dorsal view **13** Lateral view. Scale bar: 3 mm.

margin. Dorsal parts of metathorax with one seta located along its anterior margin. Coxa with one very short seta (*cs*). Each apex of femora with groups of two *fes*. Abdominal segments I–V damaged, and it was not possible to observe setae. Dorsal parts of abdominal segments VI–VIII each with one seta located posteriorly (*d1*) and five pairs (*d2–6*) located along their anterior margins; setae short, hair-like. Abdominal segments V–VIII with groups of two lateral setae and one (or two) ventral setae. Abdominal segment IX with two ventral microsetae and two short, thin setae. Pseudocerci absent.

Biological notes. *Habitats*. *Metadonus vuillefroyanus* occurs only at saline sites in the presence of its host plant. This species has been observed both at saline sites near the sea (locality Cabo de Gata less than 1 km from the seashore, Fig. 14) and at inland saline sites (Tabernas is approximately 50 km inland from the sea, Fig. 15). The population density of the host plant was not high at these localities; at Cabo de Gata



Figures 14–17. Habitat and immature stages of *Metadonus vuillefroyanus*. **14** Locality Cabo de Gata **15** Locality Tabernas **16** Mature larva on host plant, **17** Pupa on ground. Photos: Bogusch P (**13**, **14**), Pelikán J (**16**, **17**).

it was much less numerous than the dominant *Salicornia maritima*, and in Tabernas only several plants were observed. The ruderal sites containing the host plant have also been controlled but without any observations of *M. vuillefroyanus*.

Adult behaviour. Adults are only occasionally present on host plants during the day; most of them are hidden under the plant and are active at night.

Host plant. Both adults and larvae were observed feeding exclusively on *Suaeda vera* (Fig. 16). The larvae were found directly on the plants during the day, usually near the growth terminals. Adults and larvae feed on the leaves of the host plant. Larvae, with their cryptic pattern, resemble the colouration of the leaves (Fig. 16). The larvae were found on larger (more than 30 cm high) bunches of the host plant and they usually settled on those with thick, old, dry branches. The adults probably do not migrate long distances and remain under the plant or group of plants at their site.

Life cycle. At the beginning of April, 15 mature larvae and five younger larval instars were swept from host plants. At that time, the host plants had quite young fresh leaves, so the development of these individuals probably began a few days or weeks earlier in the spring so larvae would emerge on the young leaves of the host plant. We suppose the whole larval development to be short-lasting, approximately three weeks at most. The larvae were kept in small plastic containers (height 12 cm, width 4 cm) with small holes for air circulation and exchange. Four to five larvae

Figures 18–21. Cocoon, pupa and parasitoids of *Metadonus vuillefroyanus*. **18** Cocoon with two layers **19** Pupa in cocoon **20** Detail of parasitoid's cocoon **21** Cocoons of parasitoids. Photos: Bogusch P (**18–21**).

were kept in each container and no aggressive behaviour was observed, as is known in some Hyperini, e.g., *Brachypera vidua* (Gené, 1837) (Skuhrovec et al. 2015b). The larvae started pupation early, usually within 2–3 days. The bottom of each container included a 5 cm high level of substrate (collected directly at the locality under the host plants). The majority of the larvae spun their cocoons on the bottom of the container; therefore, they probably generally pupate less than 5 cm into the ground in natural conditions. However, several larvae made the cocoons just below the substrate level, so there seem to exist differences in the pupation preferences (Fig. 18). These cocoons were usually under dead chunks of *Suaeda* provided for feeding. None of the larvae pupated on or above the ground. The process of creating the cocoon took approximately 1–3 days. Subsequently, larva stayed in the cocoon until pupation, which occurred after 2–4 more days. Most of the larvae moulted into pupae within 5–7 days after entering the substrate. Adults began to emerge 12–18 days later. Altogether, seven adults emerged (five males and two females) by biting a hole in the cocoon. The new adults stayed on the ground surface or remained between a few millimetres and 3 cm above the ground. They were light and smooth at first, but soon acquired the general appearance of normal mature insects, even though it took 3–5 days for them to fully sclerotize. The adults fed on thawed host plant material brought from Spain, but the plants were damaged and did not supply sufficient

food. Due to the absence of these host plants in the Czech Republic, we did not try to breed the insects and obtain eggs for a new generation.

The pupae have a green colouration that changes to a brownish colour approximately five days after the start of pupation (Fig. 19). The colouration changes over the entire body; only the eyes are slightly darker. The adults are dark brown (in contrast to the sandy light brown of newly emerged imagines) by 10 days after pupation and are ready to emerge. The emerging adults usually stay in the cocoon for 1–2 days until they are partly sclerotized and then come out and feed on the host plant. The cocoon has two silk layers. The inner layer protects the pupa and is made only of silk. The outer layer includes ground particles.

Biotic interactions. Several of the larvae did not pupate, but larvae of parasitoids emerged and formed typical dark-brown puparia with a whitish band (Figs 20–21).

Discussion

Comparison with immature stages of other Hyperini

The larvae of 43 hyperine taxa have already been described (Anderson 1948; Baccetti 1957, 1958, 1959; Zaslavskij 1959, 1965, 1967; Scherf 1964; Lee and Morimoto 1988; May 1993; Nazarenko 2000a, b; Marvaldi 2003; Skuhrovec 2005a, 2006a, 2007; Vanin et al. 2012). The detailed description of the pupa is similar for nine hyperine taxa (Baccetti 1957, 1958, 1959; Scherf 1964; Gosik 2008; Vanin et al. 2012).

The comparison of the larvae and pupae of *Metadonus vuillefroyanus* with those described by Scherf (1964) was somewhat difficult due to the use of different terminology for morphology and chaetotaxy and/or the absence of good-quality drawings. The larvae were compared with the majority of species described and/or drawn by Anderson (1948), Zaslavskij (1959, 1965, 1967), Scherf (1964), and Nazarenko (2000a, b) which are of high or good-enough quality and very useful; however, the described characteristics are useful only for differential diagnosis. Detailed descriptions of these hyperine taxa are missing.

Only one brief description of *Metadonus distinguendus* were done by Zaslavskij (1959), being the only previously known in the genus *Metadonus*. Unfortunately, the description includes only drawings of the dorsal view of the prothorax, mesothorax and abdominal segment II. Despite these challenges, we were able to compare the morphology of these two taxa. *Metadonus vuillefroyanus* has one more minute *prn*, one more minute *prs* on mesothorax (see Fig. 8 for both) and two more *prs* (one normal size and one minute, see Fig. 9) on abdominal segment II than larvae of *M. distinguendus*. Both species have a unique characteristic in that all setae on the larval body are thorn-like and located on distinct black protuberances. This feature is also known in hyperines in all four species of the subgenus *Eririnomorphus* Capiomont, 1868, for which larvae are known (Anderson 1948; Skuhrovec 2006a, b) as well as

in *Hypera arator* (Linnaeus, 1758) of the subgenus *Kippenbergia* Alonso-Zarazaga, 2005 (Skuhrovec 2005a). Species of the subgenus *Eririnomorphus* have more points of similarity with species of the genus *Metadonus* than just these specific setae with protuberances (Skuhrovec, unpubl. data). The adults of these two groups have very similar body-scale shapes (see Skuhrovec 2008, 2009) and also share particularly harsh or extreme habitats (Skuhrovec 2009, 2012). The species of subgenus *Eririnomorphus* are more similar in these characteristics to *Metadonus* than to any other species of the genus *Hypera* (Skuhrovec, unpubl. data). This is suggestive of a close phylogenetic relationship between these taxa, a hypothesis that remains to be tested via phylogenetic analyses of the hyperines.

Various morphological characteristics of larvae of the tribe Hyperini were published by Lee and Morimoto (1988), May (1993), and Marvaldi (2003), including epipharynx and maxilla with simple setae, the third dorsal seta (*des3*) on the epicranium, the fifth frontal seta (*fs5*) longer than the fourth one (*fs4*), labial palpus one-segmented, mandible with sharp teeth, labral rods indistinct, postoccipital condyles present, pedal areas swollen to form prolegs or large lobes, head maculate and pigmented body. Vanin et al. (2012) published a detailed description of the immature stages of *Phelypera schuppeli* (Boheman, 1834) and found that the larvae have 2-segmented labial palpi unlike "typical" hyperines, but identical to *Metadonus vuillefroyanus*. A comparative summary of all recent data was provided by Oberprieler et al. (2014). Later, Nazarenko (2014) described and discussed the epipharyngeal morphology of seven hyperines. His final main epipharyngeal composition, with three pairs of *als*, one (or two) pairs of *ams* and two pairs of *mes*, completely fit the morphology of *Metadonus vuillefroyanus* (Fig. 3). The precise count of some of the setae on the epipharynx (especially *ams* and *mes*) in weevils is not completely resolved. According to Marvaldi (1998a, 1999), the standard for epipharynx setae in weevils is two *ams* and three *mes*, but when the positions of the distal *mes* are very close to the anterior margin they appear as *ams* (see different solutions within the same groups, e.g., Tychiini: Skuhrovec et al. 2014 vs. 2015b).

Knowledge of the immature stages and life histories of species can help to protect endangered species more effectively. The detailed description of larvae and pupae and their comparisons with known descriptions as reported here demonstrates that it is possible to identify this species in its immature stages, as has been accomplished for other groups (i.e., Entiminae: Gosik and Sprick (2012a, b, 2013); Gosik et al. (in press); Curculioninae, Tychiini: Skuhrovec et al. (2014, 2015b); Lixinae: Gosik and Skuhrovec 2011; Gosik and Wanat 2014; Stejskal et al. 2014; Skuhrovec and Volovnik 2015; Trnka et al. 2015). This process is particularly valuable for rare and endangered species because finding larvae is typically much simpler than finding adults. Additional detailed descriptions for hyperines, precise keys, detailed generic studies and comparisons of all groups could help in future to undertake the phylogenetic analysis of this group. It could also be very useful in different entomological fields, such as agriculture, biological control, and protection of endangered species.

Biological singularity

Hyperines are mainly known for two typical but biologically unusual features: ectophytic larvae with cryptic colouration and the ability to spin mesh cocoons. Both these features have been confirmed in *Metadonus vuillefroyanus* with specific details. Velázquez de Castro et al. (2000) presented the first biological data for *Metadonus* species, particularly for *Metadonus vuillefroyanus*, stating that this weevil occurred in salty wetlands, and that its host plant was *Suaeda vera*, which is a common plant in salty wetlands on Mediterranean seacoasts. All these data correspond with our observations.

Skuhrovec (2012) described J. Krátký's and J. Pelikán's observations of this weevil in salt wetlands in southeastern Spain. They described the colouration of larvae as similar to other Hyperini larvae – greenish with white stripes; however, these larvae have a thick yellow stripe and parallel pink to violet stripes on their dorsal parts (see Description and Fig. 16). Our observations about larval colouration are similar to previous descriptions, but the observations differ in the intensity and extent of purple stripes on the dorsum of the larvae. Some larvae were more green than purple, but some larvae were completely purple on the dorsal and lateral parts of their bodies. The colouration of the purple stripes varied from dark red to dark violet, almost black in some cases. The width of the dorsal yellowish stripes was also quite variable. A similar variation was observed in the plants, the shrubs of *Suaeda vera*: some were completely green, most were green and reddish, and some had completely reddish or violet-coloured leaves. We think that the variable colouration of the larvae corresponds with the colouration of the leaves of the host plant on which they fed, but we did not study this topic in detail (for example, whether the larvae are redder on reddish plants than on green ones). Cryptic colouration is one of the most common defensive strategies among insects and their larvae (Alcock 2009). Hyperine larvae are among the few in weevils that feed externally on the surface of their host plants, which may explain why they have evolved this protective colouration. The cryptic coloration, among other features like reduced body setae, and ambulatory ampullae, is also found in other weevil groups with ectophytic larval feeding, like *Gonipterus* and *Listroderes* (May 1993, 1994; Marvaldi 1998b).

Skuhrovec (2012) claimed that his colleagues observed that larvae of this species do not create cocoons; instead, they dig into the soil. Our observations are unambiguously different and we can rectify the previous mistake. According to the observations of the second author of this paper, the cocoon has two layers (Fig. 18). The inner layer protects the pupa and is made only of silk spun from Malpighian tubules. The outer layer is similar to the inner layer but the surface is covered with soil particles. This is different from cocoons of other similar and related European genera and species (Skuhrovec, unpublished data), which usually pupate on or above the ground and do not use substrate particles to build cocoons. The function of the outer part of the cocoon is probably a defensive tactic against parasitoids as camouflage or more likely, against very dry and/or very wet site weather conditions. The outer layer was also observed earlier by Krátký

and Pelikán (Skuhrovec 2012), but the inner layer was overlooked. Some of the larvae collected for this study were parasitized by still-unidentified braconids (they formed typical dark brown puparia with whitish bands, see Fig. 20); this observation lends more support to the hypothesis that the outer cocoon layer's function is for protection against unstable weather conditions.

Acknowledgements

We would like to thank Alena Astapenková (University of Hradec Králové, Czech Republic) for help with field collections and observations of this species. The study of the first author (JS) was supported by a grant from the Czech Ministry of Agriculture (Mze ČR) RO0415. We thank Adriana Marvaldi (Argentina) and Rafal Gosik (Poland) for commenting on the manuscript and for valuable discussions about larval and pupal chaetotaxy. The language was corrected by American Journal Experts company.

References

Alcock J (2009) Animal Behaviour, Ninth edition. Sinauer Publishers, Sunderland, 546 pp.

Alonso-Zarazaga MA, Lyal CHC (1999) A World Catalogue of Families and Genera of Curculionoidea (Insecta: Coleoptera) (Excepting Scolytidae and Platypodidae). Entomopraxis SCP Edition, Barcelona, 315 pp.

Alonso-Zarazaga MA, Lyal CHC (2002) Addenda and corrigenda to 'A World Catalogue of Families and Genera of Curculionoidea (Insecta: Coleoptera)'. Zootaxa 63: 1–7.

Anderson RS (2002) 131. Curculionidae Latreille 1802. In: Arnett RH Jr, Thomas MC (Eds) American Beetles. Volume 2. Polyphaga: Scarabaeoidea through Curculionoidea. CRC Press, Boca Raton, London, New York, Washington DC, 722–815

Anderson WH (1948) A key to the larvae of some species of *Hypera* Germar, 1817 (= *Phytonomus* Schoenherr, 1823). Proceedings of the Entomological Society of Washington 50(2): 25–34.

Baccetti B (1957) Studi sui Curculionidi italiani I. Ricerche morfologiche, etologiche ed istologiche su *Hypera trilineata* Marsham. Redia 42: 61–121.

Baccetti B (1958) Studi sui Curculionidi italiani II. *Donus crinitus* Boheman. Redia 43: 145–205.

Baccetti B (1959) Studi sui Curculionidi italiani IV. *Phytonomus philanthus* Olivier. Redia 44: 85–126.

Bouchard P, Bousquet Y, Davies AE, Alonso-Zarazaga MA, Lawrence JF, Lyal CHC, Newton AF, Reid CAM, Schmitt M, Ślipiński SA, Smith ABT (2011) Family-group names in Coleoptera (Insecta). ZooKeys 88: 1–972. doi: 10.3897/zookeys.88.807

Capiomont MG (1868) Révision de la Tribu des Hypérides, Lacordaire, et en particulier des genres *Hypera* Germ., *Limobius*, Schönh. et *Coniatus* (Germ.) Schönh. renfermant la description de plusieurs genres nouveaux et de 85 espèces nouvelles. Annales de la Société Entomologique de France Série 4 8(1): 73–160.

Gosik R (2008) Description of the pupa of *Hypera arundinis* (Paykull, 1792) with some comment on its biology. Snudebiller 9: 339–343.

Gosik R, Skuhrovec J (2011) Descriptions of mature larvae and pupae of the genus *Larinus* (Coleoptera: Curculionidae, Lixinae). Zootaxa 3019: 1–25.

Gosik R, Sprick P (2012a) Morphology and identification of the pupae of seven species of the genus *Otiorhynchus* Germar, 1822 (Coleoptera, Curculionidae, Otiorhynchini). Zootaxa 3483: 39–57.

Gosik R, Sprick P (2012b) Larval morphology of *Otiorhynchus ligustici*, *O. porcatus* and *O. salicicola* (Coleoptera, Curculionidae, Otiorhynchini). Deutsche Entomologische Zeitschrift 59: 301–316.

Gosik R, Sprick P (2013) Morphology and identification of the pupae of several species of soil-dwelling broad-nosed weevils (Coleoptera, Curculionidae, Entiminae). Zootaxa 3731(4): 445–472. doi: 10.11646/zootaxa.3731.4.2

Gosik R, Sprick P, Skuhrovec J, Deruś M, Hommes M (in press) Morphology and identification of the mature larvae of several species of the genus *Otiorhynchus* (Coleoptera, Curculionidae, Entiminae) from Central Europe with an update of life history traits. Zootaxa.

Gosik R, Wanat M (2014) Descriptions of immature stages of the weevil *Lixus punctiventris* Boheman, 1835 (Coleoptera, Curculionidae, Lixini). Zootaxa 3754(2): 159–172. doi: 10.11646/zootaxa.3754.2.5

Gunter NL, Oberprieler RG, Camron SL (2015) Molecular phylogenetics of Australian weevils (Coleoptera: Curculionoidea): exploring relationships in a hyperdiverse lineage through comparison of independent analyses. Austral Entomology. doi: 10.1111/aen.12173

Hundsdoerfer AK, Rheinheimer J, Wink M (2009) Towards the phylogeny of the Curculionoidea (Coleoptera): reconstructions from mitochondrial and nuclear ribosomal DNA sequences. Zoologischer Anzeiger 248: 9–31. doi: 10.1016/j.jcz.2008.09.001

Hunt T, Bergsten J, Levkanicova Z, Papadopoulou A, St. John O, Wild R, Hammond PM, Ahrens D, Balke M, Caterino MS, Gómez-Zurita J, Ribera I, Barraclough TG, Bocakova M, Bocak L, Vogler A (2007) A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. Science 318: 1913–1916. doi: 10.1126/science.1146954

Kenchington W (1983) The larval silk of *Hypera* spp. (Coleoptera: Curculionidae). A new example of the cross-β protein conformation in insect silk. Journal of Insect Physiology 29(4): 355–361. doi: 10.1016/0022-1910(83)90037-9

Korotyaev BA, Gültekin L, Volkovitsh MG, Dorofeyev VI, Konstantinov AS (2016) Bioindicator beetles and plants in desertified and eroded lands in Turkey. Journal of Insect Biodiversity 4(1): 1–47. doi: 10.12976/jib/2016.4.1

Kuschel G (1995) A phylogenetic classification of Curculionoidea to families and subfamilies. Memoirs of the Entomological Society of Washington 14: 5–33.

Lee CY, Morimoto K (1988) Larvae of the family Curculionidae of Japan. Part 2. Hyperinae to Cioninae (Insecta: Coleoptera). Journal of the Faculty of Agriculture, Kyushu University 33(1–2): 131–152.

Legalov AA (2007) Leaf-rolling Weevils (Coleoptera: Rhynchitidae, Attelabidae) of the World Fauna. Agro-Siberia, Novosibirsk, 523 pp.

Legalov AA (2010) Annotated checklist of species of superfamily Curculionoidea (Coleoptera) from Asian part of the Russia. Amurian Zoological Journal 2(2): 93–132.

Legalov AA (2011a) A review of weevils of the tribe Hyperini (Coleoptera: Curculionidae) of Inner Asia, with remarks on systematic [sic] and description of new taxa. Euroasian Entomological Journal 10(2): 145–156.

Legalov AA (2011b) Contribution to the knowledge of the tribe Hyperini (Coleoptera: Curculionidae) from Asia, with descriptions of new species. Amurian Zoological Journal 3(1): 35–45.

Marvaldi AE (1998a) Larvae of Entiminae (Coleoptera: Curculionidae): Tribal diagnoses and phylogenetic key, with a proposal about natural groups within Entimini. Entomologica Scandinavica 29: 89–98. doi: 10.1163/187631298x00212

Marvaldi AE (1998b) Larvae of South American Rhytirrhininae (Coleoptera: Curculionidae). The Coleopterists Bulletin 52(1): 71–89.

Marvaldi AE (1999) Morfología larval en Curculionidae (Insecta: Coleoptera). Acta zoológica Lilloana 45(1): 7–24.

Marvaldi AE, Sequeira AS, O´Brien CW, Farrell BD (2002) Molecular and morphological phylogenetics of weevils (Coleoptera, Curculinoidea): do nitche shifts accompany diversification? Systematic Biology 51: 761–785. doi: 10.1080/10635150290102465

Marvaldi AE (2003) Key to larvae of the South American subfamilies of weevils (Coleoptera, Curculinoidea). Revista Chilena de Historia Natural 76: 603–612. doi: 10.4067/s0716-078x2003000400005

Marvaldi AE, Lanteri AA (2005) Key to higher taxa of South American weevils based on adult characters (Coleoptera, Curculionoidea). Revista Chilena de Historia Natural 78: 65–87. doi: 10.4067/S0716-078X2005000100006

May BM (1977) Immature stages of Curculionidae: larvae of soil dwelling weevils of New Zealand. Journal of the Royal Society of New Zealand 72: 189–228. doi: 10.1080/03036758.1977.10427160

May BM (1993) Fauna of New Zealand, 28. Larvae of Curculionoidea (Insecta: Coleoptera): a systematic overview. Manaaki Whenua Press, Lincoln, New Zealand, 226 pp.

May BM (1994) An introduction to the immature stages of Australian Curculionoidea. In: Zimmerman EC (Ed.) Australian weevils (Coleoptera: Curculionidae) (Vol. 2). Brentidae, Eurhynchidae, Apionidae and a chapter on immature stages. CSIRO, Canberra, 365–755.

McKenna DD, Sequeira AS, Marvaldi AE, Farrell BD (2009) Temporal lag and overlap in the diversification of weevils and flowering plants. Proceedings of the National Academy of Sciences of the United States of America 106(17): 7083–7088. doi: 10.1073/pnas.0810618106

Nazarenko VYu (2000a) K morphologiyi lichinki starshego vozrasta zhuka-dolgonosika *Donus intermedius* (Coleoptera, Curculionidae) (On the morphology of old-stage larva of the weevil *Donus intermedius* (Coleoptera, Curculionidae)). Vestnik Zoologii 34: 91–94. [In Russian, English summary]

Nazarenko VYu (2000b) Opisanie lichinki starshego vozrasta zhuka-dolgonosika *Donus bucovinensis* (Coleoptera, Curculionidae) [A description of old-stage larva of the weevil *Donus bucovinensis* (Coleoptera, Curculionidae)]. Vestnik Zoologii, Supplement 14: 165–168. [In Russian, English summary]

Nazarenko VYu (2014) Epipharyngeal Morphology in Hyperini Larvae (Coleoptera, Curculionidae, Hyperinae). Vestnik Zoologii 48(4): 345–352. doi: 10.2478/vzoo-2014-0042

Oberprieler RG, Marvaldi AE, Anderson RS (2007) Weevils, weevils, weevils everywhere. Zootaxa 1668: 491–520.

Oberprieler RG, Caldara R, Skuhrovec J (2014) 3.7.2. Bagoini Thomson, 1859; Gonipterini Lacordaire, 1863; Hyperini Marseul, 1863. In: Leschen RAB, Beutel RG (Eds) Handbook of Zoology, Coleoptera, Beetles, Volume 3. De Gruyter, Göttingen, 452–476.

Petri K (1901) Monographie des Coleopteren-Tribus Hyperini. Siebenbürgischer Verlag für Naturwissenschaften zu Hermannstadt, 210 pp.

Scherf H (1964) Die Entwicklungsstadien der mitteleuropäischen Curculioniden (Morphologie, Bionomie, Ökologie). Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 506: 1–335.

Skuhrovec J (2005a) Descriptions of larvae of the tribe Hyperini (Coleoptera: Curculionidae): I. Mature larvae of the nominotypical subgenus *Hypera*. Acta Societatis Zoologicae Bohemicae 68[2004]: 245–280.

Skuhrovec J (2005b) Host plants of weevils of the genus *Hypera* (Coleoptera: Curculionidae) occurring in the Czech Republic. Klapalekiana 41: 215–255.

Skuhrovec J (2006a) Descriptions of larvae of the tribe Hyperini (Coleoptera: Curculionidae): II. Mature larvae of the subgenera *Antidonus*, *Eririnomorphus*, *Dapalinus* and *Boreohypera* of the genus *Hypera*. Entomologica Basiliensia et Collectionis Frey 28: 365–396.

Skuhrovec J (2006b) Identification of instars of *Hypera postica* by using chaetotaxy. Journal of Economic Entomology 99(6): 2216–2218. doi: 10.1603/0022-0493-99.6.2216

Skuhrovec J (2007) Descriptions of larvae of the tribe Hyperini (Coleoptera: Curculionidae): III. Mature larvae of the genus *Donus* Jekel, 1865. Zootaxa 1606: 1–28.

Skuhrovec J (2008) Taxonomic changes within the tribe Hyperini (Coleoptera: Curculionidae). Acta Entomologica Musei Nationalis Pragae 48(2): 677–690.

Skuhrovec J (2009) Digital-Weevil-Determination for Curculionoidea of West Palaearctic. Transalpina: *Hypera* / *Limobius* / *Metadonus* (Hyperinae: Hyperini). Snudebiller 10: 39–47.

Skuhrovec J (2012) Revision of the genus *Metadonus* (Coleoptera: Curculionidae, Hyperini). Snudebiller 13: 34–79.

Skuhrovec J (2013a) New nomenclatural and taxonomic acts, and comments on Hyperinae. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera. Vol. 8. Apollo Books, Stenstrup, 93–96.

Skuhrovec J (2013b) Hyperinae. In: Löbl I, Smetana A (Eds) Catalogue of Palaearctic Coleoptera. Vol. 8. Apollo Books, Stenstrup, 423–437.

Skuhrovec J, Gosik R, Caldara R (2014) Immatures of Palaearctic species of the weevil genus *Tychius* (Coleoptera, Curculionidae): new descriptions and new bionomic data with an evaluation of their value in a phylogenetic reconstruction of the genus. Zootaxa 3839(1): 1–83. doi: 10.11646/zootaxa.3839.1.1

Skuhrovec J, Gosik R, Caldara R, Košťál M (2015a) Immatures of Palaearctic species of the weevil genus *Sibinia* (Coleoptera, Curculionidae): new descriptions and new bionomic data with suggestions on their potential value in a phylogenetic reconstruction of the genus. Zootaxa 3955(2): 151–187. doi: 10.11646/zootaxa.3955.2.1

Skuhrovec J, Štys P, Exnerová A (2015b) Intraspecific larval aggression in two species of Hyperini (Coleoptera: Curculionidae). Journal of Natural History 49(19–20): 1131–1146. doi: 10.1080/00222933.2014.974704

Skuhrovec J, Volovnik S (2015) Biology and morphology of immature stages of *Lixus canescens* (Coleoptera: Curculionidae: Lixinae). Zootaxa 4033(3): 350–362. doi: 10.11646/zootaxa.4033.3.2

Stejskal R, Trnka F, Skuhrovec J (2014) Biology and morphology of immature stages of *Coniocleonus nigrosuturatus* (Coleoptera: Curculionidae: Lixinae). Acta Entomologica Musei Nationalis Pragae 54(1): 337–354.

Thompson RT (1992) Observations on the morphology and classification of weevils with a key to the major groups. Journal of Natural History 26: 835–891. doi: 10.1080/00222939200770511

Trnka F, Stejskal R, Skuhrovec J (2015) Biology and morphology of immature stages of *Adosomus roridus* (Coleoptera: Curculionidae: Lixinae). Zootaxa 4021(3): 433–446. doi: 10.11646/zootaxa.4021.3.3

Vanin SA, de Cassia Benda D, Albertoni FA (2012) Description of immature stages of *Phelypera schuppeli* (Boheman, 1834) with comments on natural history (Coleoptera: Curculionidae: Hyperinae). Zootaxa 3423: 45–60.

Velázquez de Castro A, Blasco-Zumeta J, Colonnelli E, Pelletier J, Alonso-Zarazaga MA, Sánchez-Ruiz M (2000) Weevil fauna from Los Monegros, north-east Spain (Coleoptera, curculionoidea). Bulletin de la Société Entomologique de France 105(4): 401–418.

Zaslavskij VA (1959) Materials on the study of weevil larvae of the subfamily Hyperinae (Coleoptera, Curculionidae). Zoologischeskij Zhurnal 38: 208–220. [In Russian, English summary]

Zaslavskij VA (1965) A new genus and species of weevils of the subfamily Hyperinae (Coleoptera, Curculionidae) from Middle Asia. Entomologicheskoe Obozrenie 44(1): 179–181. [In Russian]

Zaslavskij VA (1967) Novije vidy dolgonisikov roda *Hypera* Germ. (Coleoptera, Curculionidae) iz gornogo Kryma (New *Hypera* species (Coleoptera, Curculionidae) from Crimean mountains). Entomologicheskoe Obozrenie 46: 234–240. [In Russian, English title]

Zimmerman EC (1992) Australian Weevils (Coleoptera: Curculionoidea). Volume VI – Colour Plates 304–632. CSIRO Australia, Melbourne, 707 pp.

ZooKeys 589: 143–164 (2016)
doi: 10.3897/zookeys.589.8291
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Sergey gen. n., a new doryctine genus from temperate forests of Mexico and Cuba (Hymenoptera, Braconidae)

Juan José Martínez[1], Rubi Nelsi Meza Lázaro[2], Carlos Pedraza-Lara[2,3], Alejandro Zaldívar-Riverón[2]

1 *CONICET-Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de La Pampa, Santa Rosa, La Pampa, Argentina* **2** *Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, México* **3** *Licenciatura en Ciencia Forense, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad de México, México*

Corresponding author: *Alejandro Zaldívar-Riverón* (azaldivar@ib.unam.mx)

Academic editor: *M. Sharkey* | Received 2 March 2016 | Accepted 7 April 2016 | Published 16 May 2016

http://zoobank.org/D51D11EC-6FAB-475A-9BFC-C20A75F1D7F3

Citation: Martínez JJ, Lázaro RNM, Pedraza-Lara C, Zaldívar-Riverón A (2016) *Sergey* gen. n., a new doryctine genus from temperate forests of Mexico and Cuba (Hymenoptera, Braconidae). ZooKeys 589: 143–164. doi: 10.3897/zookeys.589.8291

Abstract

The new doryctine genus *Sergey* **gen. n.** is described with four new species (*S. cubaensis* Zaldívar-Riverón & Martínez, **sp. n.**, *S. coahuilensis* Zaldívar-Riverón & Martínez, **sp. n.**, *S. tzeltal* Martínez & Zalídivar-Riverón, **sp. n.**, *S. tzotzil* Martínez & Zalídivar-Riverón, **sp. n.**) from temperate forests of Mexico and Cuba. Similar to many other doryctine taxa, the new genus has a considerably elongated, petiolate basal sternal plate of the first metasomal tergite, although it can be distinguished from these by having the mesoscutum sharply declivous anteriorly with sharp anterolateral edges. The described species have been characterised molecularly based on two mitochondrial (COI, cyt *b*) and one nuclear (28S) gene markers. Based on the mitochondrial gene genealogies reconstructed, the evidence suggests the existence of incomplete lineage sorting or hybridization in the populations from Chiapas and Oaxaca assigned to *S. tzeltal* **sp. n**.

Keywords

Doryctinae, Ichneumonoidea, incomplete lineage sorting, new species, taxonomy

Introduction

The braconid wasp subfamily Doryctinae is a highly diverse, cosmopolitan group that currently comprises 198 genera and about 1,700 species (Braet 2016; Yu et al. 2012). This group gathers a wide array of genera with distinct morphologies and biologies, though most of its species appear to be idiobiont ectoparasitoids of bark-boring or xylophagous beetle larvae (Belokobylskij 1992; Marsh 1997). Previous attempts trying to elucidate the phylogenetic relationships among doryctine genera based on morphological evidence yielded poorly resolved hypotheses (Belokobylskij 1993; Belokobylskij et al. 2004). Subsequent molecular phylogenetic studies carried out for the subfamily (Zaldívar-Riverón et al. 2007, 2008) refuted most of the previously proposed tribes and subtribes (Belokobylskij 1993; Fischer 1981). These molecular phylogenies have served as a base to start building a stable higher-level classification for the group (Zaldívar-Riverón et al. 2007, 2008, 2014; Samacá-Sáenz et al. 2016).

One of the main external morphological features that was traditionally used to group genera within the Doryctinae is the relative length of the basal sternal plate of the first metasomal tergite (acrosternite *sensu* Belokobylskij 1995). This structure can be petiolate, tubular and long or sessile and short (Belokobylskij 1995; Marsh 1997). Within the Doryctinae, a long and tubular basal sternal plate has been shown to have independently evolved in various unrelated genera. Two of these genera are among the most speciose within the subfamily, the cosmopolitan, mainly Old World *Spathius* Nees, and the exclusively Neotropical *Notiospathius* Mathews & Marsh.

In a recent molecular phylogenetic study of *Notiospathius*, various species originally assigned to this genus were nested in two distantly related clades (Ceccarelli and Zaldívar-Riverón 2013). Members of these two clades have consistent external morphological features that distinguish them from each other and from the remaining doryctine genera. Species of one of these clades were placed in the newly described genus *Bolivar* Zaldívar-Riverón & Rodríguez-Jimenez (Zaldívar-Riverón et al. 2013).

In this work, a new doryctine genus, *Sergey* gen. n., is erected to include the species of the second clade, and four new species are described. Three of these species were collected in cloud forests from México and Cuba, whereas the remaining one was collected in a submontane forest in Coahuila, northeast Mexico. Members of the new genus are morphologically distinct from other doryctine genera with petiolate first metasomal tergite by having the anterolateral corners of mesoscutum sharply pointed and a different pattern of ornamentation in the propodeum, with two divergent carinae that sometimes enclose a more or less distinguishable areola. The phylogenetic relationships within the new genus have been assessed based on separate analyses of one nuclear and two mitochondrial (mt) markers, and provide evidence that suggests the existence of incomplete lineage sorting between two populations of one of the described species.

Material and methods

Specimens and terminology

Specimens were collected in four different localities in Mexico and Cuba, preserved in 100% ethanol, kept at 20°C until they were processed for DNA sequencing, and subsequently dried, labelled and mounted. The examined specimens are deposited in the Colección Nacional de Insectos, Instituto de Biología, Universidad Nacional Autónoma de México (IB-UNAM), Mexico City, Mexico, and the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN), Buenos Aires, Argentina.

The morphological terminology follows Sharkey and Wharton (1997), except for the sculpture characters, which follow Harris (1979), and the term precoxal sulcus, which replaces the term sternaulus according to Wharton (2006). Digital colour images were taken with a Leica® Z16 APO-A stereoscopic microscope, a Leica® DFC295/ DFC290 HD camera, and the Leica Application Suite® program. Digital SEM images were taken with a FEI® INSPECT (Oregon, USA) and a Hitachi® SU1510 SEM microscopes in low vacuum at the Museo Nacional de Ciencias Naturales (CSIC, Madrid, Spain) and the IB-UNAM, respectively.

Gene genealogies

Sequences of three gene markers have been examined for specimens belonging to the new genus. These included 34 and 26 sequences that were previously published in Ceccarelli and Zaldívar-Riverón's (2013) phylogenetic study of *Notiospathius* belonging to the cytochrome oxidase I (COI; 531 bp) mt and the second and third domain regions of the 28S nuclear ribosomal (r) (~617 bp;) DNA genes, respectively. Moreover, COI and 28S sequences were generated of additional specimens of this genus, as well as sequences of a 371 bp fragment of the cytochrome *b* mt DNA gene for a subset of the examined specimens. Sequences of *Heterospilus tauricus* Telenga (DNA voucher number CNIN884; GenBank accession nos. KC822008, 36, 72 for COI, 28S and cyt *b*, respectively) were also included to root the trees. *Heterospilus* was closely related to the newly described genus in the above molecular phylogenetic study. The sequenced ingroup specimens, their localities and GenBank accession numbers are provided in the description section.

Corrected pairwise genetic distances for the three gene markers were calculated using the K2P model with MEGA version 6 (Kimura 1980; Tamura et al. 2013). Separate gene genealogies were carried out with the program MrBayes version 3.2.6 (Ronquist et al. 2012) in the Cipres Science Gateway (Miller et al. 2010). Each analysis consisted of two independent runs of 20 million generations each, used uniform priors and sampled trees every 1000 generations. The following evolutionary models selected for each partition were obtained using the Bayesian criterion with JMODELTEST2 (Darriba et al. 2012): 28S.- K2; CytB.- 1st pos, TrN + G, 2nd pos, HKY

+ I, 3rd pos, GTR + G; COI.- 1st pos, F81, 2nd pos F81, 3rd pos HKY. Burn-in was determined assessing convergence between runs verifying the potential scale reduction factors (PSRF) and the estimated sample size (ESS) for all tested parameters. The burn-in fraction was set to 0.25, which corresponded to 5,000 trees (5×10^6 generations) in all analyses. The remaining trees from the two independent runs were employed to reconstruct a majority rule consensus tree using the 'halfcompat' option implemented in MrBayes. Clades were regarded as significantly supported if they had a posterior probability 0.95 (Ronquist et al. 2012).

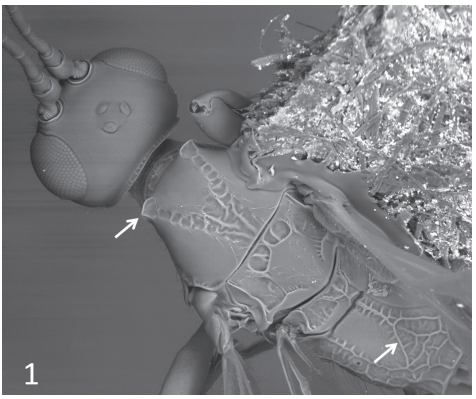
Results and discussion

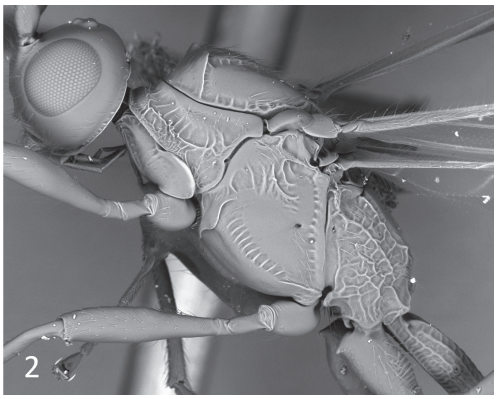
***Sergey* gen. n.**
http://zoobank.org/0C1D768E-779F-42A9-BC6A-520BA2447E06
Figs 1–2

Diagnosis. Species of this new genus can be distinguished from members of the remaining doryctine genera with long, petiolate first metasomal tergite (*e.g. Bolivar*, *Notiospathius*, *Pecnobracon* Kieffer et Jöergensen, *Spathius*, *Trigonophasmus* Enderlein) by having the mesoscutum sharply declivous anteriorly with sharp anterolateral corners. *Sergey* could be included in the key to dorcytine genera of the New World (Marsh 1997) as follows:

69 (65) First subdiscal cell of fore wing open at apex, 2cu-a absent, occasionally an infuscate spot or short line present between 2–1A and 2CUa but no distinct vein present (Fig. 28) **70**
- First subdiscal cell closed at apex, 2cu-a present and distinctly meeting 2–1A (Fig. 30) **75**
70 (69) Anterolateral corners of mesoscutum sharply pointed into two flanges, metasoma petiolated ..... ***Sergey* gen. n.**
- Anterolateral corners of mesoscutum not sharply pointed anteriorly, the mesoscutum may be sharply raised anteriorly with respect to the pronotum, but flanges are not present, metasoma variable ..... **70'**
70'(70) Most of mesosoma smooth and shining, mesonotum occasionally coriaceous, propodeum occasionally rugose **71**
- Most of mesosoma sculptured, rugose or coriaceous, at most mesopleuron smooth below sternaulus (precoxal sulcus) **73**

Description. *Head*: not depressed. Ocelli arranged in almost equilateral triangle. Frons not distinctly excavated, without a median keel between antennal sockets. Occipital carina complete, fused with hypostomal carina before mandible. Malar suture absent. Clypeus not high, delineated from face by distinct furrow, with fine lower flange. Hypoclypeal depression wide, round. Postgenal bridge narrow. Maxillary palpi





Figures 1–2. SEM images of *Sergey tzeltal* sp. n.; **1** head and mesosoma in dorsal view **2** head and mesosoma in lateral view. Arrows indicate anterolateral sharp edges/flanges on mesoscutum and divergent carinae on propodeum.

5-segmented, apical segment longer than fourth segment; labial palpi short, 4-segmented, third segment not shortened. Scape of antenna wide and rather short, without flange apically and ventroapical lobe, without basal constriction; ventral margin of scape shorter than dorsal margin in lateral view. First flagellar segment about the same length as second segment, usually several apical or subapical segments whithish. Apical segment more or less pointed apically, without "spine".

Mesosoma: not depressed. Neck of prothorax short but visible in dorsal view. Pronotum dorsally weakly convex (lateral view), with a transverse carina and a scrobiculate pronotal sulcus. Pronope absent. Propleural dorsoposterior flange rather short. Mesonotum distinctly elevated above pronotum. Anterolateral corners of mesoscutum projected in two flanges (Figs 1–2). Notauli present and complete, scrobiculate, obscured in rugose median area of mesoscutum. Scuto-scutellar (transscutal) suture distinct and complete. Prescutellar depression, with 3–5 high carinae. Scutellum slightly convex, subtriangular in dorsal view, about as long as wide, without lateral carinae. Subalar depression distinct. Mesopleuron with subalar sulcus, sternaulus and posterior mesopleural sulcus coarsely sculptured, otherwise smooth and polished. Mesopleural pit distinct. Precoxal sulcus (sternaulus) rather deep, wide, and scrobiculate, extended at least two thirds length of mesopleuron. Prepectal carina distinct and complete, laterally reaching anterior margin of subalar depression. Propodeum with two dorsolateral areas delimited by distinct carinae; sometimes these divergent carinae suggest an areola enclosing a rugose area (Fig. 1), in other cases the propodeum is uniformly rugose-areolate beyond the dorsolateral areas. Propodeal bridge absent. Propodeal spiracles small and round. Metapleuron slightly convex, entirely sculptured, rugose-areolate.

Wings: veins RS and r-m present, thus first and second submarginal cells entirely closed. Second submarginal cell rather long and narrow. First subdiscal cell open postero-apically, vein 2cu-a absent. Veins 1a and 2a absent. Hind wing with vein C+Sc+R

longer than vein SC+R. Vein RS arising from vein R far from vein r-m. Marginal cell more or less distinctly narrowed towards apex, without vein r. Vein cu-a present. Vein M+CU about 0.6-0.7 times as long as 1M; vein m-cu straight. Male hind wing without stigma-like swelling of basal veins.

Legs: Fore tibia on inner surface with several long and slender spines arranged along its anterior margin in almost single vertical line. Hind coxa long and narrow, with basoventral tubercle. Claws simple.

Metasoma: first tergite petiolate, long and narrow, usually striate-coriaceous, with some transverse carinae basally, these carinae sometimes reduced. Basal sternal plate (acrosternite) of first tergite long, 0.6–0.7 times as long as first tergite, extended distinctly beyond level of spiracles. Dorsope of first tergite small and shallow; spiracular tubercles indistinct, situated in basal 0.3 of tergite. Second tergite without distinct furrows and areas. Second suture considerably shallow, complete, almost straight in females and distinctly curved in males. Third tergite without transverse furrow and basal area. Tergites behind second with a single transverse line of sparse long erect setae. Ovipositor distinctly darkened apically, with two distinct subapical nodes. Ovipositor sheaths long, about as long as metasoma or slightly longer.

Etymology. We are very pleased to name this genus after our dear friend and colleague Dr. Sergey A. Belokobylskij, for his great contribution to the taxonomic knowledge of the braconid subfamily Doryctinae. Gender is to be considered masculine.

Type species. *Sergey tzeltal* sp. n.

Key to species of *Sergey*

1 Eyes small, their height about as long as malar space (Fig. 4); first metasomal tergite 1.5 times longer than its apical width (Fig. 8) (state of Coahuila, Mexico) ..... ***S. coahuilensis* sp. n.**

– Eyes big, their height distinctly longer than malar space (Figs 21, 31); first metasomal tergite slender, at least two times longer than its apical width 2

2 Head and mesoscutum distinctly sculptured, transversally striate (Figs 13, 17; fore wing with vein m-cu reaching vein RS+M basally to 2RS, thus vein (RS+M)b present and distinct (Fig. 18) (Cuba) ..... ***S. cubaensis* sp. n.**

– Head and mesoscutum mostly smooth and polished (Figs 9, 22, 32, 33); fore wing with vein m-cu reaching vein RS+M, interstitial with respect to vein 2RS, thus vein (RS+M)b absent (Figs 7, 28) (Mexico) **3**

3 Antenna with a white apical or subapical band composed of 3-7 (rarely two) flagellomeres in females (Figs 24, 26); males either with two apical flagellomeres whithish (Fig. 25), or with antenna entirely brown (Fig. 27) (states of Oaxaca and Chiapas) ..... ***S. tzeltal* sp. n.**

– Antenna of females with a subapical band only composed of the articulation between the 19th and 20th flagellomeres, five apical flagellomeres brown (Fig. 34), males with antenna entirely brown (state of Chiapas) .... ***S. tzotzil* sp. n.**

***Sergey coahuilensis* Zaldívar-Riverón & Martínez, sp. n.**
http://zoobank.org/A27FD56A-EB2D-42B1-A918-EA9F9CF1AD8A
Figs 3–10

Diagnosis. This is the most distinctive species of the genus. It can be distinguished from the remaining members of *Sergey* by having the eyes considerably smaller, their height about as long as malar space (distinctly longer than malar space in the remaining species); and the first metasomal tergite broad, 1.5 times longer than its apical width (slender, at least 2.1 times longer than its apical width in the remaining species).

Description. Body length 2.1mm (Fig. 3), fore wing 1.5mm; ovipositor sheaths 1.1mm. Colour: head excluding antennae, mesoscutum and mesopleuron brown, otherwise uniformly honey yellow. *Head*: about as high as wide (anterior view) (Fig. 4), 0.7 times as long as wide (dorsal view). Clypeus, face, frons and vertex largely smooth and shining (Fig. 5), with a few shallow rugae near the mandible insertion and antennal sockets; temple smooth. Eye small 1.5 times higher than wide. Malar space height/eye height ratio 1.1 (Fig. 4). Temple/eye length ratio (dorsal view) 0.6. Antenna incomplete, only with nine basal flagellomeres; first flagellomere about four times longer than wide and as long as second.

Mesosoma: 2.0 times longer than wide and 1.9 times longer than high (Fig. 6). Pronotal groove wide, and scrobiculate, pronotal carina distinct. Propleuron rugose on median third. Mesoscutum transverse, 0.6 times as long as wide. Mesoscutal lobes smooth, notauli deep and scrobiculate, obscured in an irregularly rugose median area before reaching the scuto-scutellar suture (Fig. 9). Prescutellar sulcus with three carinae, the median one straight, and the lateral ones irregular. Scutellar disc smooth and triangular. Mesopleuron smooth. Precoxal sulcus, deep, wide and scrobiculate-rugose, running along the entire length of mesopleuron. Subalar sulcus deep and rugose. Metanotum with a median carina but without a distinct projection. Metapleuron entirely areolate rugose. Propodeum with two divergent carinae running from median anterior edge delimiting two smooth dorsolateral areas, beyond these carinae it is almost uniformly areolate rugose.

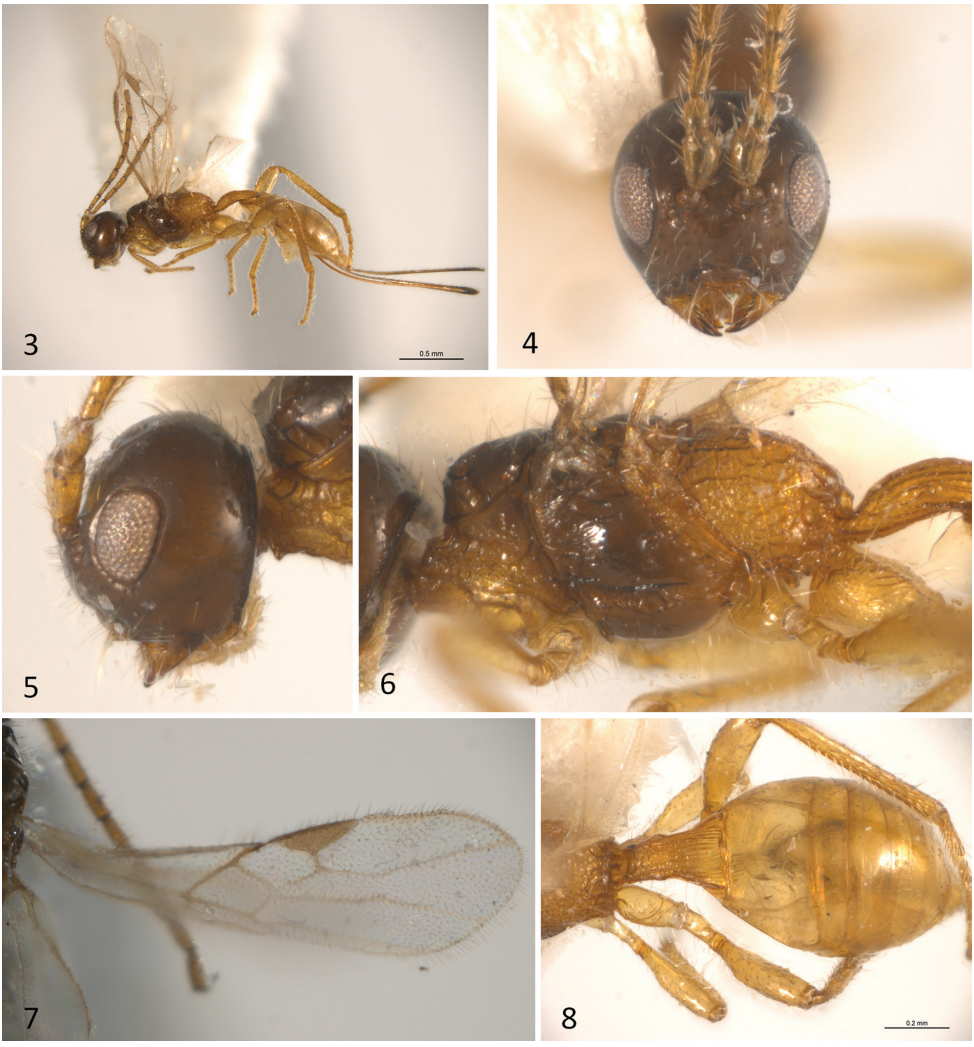
Wings: fore wing (Fig. 7) length 3.0 mm, length/width ratio 3.85; vein 1cu-a postfurcal to vein 1M; veins 2RS/2M ratio 0.5.

Legs: fore tibia with a row of spines. Hind coxa slightly striate dorsally, smooth ventrally, with a small but distinct basoventral tubercule.

Metasoma: basal sternal plate/length of first tergum 0.6. First metasomal tergite 2.8 times longer than apically wide (Figs 8, 10). Second median tergite longitudinally striate on basal one fifth, smooth apically. Suture between second and third median tergites slightly sinuate. Remaining terga smooth and polished. Ovipositor length 1.2mm, 1.1 times as long as metasoma.

Male. Unknown.

Distribution. Known only from a submontane forest at the type locality in Coahuila, Mexico.

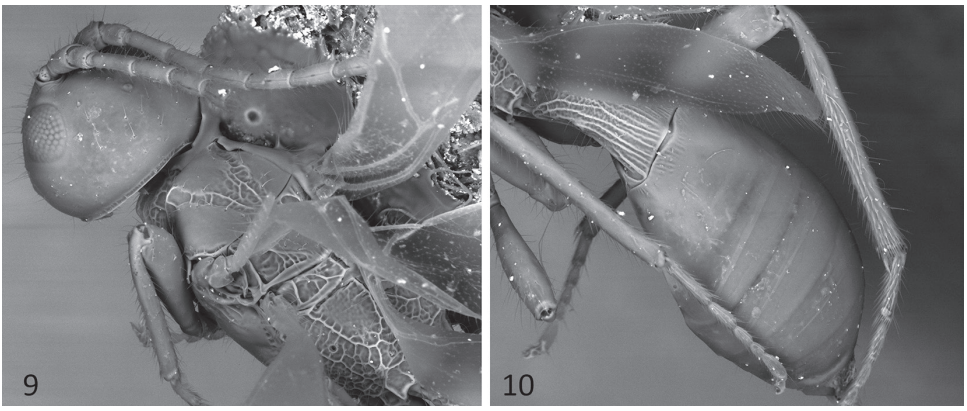


Figures 3–8. *Sergey coahuilensis* sp. n.; **3** habitus of female in lateral view **4** head in anterior view **5** head in lateral view **6** head and mesosoma in lateral view **7** forewing **8** metasoma in dorsal view.

Biology. Unknown.

Etymology. The specific epithet refers to Coahuila, the Mexican state where the type locality of this species is located.

Material examined. Holotype: female (CNIN), Mexico, Coahuila, Mpio. Torreón, Sierra de Jumillo, Arroyo Palos, 09-11/X/2009, 25.13 N - 103.27 O, 2006 msnm, DNA voucher no. IB-CNIN-637, GenBank accession nos. JN870454 (COI), JN870613 (cyt *b*), KC822013 (EF-1alpha; not included in this work), JN870735 (*wingless*, not included in this work).



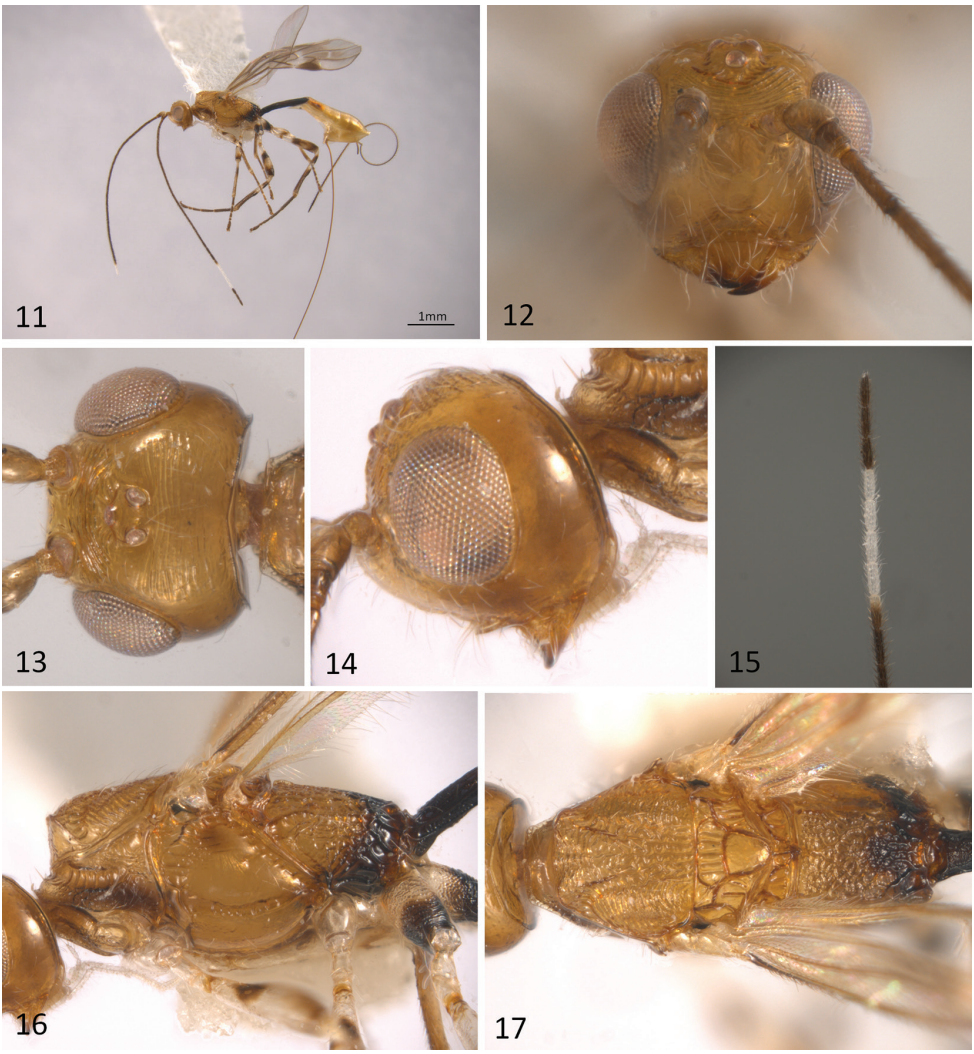
Figures 9–10. SEM images of *Sergey coahuilensis* sp. n.; **9** head and mesosoma in dorsal view **10** metasoma in dosolateral view.

***Sergey cubaensis* Zaldívar-Riverón & Martínez, sp. n.**
http://zoobank.org/4EB92C3F-9FE0-477A-BB34-A838A602D43B
Figs 11–19

Diagnosis. This distinctive species can be distinguished from the remaining species of *Sergey* by having: 1) a mostly yellow body colour (brown to black in the remaining species); 2) head and mesoscutum distinctly sculptured, transversally striate (entirely smooth and polished in the remaining species); and 3) fore wing with vein m-cu reaching vein RS+M basally to 2RS, thus vein (RS+M)b present and distinct (m-cu reaching vein RS+M interstitial with respect to vein 2RS, thus vein (RS+M)b absent in the remaining species).

Description. Body length 3.1mm (Fig. 11), fore wing 2,5 mm; ovipositor sheaths 3.5 mm. *Colour*: most part of the body yellow; apical third of propodeum and first metasomal tergite dark brown, second metasomal tergite yellow with lateral areas brown; antennae honey yellow, gradually darkening toward apex, subapical 20th to 23rd segments white (Fig. 15), apical three segments dark brown; fore and middle coxae pale yellow; fore and middle tibiae brown to dark brown; trochanter and trochantellus pale yellow; tarsi brown to dark brown; hind coxa pale yellow basally, dark brown apically; hind femur and tibia with four alternate yellow and dark brown transversal bands. Wings hyaline; pterostigma and veins brown. Ovipositor sheaths yellow to honey yellow.

Head: 0.7 times as high as wide in anterior view (Fig. 12), 0.6 times as long as wide in dorsal view (Fig. 13). Vertex and frons distinctly striate; face, temple and gena smooth (Fig. 14); clypeus transversally striate. Eye 1.2 times higher than wide. Malar space height/eye height ratio 0.3 (Fig. 12). Temple/eye length ratio (dorsal view) 0.4. Antenna with 26 flagellomeres, first flagellomere about four times longer than wide and as long as second.



Figures 11–17. *Sergey cubaensis* sp. n.; **11** habitus of female in lateral view **12** head in anterior view **13** head in dorsal view **14** head in lateral view **15** female apical flagellomeres **16** mesosma in lateral view **17** mesosoma in dorsal view.

Mesosoma: about 1.9 times longer than wide and 2.0 times longer than high (Figs 16–17). Pronotal groove wide, deep, and scrobiculate, pronotal carina distinct. Propleuron smooth to slightly rugose. Mesoscutum slightly transverse, 0.7 times as long as wide. Mesoscutal lobes transversally striate with coriaceous microsculpture, notauli deep, complete and scrobiculate (Fig. 17), not joining, reaching the end of mesoscutum, obscuring in an irregular longitudinal rugose median area before reaching the scuto-scutellar suture. Prescutellar sulcus with four distinct carinae. Scutellar disc smooth and triangular. Mesopleuron smooth. Precoxal sulcus, deep and scrobiculate,

Figures 18–19. *Sergey cubaensis* sp. n.; **18** fore wing **19** metasoma in dorsal view.

running along the entire length of mesopleuron. Subalar sulcus deep and scrobiculate. Metanotum with a distinct median carina-like projection. Metapleuron entirely areolate-rugose. Propodeum uniformly areolate-rugose, with two longitudinal carinae joined basally and that immediately diverge forming an areola-like structure.

Wings: fore wing length 3.6 mm, length/width ratio 3.7; vein 1cu-a slightly postfurcal to vein 1M, thus vein (RS+M)b present (Fig. 18); veins 2RS/2M ratio 0.5.

Legs: fore tibia with a row of spines. Hind coxa transversally striate-rugose, with a small but distinct basoventral tubercle..

Metasoma: Basal sternal plate/length of first tergum 0.6. First metasomal tergite 2.5 times longer than apically wide (Fig. 19). Second median tergite longitudinally costate on basal three fourths, smooth on apical fourth. Suture between second and third median tergites sinuate laterally. Remaining terga smooth and polished. Ovipositor length 3.5 mm, 1.8 times longer than metasoma.

Variation. Body length 3.4–4.3 mm. Temple/eye length ratio in dorsal view 0.4–0.5. Antenna with 26–28 flagellomeres. Prescutellar sulcus with four or five carinae. Fore wing length 3.5–3.6 mm, length/width ratio 3.7–3.8 times its maximum width. Ovipositor length 3.5–4.3 mm, 1.8-2.0 times longer than metasoma.

Males. Unknown.

Distribution. Known only from the type locality in southern Cuba.

Biology. Unknown.

Etymology. This species is named after the Caribbean country where it occurs, Cuba.

Material examined. Holotype (CNIN): Female, Cuba, Santiago, Gran Piedra Isabélica, 06-14/VII/1995, FIT, Cloud Forest, 1100m, S.B. Peck, DNA voucher number CNIN413, GenBank accession numbers JN870310 (COI), JN870491 (cyt *b*), KC822012 (EF-1alpha; not included in this work), KC822095 (*wingless*; not included in this work). Paratype (CNIN): one female, same data as holotype; DNA voucher number CNIN414, GenBank accession numbers JN870311 (COI), JN870492 (cyt *b*), JN870651 (*wingless*; not included in this work).

Sergey tzeltal Martínez & Zaldívar-Riverón, sp. n.

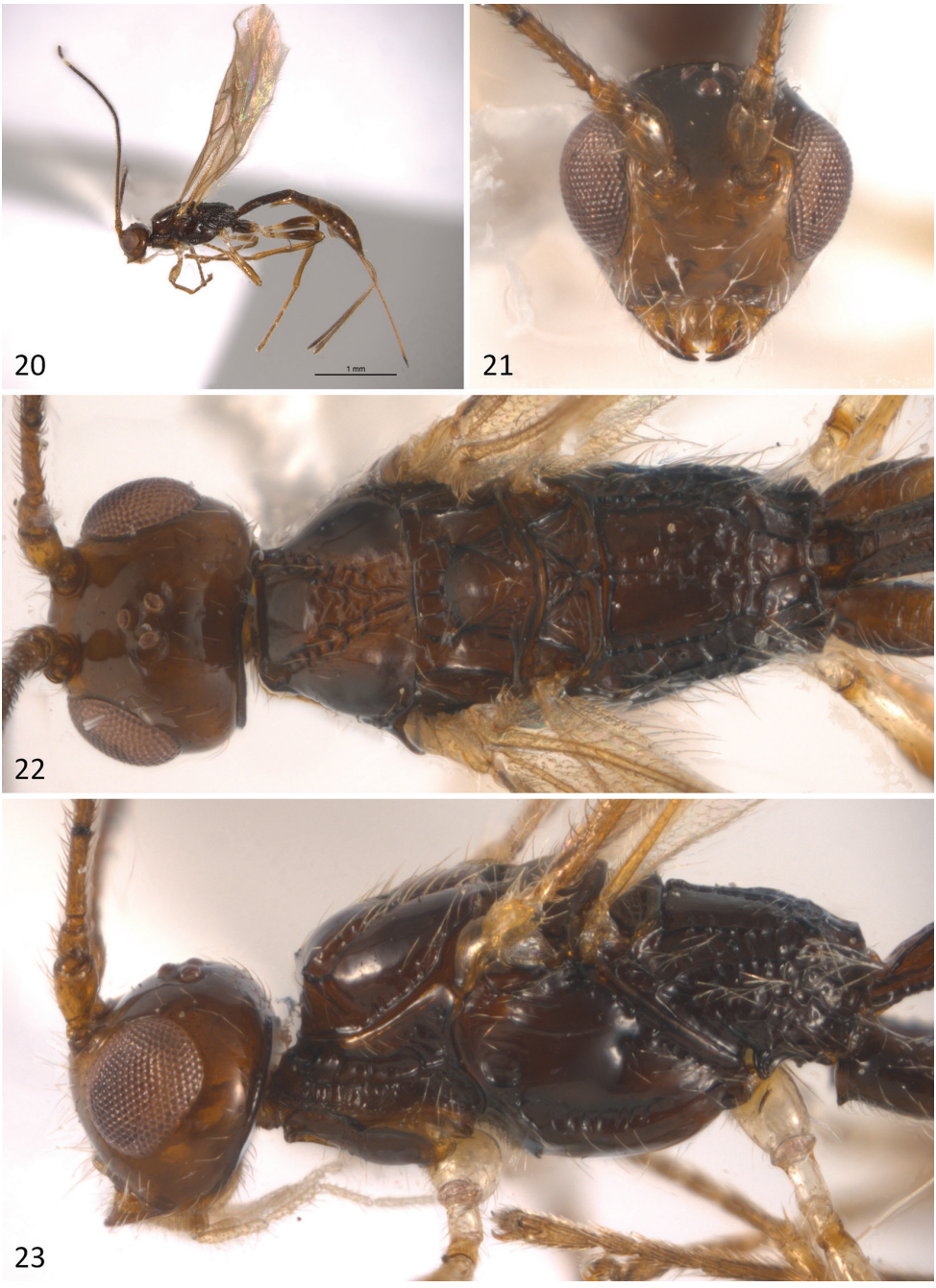
http://zoobank.org/7ECECEFC-2CB3-44C2-B544-D5A5FBB18FBF
Figs 20–29

Diagnosis. This species is similar to *S. tzotzil*, but it can be distinguished from the latter species by the colour pattern of the white band on the female antenna. In *S. tzeltal*, the white band is either apical or subapical and is composed of at least two entire whitish flagellomeres, usually more, with at most three apical flagellomeres brown. In *S. tzotzil*, the white band is subapical and consists only of the lighter color on the articulation between the 19th and 20th flagellomeres, and with the five apical flagellomeres brown.

Description. Body length 3.1 mm (Fig. 20), fore wing 2.7 mm; ovipositor sheaths 1.5mm. *Colour*: head uniformly brown, antenna brown, gradually darkening towards apex, except for a subapical white band composed of 2–4 flagellomeres (apical in Oaxaca population composed of 6-8 flagellomeres, see below) (Figs 24, 26). Mesosoma uniformly dark brown, except for a slightly lighter area on the median area of mesoscutum. Metasoma brown. Legs light brown, except fore and middle coxae, trochanters and trochantelli and hind trochantellus, which are pale yellow; hind coxa and apical three fourths of hind femur dark brown. Wings hyaline; pterostigma and veins brown. Ovipositor sheaths brown.

Head: 0.8 times as high as wide in anterior view (Fig. 21), and 0.7 times as long as wide in dorsal view (Fig. 22). Clypeus, face, frons and vertex smooth and polished; temple smooth. Eye 1.3 times higher than wide. Malar space height/eye height ratio 0.3. Temple/eye length ratio (dorsal view) 0.6. Antenna with 24 flagellomeres, first flagellomere about 4.0 times longer than wide, as long as second one.

Mesosoma: 2.0 times longer than wide (Fig. 22), 2.1 times longer than high (Fig. 23). Pronotal groove wide, deep and scrobiculate, pronotal carina distinct. Propleuron smooth. Mesoscutum slightly transverse, 0.7 times as long as wide. Mesoscutal lobes smooth, notauli deep and scrobiculate, obscured in an irregularly rugose median area before reaching the scuto-scutellar suture. Prescutellar sulcus with three distinct carinae. Scutellar disc smooth and triangular. Mesopleuron smooth. Precoxal sulcus deep and scrobiculate, running along basal two-thirds of mesopleuron. Subalar sulcus deep and rugose. Metanotum with a distinct median carina-like projection. Metapleuron entirely areolate-rugose. Propodeum with two divergent carinae running from median anterior edge delimiting two smooth dorsolateral areas; area beyond these carinae almost uniformly areolate-rugose.



Figures 20–23. *Sergey tzeltal* sp. n.; **20** habitus of female in lateral view **21** head in anterior view **22** head and mesosoma in dorsal view **23** head an mesosoma in lateral view.

Wings: fore wing (Fig. 28) length 3.0 mm, length/width ratio 3.85; vein 1cu-a postfurcal to vein 1M; veins 2RS/2M ratio 0.5.

Legs: fore tibia with a row of spines. Hind coxa transversally striate dorsally, smooth ventrally, with a distinct basoventral tubercule.

Metasoma: Basal sternal plate/length of first tergum 0.6. First metasomal tergite 2.2 times longer than apically wide (Fig. 29). Second median tergite longitudinally costate on basal three fourths, smooth apically. Suture between second and third median tergites slightly sinuate. Remaining tergites smooth and polished. Ovipositor length 1.4 mm, 0.9 times as long as metasoma.

Variation. Body length 2.6–3.8 mm. Temple/eye length ratio in dorsal view 0.16–0.33. Antenna with 22-27 flagellomeres, white subapical band composed of two to four flagellomeres. In smaller specimens, rugose median area of mesoscutum reduced, though notauli never clearly distinguishable at posterior edge of mesoscutum, obscured among rugosities. Prescutellar sulcus sometimes with para-median carinae reduced, thus only the median carina is clearly distinguishable. Fore wing length 2.2–2.9 mm, length/width ratio 2.9–3.9 times its maximum width. Veins 2RS/2M ratio 0.5–0.55. Basal sternal plate 0.53–0.68 times length of first metasomal tergum. Ovipositor length 2.0–2.1 mm.

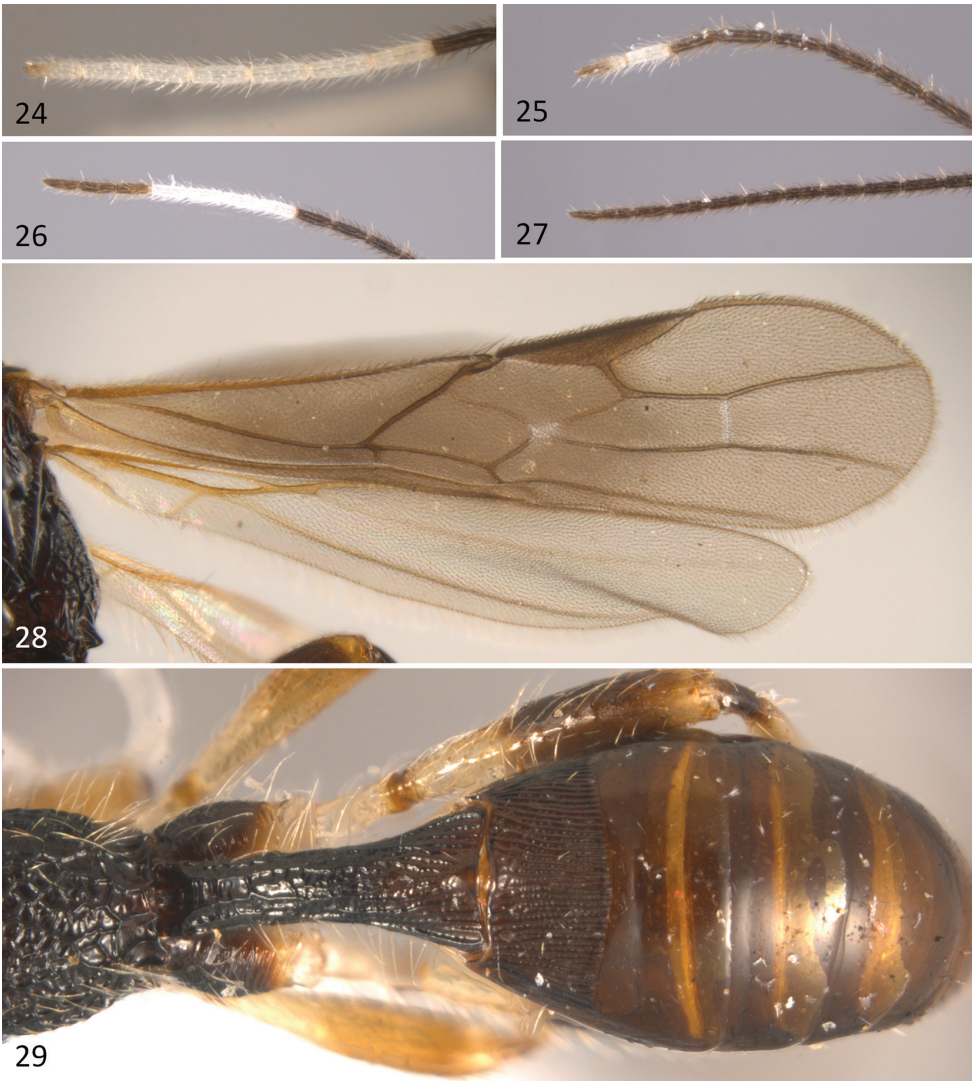
Males. Body length 2.5–3.5 mm. Malar space 0.28. Temple/eye length ratio (dorsal view) 0.29–0.39. Flagellomeres 21–26 either entirely brown (Chiapas) (Fig. 27) or with two apical flagellomeres whitish (Oaxaca) (Fig. 25). Median apex of mesoscutum slightly coriaceous. Venter of mesosoma coriaceous to slightly coriaceous. Metapleuron coriaceous, coriaceous to slightly rugose distally. Fore wing 1cu-a vein slightly postfurcal to vein1M.2RS/2Mratio 0.47–0.51. Basal sternal plate 0.54 times length of first metasomal tergite.

Distribution. This species is known from cloud forests located in the Reserva el Triunfo, Chiapas, and Santiago Comaltepec, Oaxaca, in southeast Mexico.

Biology. Unknown.

Comments. This species has a considerable variation in the antennal color pattern. We had originally grouped the specimens assigned to this taxon in two morphospecies, each represented by the specimens from Chiapas and Oaxaca, respectively. Females from Oaxaca have a distinct apical white band composed of 6–8 flagellomeres (Fig. 24), whereas in males this apical band is smaller (Fig. 25). On the other hand, most females from Chiapas have a white antennal band that is subapical and is only composed of 2-4 flagellomeres (Fig. 26). However, one female that could not be sequenced has an apical band similar to the specimens from Oaxaca. Other external morphological features (*e.g.* sculpture of propodeum and first metasomal tergite) also varied but we could not find any correlation with the geographical provenance of the specimens.

There was no concordance between the corrected COI distances and the geographic provenance and morphological variation for the above specimens. Some of the specimens from Oaxaca had lower COI distances with those from Chiapas than with the remaining specimens from the same locality (0.38–0.76 and 1.7–1.9%, respectively). This incongruence suggests that the existence of incomplete lineage sorting or



Figures 24–29. *Sergey tzeltal* sp. n.; **24** antenna of female (Oaxaca) **25** antenna of male (Oaxaca) **26** antenna of female (Chiapas) **27** antenna of males (Chiapas) **28** fore and hind wings **29** metasoma in dorsal view.

hybridization between two recently diverged, sympatric species (see below). We have followed a conservative approach and consider the members of the populations from Chiapas and Oaxaca as a single species. One of the specimens from Oaxaca (DNA voucher number CNIN573) has considerably higher COI distances compared with the remaining conspecific specimens (3.6–4.7%). However, it is morphologically undistinguishable and we thus placed it within *S. tzeltal*.

Etymology. The name of this species refers to the Tzeltal ethnic group, descendant from the Mayans that inhabits Los Altos, a mountain region located in central Chiapas

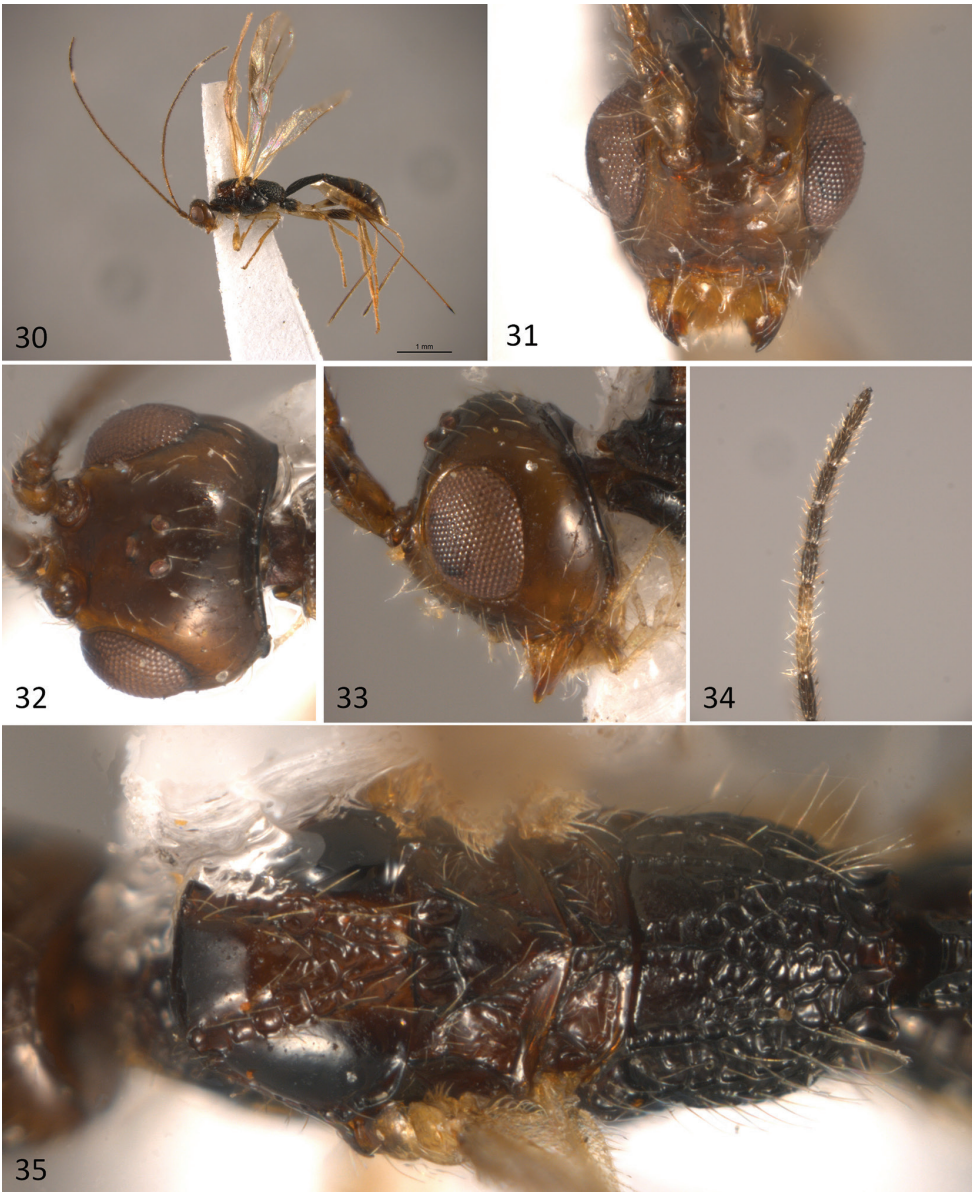
Material examined. *Holotype* (CNIN): female, Mexico, Chiapas, Mpo. Albino Corzo, Reserva el Triunfo, 15°39.428N, 92°48.67W, YPT, 16/XI/2001, Kovarik col., DNA voucher number CNIN-711, GenBank accession numbers KC821997 (COI), KC822257 (cyt *b*), KC822078 (EF-1alpha; not included in this work), KC822122 (*wingless;* not included in this work). *Paratypes* (CNIN, MACN): one female, five males, same data as holotype, DNA voucher numbers CNIN712-15, 18, GenBank accession numbers KX074181-84, 87 (COI), KX074190-93 (cyt *b*), KX074195-97, 200 (28S); two females, same data as holotype except 15°39.447N, 92°48.40W, 17-20/XI/2001, DNA voucher numbers CNIN720-21, GenBank accession numbers KX074188-89 (COI); two females, five males, Mexico, Oaxaca, Mpio. Santiago Comaltepec, 17.62836 -96.4672; 6-8/VI/2009, YPT, 1495m, A. Zaldívar, H.Clebsch, DNA voucher numbers CNIN457-59, 461-64, GenBank accession numbers JN870332-34, 36-39 (COI), JN870515, 17-19 (cyt *b*), JN870673-74, 76-79 (*wingless*; not included in this work); one female, four males, Mexico, Oaxaca, Mpio. Santiago Comaltepec, 17.59056 -96.39902; 8/VI/2009, bosque mesófilo, 1998-2141m, A. Zaldívar, H.Clebsch, DNA voucher numbers CNIN468-71, GenBank accession numbers JN870343-46 (COI), JN870524 (cyt *b*), JN870681-84 (*wingless*; not included in this work); one male, Mexico, Oaxaca, Mpio. Santiago Comaltepec, La Esperanza, 17.62661 -96.36950; 8/VI/2009, cloud forest, 1600m, A. López, DNA voucher number CNIN479, GenBank accession number JN870352 (COI), KX074194 (cyt *b*), JN870689 (*wingless*; not included in this work); two females, five males, Mexico, Oaxaca, Mpio. Santiago Comaltepec, 17.62334 -96.34669; 6/V/2009, bosque mesófilo, 1460m, A. Zaldívar, DNA voucher numbers CNIN379-80, 446-48, 452-53, GenBank accession number JN870324-26, 295-96 (COI), JN870509-11, 466 (cyt *b*), JN870630-31, 64-65 (*wingless*; not included in this work), KC822056-57 (EF-1alpha; not included in this work).

***Sergey tzotzil* Martínez & Zaldívar-Riverón, sp. n.**
http://zoobank.org/7910978A-AFB7-41D2-B064-BBE99700B5E8
Figs 30–37

Diagnosis. See diagnosis of *S. tzeltal*.

Description. Body length 3.7mm (Fig. 30), fore wing 3.2mm; ovipositor sheaths 2.3mm. *Colour*: head uniformly brown, antenna brown, gradually darkening towards apex, except for a light band composed of most of the 19th and the basal half of the 20th flagellomeres (Fig. 34). Mesosoma uniformly dark brown, except for a slightly lighter area on median area of mesoscutum. Metasoma brown. Legs light brown, except fore and middle coxae, trochanters and trochantelli and hind trochantellus which are pale yellow; hind coxa and apical three fourths of hind femur dark brown. Wings hyaline; pterostigma and veins brown. Ovipositor sheaths brown.

Head: in anterior view 0.9 times as high as wide (Fig. 31), and 0.6 times as long as wide in dorsal view (Fig. 32). Clypeus, face, frons and vertex smooth and shining; temple smooth (Fig. 33). Eye 1.3 times higher than wide. Malar space height/eye height



Figures 30–35. *Sergey tzotzil* sp. n.; **30** habitus of female in dorsal view **31** head in anterior view **32** head in dorsal view **33** head in lateral view **34** female apical flagellomeres **35** mesosoma in dorsal view.

ratio 0.4. Temple/eye length ratio (dorsal view) 0.6. Antenna with 25 flagellomeres, first flagellomere five times longer than wide and about as long as the second one.

Mesosoma: 2.1 times longer than wide (Fig. 35) and 2.0 times longer than high (Fig. 36). Pronotal groove wide, deep, and scrobiculate, pronotal carina distinct. Propleuron smooth. Mesoscutum slightly transverse, 0.7 times as long as wide. Mesoscutal

lobes smooth, notauli deep and scrobiculate, obscured in an irregularly rugose median area before reaching the scuto-scutellar suture. Prescutellar sulcus with three distinct carinae. Scutellar disc smooth and triangular. Mesopleuron smooth. Precoxal sulcus, deep and scrobiculate, running along basal two-thirds of mesopleuron. Subalar sulcus deep and scrobiculate-rugose. Metanotum with a distinct median projection. Metapleuron entirely areolate rugose. Propodeum with two divergent carinae running fron median anterior edge delimiting two dorsolateral areas, these areas are mostly smooth, but turn rugose areolate near carinae; beyond these carinae the propodeum almost uniformly areolate-rugose.

Wings: Fore wing length 2.9 mm, length/width ratio 3.3; vein 1cu-a slightly postfurcal to vein 1M; veins 2RS/2M ratio 0.5.

Legs: Fore tibia with a row of spines. Hind coxa transversally striate dorsally, smooth ventrally, with a distinct basoventral tubercle.

Metasoma: Basal sternal plate/length of first tergum 0.6. First metasomal tergite 2.1 times longer than apically wide (Fig. 37). Second median tergite longitudinally costate on basal three fourths, smooth apically. Suture between second and third median tergites almost straight. Remaining terga smooth and polished. Ovipositor length 2.4 mm, 1.1 times as long as metasoma.

Males. Similar to female, slightly smaller and with antenna uniformly brown.

Distribution. Known only from the type locality in El Triunfo, Chiapas, Mexico.

Biology. Unknown.

Comments. This species and *S. tzeltal* were collected in the same locality in Chiapas.

Etymology. The name of this species refers to the Tzotzil ethnic group, descendant from the Mayans, who inhabits the Altos, a mountain region located in central Chiapas.

Material examined. *Holotype* (CNIN): female, Mexico, Chiapas, Mpo. Albino Corzo, Reserva el Triunfo, 15°39.428N, 92°48.67W, YPT, 16/XI/2001, Kovarik col., DNA voucher number CNIN717, GenBank accession nos. KC821999 (COI), KC822259 (cyt *b*), KX074199 (28S), KC822124 (wingless, not included in this work), KC822080 (EF-1alpha; not included in this work). Paratype (CNIN): one male, same data as holotype; DNA voucher number, CNIN716, DNA voucher nos. KC821998 (COI), KC822258 (cyt *b*), KX074198 (28S), KC822123 (*wingless*, not included in this work), KC822079 (EF-1alpha; not included in this work).

Gene genealogies. Intraspecific corrected genetic divergences varied from 0 to 2.1 (excluding CNIN573), 0.27 to 3.18 and 0 to 0.33% for COI, cyt *b* and 28S, respectively. Interspecific distances within *Sergey* on the other hand ranged from 7.99 to 15.28, 12.64 to 13.6 and 0.17 to 0.5% for COI, cyt *b* and 28S, respectively.

The Bayesian phylograms derived from the separate COI and cyt *b* analyses are included in the Figure 38. The COI bayesian phylogram significantly supported the monophyly of the three described species. *Sergey coahuilensis* was recovered as sister to *Heterospilus* but without statistical support (PP = 0.5). A clade with the remaining species of *Sergey* (PP = 0.5) recovered *S. cubaensis* from Cuba (PP = 0.5) as sister to a *S. tzotzil* (Chiapas) + *S. tzeltal* (Chiapas and Oaxaca) clade (PP = 1.0). Within *S. tzeltal*,

Figures 36–37. *Sergey tzotzil* sp. n.; **36** mesosoma in lateral view **37** metasoma in dorsal view.

there were three non-significantly supported, subclades, two of which were composed of specimens from Comaltepec, Oaxaca, but with one of them being more closely related to the subclade containing the specimens from El Triunfo, Chiapas.

The bayesian phylogram derived from the cyt *b* sequences yielded similar relationships with the COI topology. Again, some of the specimens of S. *tzeltal* from Comaltepec, Oaxaca were more closely related to the ones from El Triunfo, Chiapas (PP = 0.6) than with the remaining specimens from the same locality. The 28S tree was largely unresolved (phylogram not shown), with the sequenced specimens *S. tzeltal* and *S. tzotzil* grouped together (PP = 1.0). The reconstructed mt gene genealogies, together with the geographic provenance and morphological variation found in the specimens of *S. tzeltal* from Oaxaca and Chiapas suggests that this taxon could consist of two sympatric, recently derived lineages in which there is incomplete lineage sorting or hybridization. Further morphological and genetic studies will help to confirm the taxonomic status of the populations of *S. tzeltal* from the latter two Mexican regions.

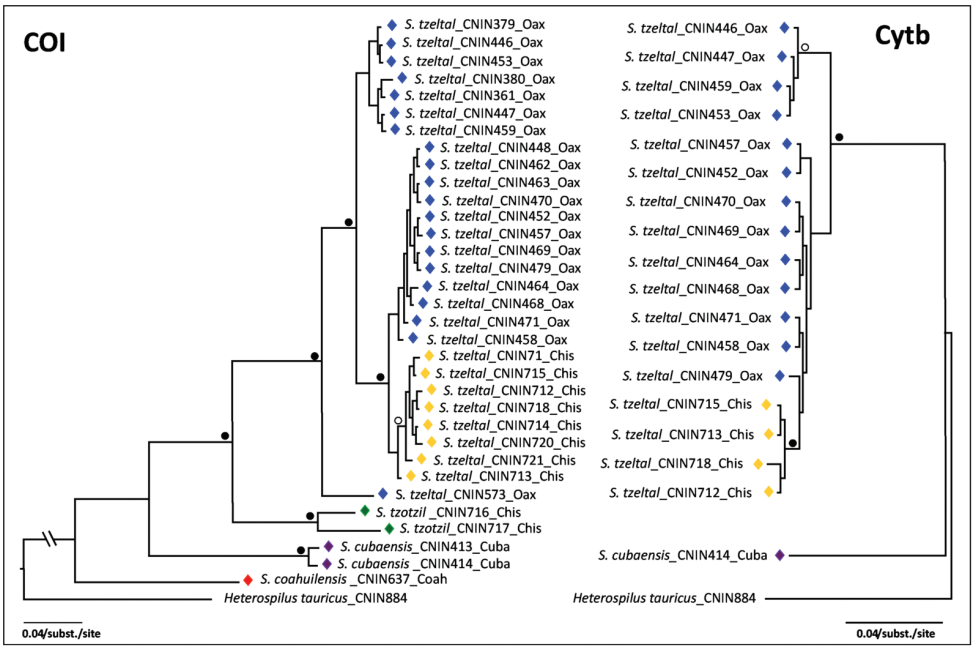


Figure 38. Bayesian phylograms showing the relationships recovered by the separate COI and cyt *b* analyses. Black circles near branches indicate clades supported by posterior probabilities ≥ 95%; hollow circles near branches indicate clades supported by posterior probabilities ≥ 90 and ≤ 94%.

Acknowledgments

We thank Marysol Trujano for collecting the specimen of *S. coahuilensis*; Mike Sharkey for providing the two specimens of *S. cubaensis*, C. Mayorga and G. Ortega for their help with the curation of specimens, Andrea Jiménez and Laura Márquez for their help in the laboratory, S. Guzman for her help taking the digital pictures, and Mario García for his help during AZR's sabbatical stay at the MNCN-CSIC. This study was supported by grants given by the Consejo Nacional de Ciencia y Tecnología (CONACyT: convocatoria SEP-Ciencia Básica 2014 No. 220454; Red Temática del Código de Barras de la Vida 2015; convocatoria fortalecimiento de infraestructura 2014 No. 224743) and UNAM (DGAPA-PASPA, convocatoria 2015; DGAPA-PAPIIT convocatoria 2016) to AZR, by a grant given by the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICT 2012-0617) to JJM, and by a grant given by CONACyT (proyecto de cooperación bilateral CONACyT-CONICET convocatoria 2014) to JJM and AZR. Fieldwork in Coahuila, Mexico, was supported by grants given by Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO, proyecto GT008) to C. García de la Peña and by a Theodore Roosevelt Memorial Fund grant given by the American Museum of Natural History to M. Trujano-Ortega and U. García-Vázquez.

References

Belokobylskij SA (1992) On the classification and phylogeny of the Braconid wasps subfamilies Doryctinae and Exothecinae (Hymenoptera: Braconidae). Part I. Classification, 1. Entomologicheskoe Obzrenie 71: 900–928. [In Russian, with English translation in Entomological Review, 1993, 72: 109–137]

Belokobylskij SA (1993) On the classification and phylogeny of the Braconid wasps subfamilies Doryctinae and Exothecinae (Hymenoptera: Braconidae). II. Phylogeny. Entomologicheskoe Obzrenie 72: 891–914. [In Russian, with English translation in Entomological Review, 1994, 73: 1–27]

Belokobylskij SA (1995) Principal evolutionary transformations of morphological structures in subfamilies Doryctinae and Exothecinae (Hymenoptera: Braconidae). Entomologicheskoe Obzrenie 74: 153–176. [In Russian, with English translation in Entomological Review, 1996, 75(3): 56–81]

Belokobylskij SA, Zaldivar-Riverón A, Quicke D (2004) Phylogeny of the genera of the parasitic wasps subfamily Doryctinae (Hymenoptera: Braconidae) based on morphological evidence. Zoological Journal of the Linnean Society of London 142: 369–404. doi: 10.1111/j.1096-3642.2004.00133.x

Braet Y (2016) Key to the genera of Doryctinae of the world. www.doryctinaekey.myspecies.info

Ceccarelli FS, Zaldívar-Riverón A (2013) Broad polyphyly and historical biogeography of the neotropical wasp genus *Notiospathius* (Braconidae: Doryctinae). Molecular Phylogenetics and Evolution 69: 142–152. doi: 10.1016/j.ympev.2013.05.001

Ceccarelli FS, Sharkey MJ, Zaldívar-Riverón A (2012) Species identification in the taxonomically neglected, highly diverse Neotropical parasitoid wasp genus *Notiospathius* (Braconidae: Doryctinae) based on an integrative molecular and morphological approach. Molecular Phylogenetics and Evolution 62: 485–495. doi: 10.1016/j.ympev.2011.10.018

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772. doi: 10.1038/nmeth.2109

Fischer M (1981) Versuch einer systematischen gliederung der Doryctinae, insbesondere der Doryctini, und redeskription nach material aus den Naturwissenschaftlichen Museum in Budapest (Hymenoptera, Braconidae). Polskie Pismo Entomologiczne 51: 41–99.

Harris RA (1979) A glossary of surface sculpturing. Occasional Papers of Entomology, California Department of Food and Agriculture 28: 1–31.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. doi: 10.1007/BF01731581

Marsh PM (1997) Subfamily Doryctinae. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) Manual of the New World genera of the family Braconidae (Hymenoptera). Special publication No. 1, International Society of Hymenopterists, Washington, DC, 206–233.

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), New Orleans, LA, 1–8. doi: 10.1109/GCE.2010.5676129

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. doi: 10.1093/sysbio/sys029

Samacá-Sáenz E, Belokobylskij SA, Quicke DLJ, Zaldívar-Riverón (2016) Systematics of the Neotropical braconid wasps of the *Pedinotus* genus group (Doryctinae). Systematic Entomology. doi: 10.1111/syen.12169

Sharkey MJ, Wharton RA (1997) Morphology and Terminology. In: Wharton RA, Marsh PM, Sharkey MJ (Eds) Manual of the New World genera of the familiy Braconidae (Hymenoptera). Special Publication of the International Society of Hymenopterists 1: 19–37.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729. doi: 10.1093/molbev/mst197

Wharton R (2006) The Species of *Sternaulopius* Fischer (Hymenoptera: Braconidae, Opiinae) and the Braconid Sternaulus. Journal of Hymenoptera Research 15: 317–347.

Yu D, van Achterberg C, Horstmann K (2012) Taxapad 2012, Ichneumonoidea 2011. Ottawa, Ontario. http://www.taxapad.com/

Zaldívar-Riverón A, Belokobylskij SA, León-Regagnon V, Briceño R, Quicke DLJ (2008) Molecular phylogeny and historical biogeography of the cosmopolitan parasitic wasp subfamily Doryctinae (Hymenoptera: Braconidae). Invertebrate Systematics 22: 345–363. doi: 10.1071/IS07028

Zaldívar-Riverón A, Belokobylskij SA, León-Regagnon V, Martínez JJ, Briceño R, Quicke DLJ (2007) A single origin of gall association in a group of parasitic wasps with disparate morphologies. Molecular Phylogenetics and Evolution 44: 981–992. doi: 10.1016/j.ympev.2007.05.016

Zaldívar-Riverón A, Martínez JJ, Belokobylskij SA, Pedraza Lara C, Shaw S, Hanson PE, Varela Hernández F (2014) Systematics and evolution of gall formation in the plant-associated genera of the wasp subfamily Doryctinae (Hymenoptera: Braconidae). Systematic Entomology 39: 633–659. doi: 10.1111/syen.12078

Zaldívar-Riverón A, Rodríguez-Jiménez A, Sarmiento CE, Pedraza-Lara C, López-Estrada K (2013) Phylogenetic relationships and description of *Bolivar*, a new genus of Neotropical doryctine wasps (Hymenoptera : Braconidae). Invertebrate Systematics 27: 673–688. doi: 10.1071/IS13021