

Two new cestode species of *Tetragonocephalum* Shipley & Hornell, 1905 (Lecanicephalidea, Tetragonocephalidae) from *Himantura randalli* Last, Manjaji-Matsumoto & Moore (Myliobatiformes, Dasyatidae) from the Gulf of Oman

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Abstract

The original description of the genus *Tetragonocephalum* was published more than one hundred years ago but its taxonomic status was clarified only recently. To date, approximately 30 nominal species of this genus have been described, mostly from the northern Indian Ocean, but nearly half of them are invalid and only 14 species are recognized as valid. In the present study two new species of *Tetragonocephalum* are described from the spiral intestine of *Himantura randalli* from off Jod, on the northern coast of the Gulf of Oman. *Tetragonocephalum sabae* **sp. n.** is distinguishable from the valid species of *Tetragonocephalum* based on number of proglottids (43–53), number of testes (42–50), and size of scolex (401–453×328–455), acetabula (87–109×72–116), mature proglottids (802–1,333×226–336), cirrus sac (92–160×103–154), and eggs (16–19×11–13). *Tetragonocephalum salarii* **sp. n.** can be distinguished from *T. sabae* **sp. n.** and all other valid species of *Tetragonocephalum* based on number of proglottids (77–86). Furthermore, it differs from its congeners based on a combination of some characteristics, including the number of mature (3–7) and gravid (18–20) proglottids, the number of testes (30–38), and the size of acetabula (84–111×80–96), mature proglottids (497–833×334–403), gravid proglottids (1,036–1,482×440–575), testes (20–34×31–50), ovary (123–215×210–278), and eggs (24–45×13–21).

Keywords

Tetragonocephalum sabae sp. n., *Tetragonocephalum salarii* sp. n., *Himantura randalli*, Gulf of Oman

Introduction

Shiple and Hornell (1905) erected *Tetragonocephalum* and described two new species, the type species *T. trygonis* Shiple & Hornell, 1905 from *Himantura walga* (Müller & Henle) (as *Trygon walga*) and *T. aetiobatidis* Shiple & Hornell, 1905 from *Aetobatus ocellatus* (Kuhl) (as *Aetiobatis* [sic] *narinari*), collected from fishes taken from the Gulf of Manaar off the coast of Ceylon (now Sri Lanka). Later, a number of species of this genus were described from several different localities around the world, mostly from the Northern Indian Ocean (Jensen 2005).

Jensen (2005), who revised the order Lecanicephalidea, resolved the taxonomic status of *Tetragonocephalum*, which she considered to include 14 valid species (13 listed and a type species), i.e., *T. alii* Deshmukh & Shinde, 1979, *T. aurangabadensis* Shinde & Jadhav, 1990, *T. bhagawatii* Shinde, Mohekar & Jadhav, 1985, *T. madhulatae* (Andhare & Shinde, 1994) Jensen, 2005, *T. madrasensis* (Andhare & Shinde, 1994) Jensen, 2005, *T. passeyi* Jensen, 2005, *T. raoi* Deshmukh & Shinde, 1979, *T. ratnagiriensis* Shinde & Jadhav, 1990, *T. sephenis* Deshmukh & Shinde, 1979, *T. shiplei* Shinde, Mohekar & Jadhav, 1985, *T. simile* (Pintner, 1928) Ivanov & Campbell, 2000, *T. trygonis*, *T. uarnak* (Shiple & Hornell, 1906) Pintner, 1928, and *T. yamagutii* Muralidhar, 1988; in addition, she recognized three *species inquirendae*, and five species as *nomina nuda*. Since 2006, some new species of *Tetragonocephalum* have been proposed, i.e., *T. govindi* Khamkar & Shinde, 2012 (Khamkar and Shinde 2012); *T. panjiensis* Khamkar, 2011 (Khamkar 2011); *T. pulensis* Kankale, 2014 (Kankale 2014); *T. ratnagiriensis* Khamkar, 2012 (Khamkar 2012); *T. sepheni* Lanka, Hippargi & Patil, 2013 (Lanka et al. 2013). These species do not follow the rules of ICZN, and especially violate Article 16, hence they are unavailable (ICZN 1999).

The only study on *Tetragonocephalum* from the Gulf of Oman was conducted by Golestaninasab et al. (2014) who showed that the genus *Tetragonocephalum* can act as a heavy metal bioindicator in the marine environment.

The present article is the first taxonomic study of the genus *Tetragonocephalum* from the Gulf of Oman. We describe two new species of this genus collected from the spiral intestine of the Arabian banded whiplay, *Himantura randalli* Last, Manjaji-Matsumoto & Moore, 2012.

Materials and methods

A total of 36 specimens of *H. randalli* was collected from northern waters of the Gulf of Oman, 29 individuals in May 2011 and seven in October 2012. They were caught by local fishermen off Jod, Zarabad, Iran (25°26'58"N, 59°30'29"E). Each specimen

was given a unique collection number for author reference. All host individuals were photographed and morphometric and morphological characteristics were recorded to facilitate species identification. Species identity was confirmed using Naylor et al. (2012), Last et al. (2012), and Henderson et al. (2015).

Host specimens were dissected along the mid-ventral line; spiral intestines were removed and opened by a longitudinal incision. Subsequently, spiral intestines were fixed in 10% seawater buffered formalin, shaken vigorously, and held for approximately seven days. The samples were then transferred to the Zoology Laboratory, School of Biology, University of Tehran for detailed examination of parasitic infection.

Spiral intestines and intestinal contents were examined under a stereomicroscope. Tapeworms were carefully removed from the spiral intestine and washed in distilled water for about one hour before being preserved in 70% ethanol. Parasite specimens were prepared as whole mounts for light microscopy observation according to Koch et al. (2012).

Whole mounts were studied using a Leica DM500 light microscope. Images of *Tetragonocephalum* specimens were taken using a Leica ICC50 HD color digital camera mounted on the Leica DM500 light microscope (Buffalo Grove, Illinois, United States) and measurements were taken using the image analysis software Leica Application Suite (LAS EZ v.3.0.0) (Leica 2013). Measurements were analyzed in IBM® SPSS® Statistics Package v.22 (IBMCorp. 2013). All measurements of the reproductive organs were taken from mature proglottids. Measurements are given in micrometers (μm) unless otherwise indicated; they are given as the range followed in parentheses by the mean, number of worms examined, and the total number of measurements if more than one measurement was taken per worm. Illustrations were prepared with Adobe® Illustrator® CC (Adobe Incorp. 2013) based on the drafts sketched under a Reichert Biovar microscope with the aid of a drawing tube.

Some scoleces were prepared for ultrastructural studies using SEM following the protocol of Jensen (2005). The specimens were sputter coated with approximately 10 nm of gold/palladium, and examined with a field emission scanning electron microscope (HIT4160102, Hitachi, Tokyo, Japan) at the School of Electrical and Computer Engineering (ECE), University of Tehran. Microthrix terminology follows Chervy (2009).

Type and voucher specimens are deposited at The Zoological Museum, University of Tehran, Tehran, Iran (ZUTC), and The Natural History Museum, London, England (BMNH).

Results

Tetragonocephalum sabae sp. n.

<http://zoobank.org/5FA0CE20-885D-4433-9B69-1C830667FD86>

Type-materials. Holotype: Slide. Original label: “*Tetragonocephalum sabae*; Roohi Aminjan & Malek; Holotype; Canada balsam; ID: A. Roohi Aminjan & M. Malek;

Scolex, proglottid & worm drawn; ZUTC Platy. 1500; (AR-1009u); ex *Himantura randalli*; spiral intestine; Gulf of Oman, Iran; Coll. May 2011"; Zoological Museum, University of Tehran, Tehran (ZUTC). **Paratypes:** Two slides, ZUTC Platy. 1501 and ZUTC Platy. 1503, from the same host individual as the holotype. **Other materials:** Two scoleces for SEM, ZUTC Platy. 1502s and ZUTC Platy. 1504s (two stubs) and their whole-mounted vouchers, ZUTC Platy. 1502v and ZUTC Platy. 1504v (two slides), from the same host individual as the holotype.

Type locality. Off Jod (25°26'58"N; 59°30'29"E), Zarabad, Iran, Gulf of Oman. Other localities: None.

Type host. *Himantura randalli* Last, Manjaji-Matsumoto & Moore, 2012, the Arabian banded whipray (Myliobatiformes, Dasyatidae) [host no. MM1009]. Additional hosts: None. Site of infection: Spiral intestine. Prevalence: 2.78% (one of 36 individuals examined). Intensity: Five specimens.

Diagnosis. *Tetragonocephalum sabae* sp. n. can be distinguished from all the other valid species of *Tetragonocephalum* by the number of proglottids and testes, the size of scolex, acetabula, mature proglottids, cirrus sac, and eggs.

Description (Figures 1 and 3a–d). Based on three whole mounts of gravid specimens and two scoleces prepared for SEM and their vouchers (partially measured).

Worms 23.2–32.4 (27.5; 3) mm long, apolytic; maximum width 398–489 (425; 5) at posterior-most gravid proglottid; with 42–53 (48; 3) proglottids (Figure 1a). Scolex 401–453 (426; 5) long by 328–455 (383; 5) wide, consisting of scolex proper and apical organ. Scolex proper 197–267 (234; 5) long by 328–455 (383; 5) wide, bearing four acetabula. Acetabula sucker-like in form, 87–109 (94; 3; 12) long by 72–116 (98; 3; 12) wide. Apical modification of scolex proper cylindrical, bearing apical organ. Apical organ muscular, with glandular surface, 178–216 (198; 5) long by 321–352 (344; 5) wide, non-invaginate, non-retractable (Figures 1b–c and 3a).

Apical organ covered with tubercles suggestive of glandular surface (Figure 3b). Microtriches on apical modification of scolex proper and scolex proper not observed (Figure 3c). Strobila covered with capilliform filitriches (Figure 3d).

Cephalic peduncle absent. Proglottids acraspedote. Immature proglottids 32–41 (36; 3) in number, initially wider than long, gradually becoming longer than wide (5–8 [7; 3] of immature proglottids longer than wide); two posterior-most immature proglottids 583–857 (746; 5; 10) long by 245–314 (278; 5; 10) wide. Mature proglottids 2 (2; 5) in number; two posterior-most mature proglottids 802–1,333 (1,074; 5; 10) long by 226–336 (287; 5; 10) wide (Figure 1d). Gravid proglottids 8–10 (9; 4) in number; two posterior-most gravid proglottids 2,311–2,910 (2,522; 4; 8) long by 398–489 (425; 4; 8) wide (Figure 1e).

Testes oval to spherical, 42–50 (46; 5; 10) in number, 28–47 (40; 5; 30) long by 37–57 (47; 5; 30) wide, extending from anterior margin of proglottid to anterior margin of cirrus sac, in multiple irregular columns in dorso-ventral view, four layers deep in cross section. Vas deferens extending from level of anterior margin of ovary to cirrus sac, entering cirrus sac at distal end (Figure 1d). External seminal vesicle absent. Internal seminal vesicle present, visible in gravid proglottids. Cirrus sac oval or

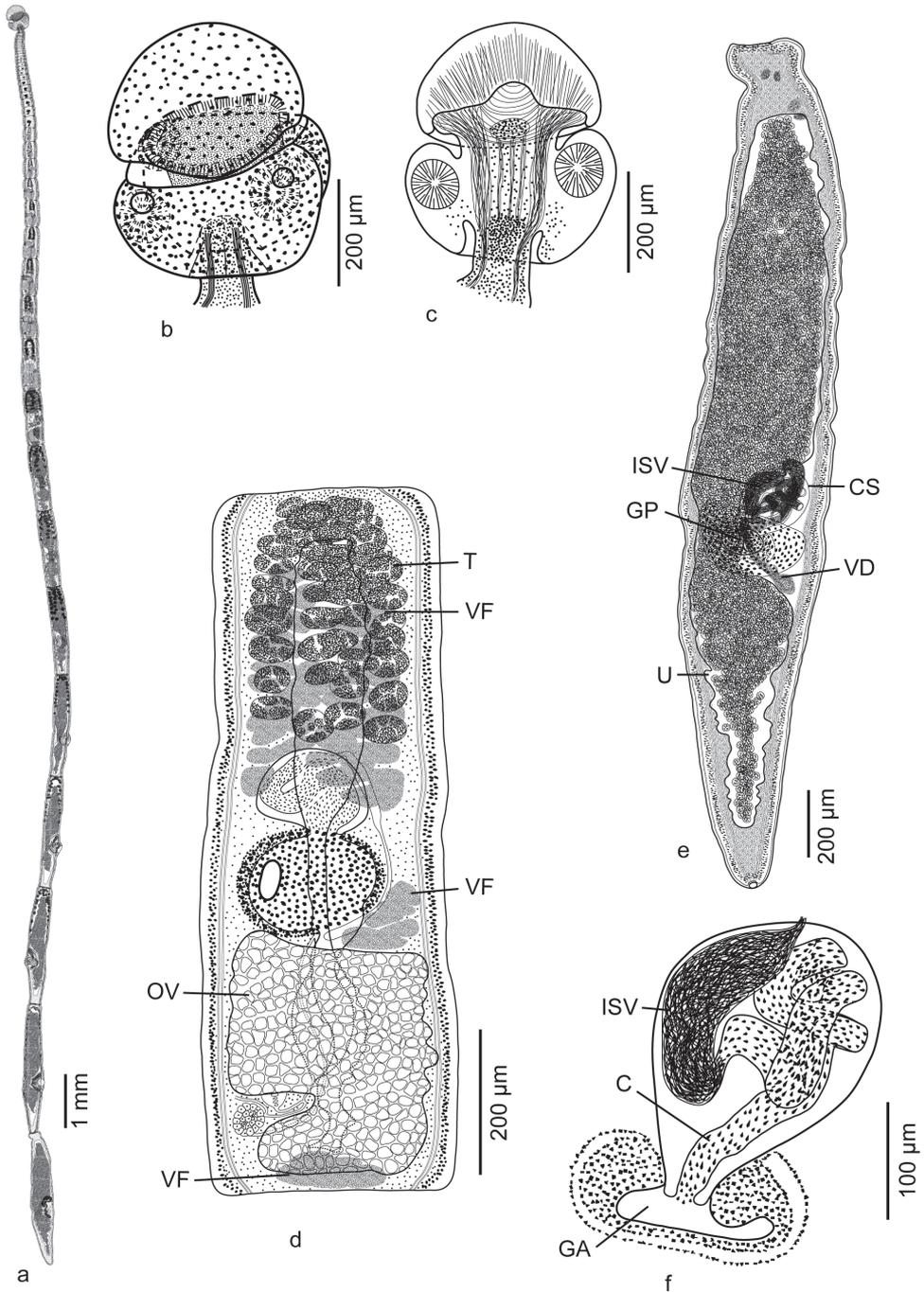


Figure 1. Line drawings of *T. sabae* sp. n. from *H. randalli*. **a** Whole worm **b** Whole structure of scolex **c** Internal details of scolex **d** Mature proglottid **e** Gravid proglottid **f** Cirrus sac. Abbreviations: C, Cirrus; CS, Cirrus Sac; GA, Genital Atrium; GP, Genital Pore; ISV, Internal Seminal Vesicle; T, Testis; OV, Ovary; U, Uterus; VD, Vas Deferens; VF, vitelline follicle.

spherical in form, angled anteriorly, 92–160 (123; 5; 10) long by 103–154 (124; 5; 10) wide, containing coiled cirrus. Cirrus armed with spinitriches (Figure f). Ovary oblong in dorso-ventral view, incomplete ring-shaped in cross-section, 290–428 (357; 5; 10) long by 175–279 (217; 5; 10) wide, symmetrical. Mehlis' gland posterior to ovarian bridge, 41–83 (60; 5; 10) long by 43–76 (59; 5; 10) wide. Vagina extending along median line from ootype to genital atrium, opening into genital atrium posterior to cirrus sac; vaginal sphincter absent. Genital pores lateral (sub-marginal in some segments, Figure 1d), irregularly alternating, 37–43% (40; 5; 10) of proglottid length from posterior end. Genital atrium expanded, conspicuous. Uterus bisaccate, extending along median line of proglottid from posterior margin of ovary to anterior margin of proglottid, constricted at level of genital atrium; uterine duct entering uterus at level of posterior margin of genital atrium. Vitellarium follicular; vitelline follicles medullary, 28–40 (33; 5; 30) long by 57–73 (64; 5; 30) wide, in three fields; anterior field anterior to genital atrium stopping short of anterior margin of proglottid; middle field between genital atrium and ovary; posterior field posterior to ovary (Figure 1d). Osmoregulatory ducts in two lateral pairs. Eggs single, lacking polar filaments, 16–19 (17; 4; 12) long by 11–13 (12; 4; 12) wide, adhering to one another in uterus; embryonated in older gravid proglottids.

Etymology. This species is named in honor of the first author's wife, Saba Saadati Safa, for her unconditional support and patience over the last five years.

Remarks. The possession of a bisaccate uterus and other characteristics clearly place this new species in the genus *Tetragonocephalum*. With respect to the 14 valid species of *Tetragonocephalum*, *T. sabae* sp. n. has a greater number of testes (42–50) than *T. bhagawatii* (37–38), *T. sephenis* (36–38), *T. shipleyi* (12) and fewer than *T. aurangabadensis* (105–110), *T. madrasensis* (125–130), *T. passeyi* (54–73), *T. raoi* (50–55), and *T. yamagutii* (54–56). It possesses more proglottids (42–53) than *T. uarnak* (30–40) and fewer than *T. alii* (55–60), *T. simile* (75), and *T. trygonis* (60). *Tetragonocephalum sabae* sp. n. differs from *T. madhulatae* in the size of the mature proglottids (802–1,333×226–336 *vs* 1,359–1,455×295–334) and eggs (16–19×11–13 *vs* 52–57×38–43); and from *T. ratnagiriensis* in the size of the scolex (401–453×328–455 *vs* 843×469–537), acetabula (87–109×72–116 *vs* 130×111), and cirrus sac (92–160×103–154 *vs* 213×86–188).

***Tetragonocephalum salarii* sp. n.**

<http://zoobank.org/5053B3A5-A29C-4E04-B2C6-D3AE518C31ED>

Type-materials. Holotype: Slide. Original label: “*Tetragonocephalum salarii*; Roohi Aminjan & Malek; Holotype; Canada balsam; ID: A. Roohi Aminjan & M. Malek; Scolex, proglottid & worm drawn; ZUTC Platy. 1546; (AR-1245c); ex *Himantura randalli*; spiral intestine; Gulf of Oman, Iran; Coll. Oct 2012”; Zoological Museum, University of Tehran, Tehran (ZUTC). **Paratypes:** Two slides, ZUTC Platy. 1547 and ZUTC Platy. 1548, from the same host individual as the holotype. **Other materials:**

One scolex for SEM, ZUTC Platy. 1549s (one stub) and its whole-mounted voucher, ZUTC Platy. 1549v (one slide), from the same host individual as the holotype.

Type locality. Off Jod (25°26'58"N; 59°30'29"E), Zarabad, Iran, Gulf of Oman. Other localities: None.

Type host. *Himantura randalli* Last, Manjaji-Matsumoto & Moore, 2012, the Arabian banded whipray (Myliobatiformes, Dasyatidae) [host no. MM1245]. Additional hosts: none. Site of infection: spiral intestine. Prevalence: 2.78% (one of 36 individuals examined). Intensity: Four specimens.

Diagnosis. *Tetragonocephalum salarii* sp. n. can be distinguished from *T. sabae* sp. n. and all other valid species of *Tetragonocephalum* by the number of mature and gravid proglottids, the number of testes; and the size of acetabula, mature proglottids, gravid proglottids, testes, ovary, and eggs.

Description (Figures 2 and 3e–h). Based on three whole mounts of gravid specimens and one scolex prepared for SEM and its voucher (partially measured).

Worms 23.5–35.9 (27.8; 3) mm long, apolytic; maximum width 440–575 (517; 3) at posterior-most gravid proglottid; with 76–86 (80; 3) proglottids (Figure 2a). Scolex 453–501 (472; 3) long by 406–480 (441; 3) wide, consisting of scolex proper and apical organ. Scolex proper 258–300 (276; 3) long by 390–457 (426; 3) wide, bearing four acetabula. Acetabula sucker-like in form, 84–111 (96; 3; 12) long by 80–96 (90; 3; 12) wide. Apical modification of scolex proper cylindrical, bearing apical organ. Apical organ muscular, with glandular surface, 178–233 (201; 3) long by 406–480 (441; 3) wide, non-invaginable, non-retractable (Figures 2b–c and 3e).

Apical organ covered with tubercles suggesting glandular surface (Figure 3f). Microtriches not observed on apical modification of scolex or on scolex proper (Figure 3g). Strobila covered with capilliform filitriches (Figure 3h).

Cephalic peduncle absent. Proglottids acraspedote. Immature proglottids 55–62 (58; 3) in number, initially wider than long, gradually becoming longer than wide (7–21 [14; 3] immature proglottids longer than wide); two posterior-most immature proglottids 464–696 (523; 4; 8) long by 295–393 (346; 4; 8) wide. Mature proglottids 3–7 (5; 4) in number; two posterior-most mature proglottids 497–833 (672; 4; 8) long by 334–403 (365; 4; 8) wide (Figure 2d). Gravid proglottids 18–20 (19; 3) in number; two posterior-most gravid proglottids 1,036–1,482 (1,239; 3; 6) long by 440–575 (517; 3; 6) wide (Figure 2e).

Testes oval, 30–38 (34; 4; 8) in number, 20–34 (26; 4; 24) long by 31–50 (40; 4; 24) wide, restricted to anterior quarter of proglottid, in multiple irregular columns in dorso-ventral view, four layers deep in cross section. Vas deferens extending from level of anterior margin of ovary to cirrus sac, entering cirrus sac at distal end (Figure 2d). External seminal vesicle absent. Internal seminal vesicle present. Cirrus sac oval in form, angled anteriorly, 53–88 (68; 4; 8) long by 120–160 (141; 4; 8) wide, containing coiled cirrus (Figures 2d–e). Cirrus armed with spinitriches. Ovary oblong to quadrate in dorso-ventral view, incomplete ring-shaped in cross-section, 123–215 (182; 4; 8) long by 210–278 (240; 4; 8) wide, symmetrical. Mehlis' gland posterior to ovary, 39–59 (48; 4; 8) long by 48–62 (54; 4; 8) wide. Vagina extending along median

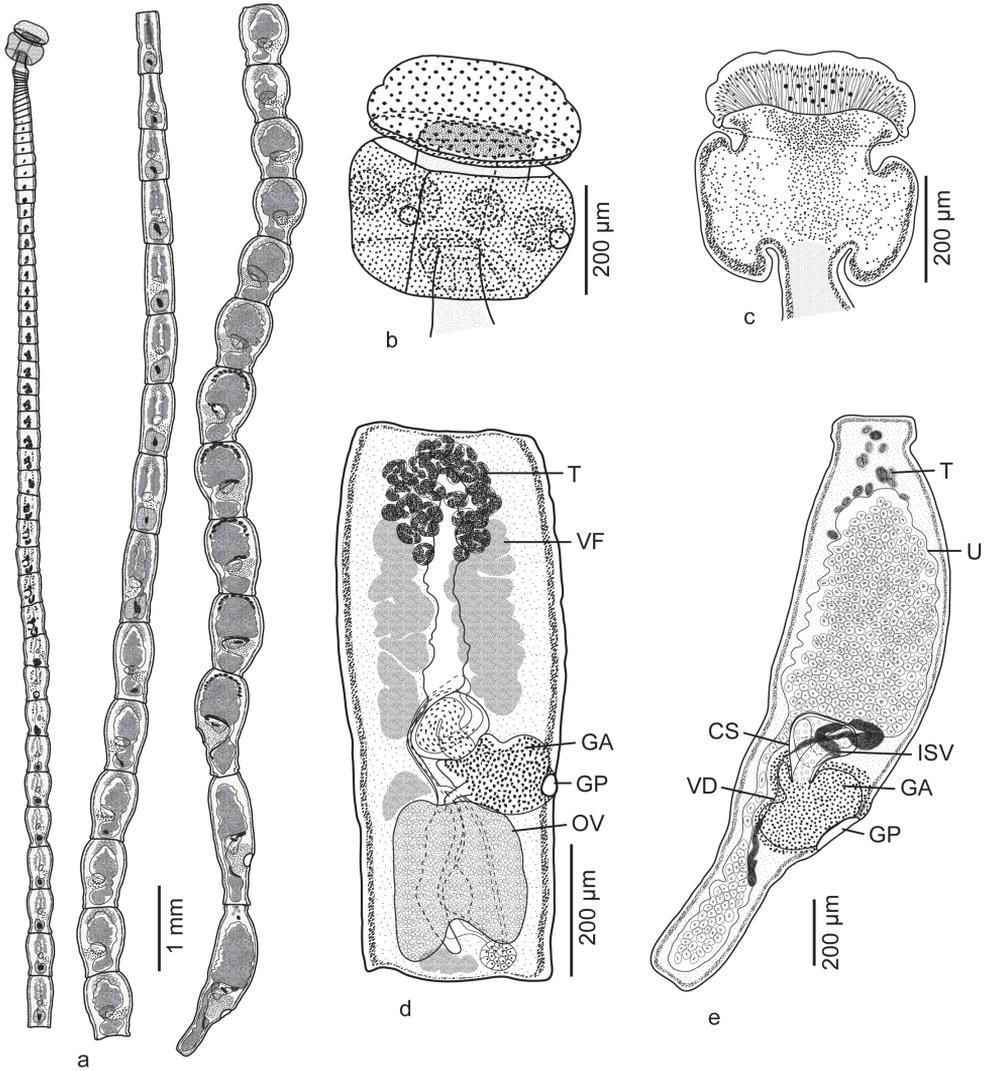


Figure 2. Line drawings of *T. salarii* sp. n. from *H. randalli*. **a** Whole worm **b** Whole structure of scolex **c** Internal details of scolex **d** Mature proglottid **e** Gravid proglottid. Abbreviations: CS, Cirrus Sac; GA, Genital Atrium; GP, Genital Pore; ISV, Internal Seminal Vesicle; T, Testis; OV, Ovary; U, Uterus; VD, Vas Deferens; VF, vitelline follicle.

line from ootype to genital atrium, opening into genital atrium posterior to cirrus sac; vaginal sphincter absent. Genital pores lateral, irregularly alternating, 30–36% (33; 4; 8) of proglottid length from posterior end. Genital atrium massive, conspicuous. Uterus bisaccate, extending along median line of proglottid from posterior margin of ovary to anterior margin of proglottid, constricted at level of genital atrium; uterine duct entering uterus at level of posterior margin of genital atrium. Vitellarium follicular; vitelline follicles medullary, 38–48 (44; 4; 24) long by 57–69 (65; 4; 24) wide, in

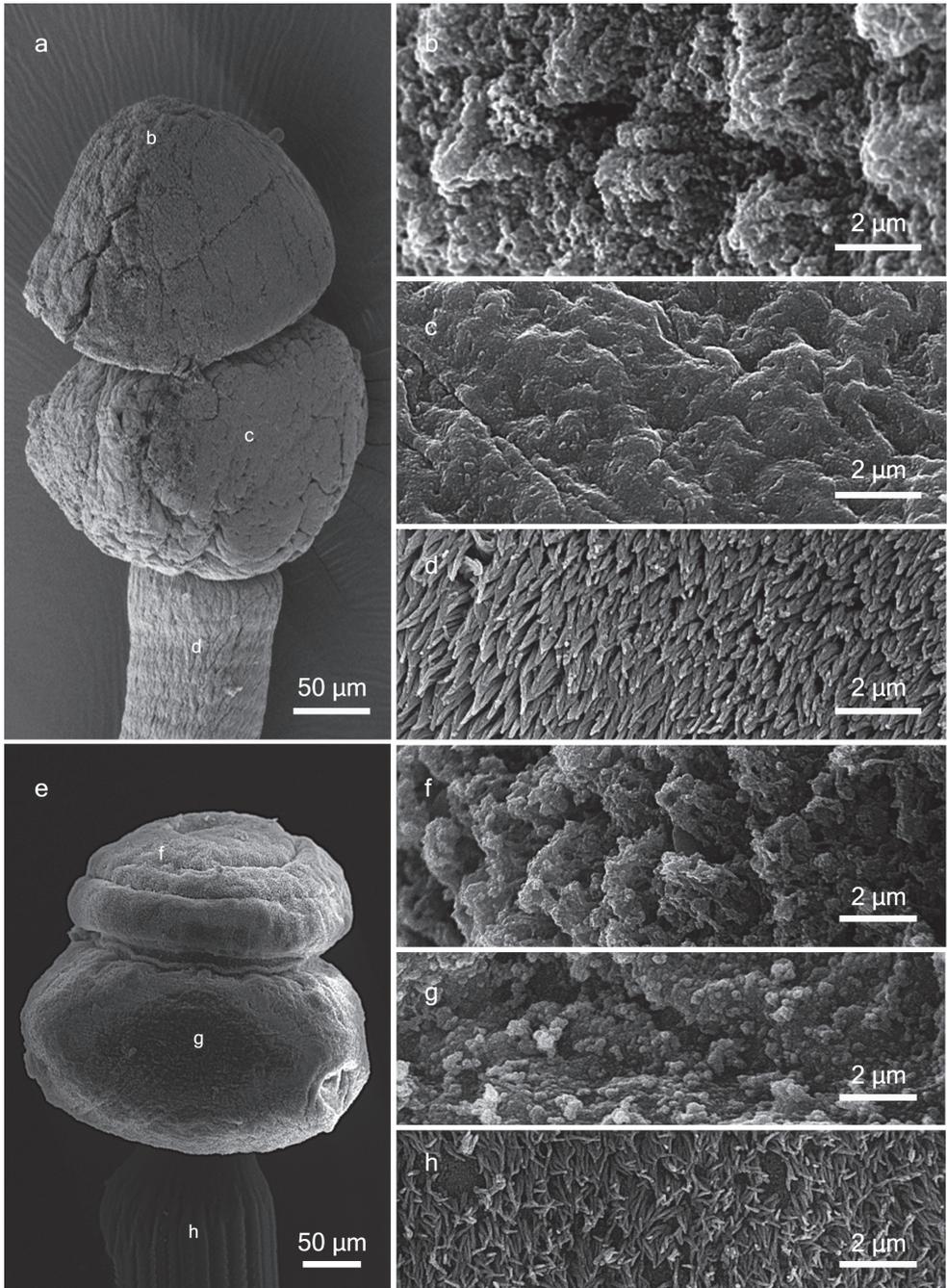


Figure 3. SEM micrographs of *T. sabae* sp. n. (**a-d**) and *T. salarii* sp. n. (**e-h**) from *H. randalli*. **a, e** Scolex **b, f** Tubercles on apical organ surface **c, g** Surface of scolex proper **d, h** Capilliform filitriches on strobila.

three fields; anterior field anterior to genital atrium stopping short of anterior margin of proglottid; middle field generally between genital atrium and ovary; posterior field posterior to ovary (Figure 2d). Osmoregulatory ducts in two lateral pairs. Eggs single, lacking polar filaments, 24–45 (34; 3; 9) long by 13–21 (17; 3; 9) wide, adhering to one another in uterus; embryonated of older gravid proglottids.

Etymology. This species is named in honor of Mr. Naser Salari in gratitude for his assistance with the collection of host specimens.

Remarks. *Tetragonocephalum salarii* sp. n. possesses the characteristics of the genus and can be distinguished from *T. sabae* sp. n. and all 14 valid congeners based on the following characteristics. *Tetragonocephalum salarii* sp. n. differs from *T. sabae* sp. n. in the total number of proglottids (77–86 *vs* 42–53), mature proglottids (3–7 *vs* 2), gravid proglottids (18–20 *vs* 8–10), and testes (30–38 *vs* 42–50); and in the size of mature proglottids (497–833×334–403 *vs* 802–1,333×226–336), gravid proglottids (1,036–1,482×440–575 *vs* 2,311–2,910×398–489), and eggs (24–45×13–21 *vs* 16–19×11–13). It has a greater number of testes (30–38) than *T. shingleyi* (12) and fewer than *T. alii* (40–45), *T. aurangabadensis* (105–110), *T. madhulatae* (45), *T. maderasensis* (125–130), *T. passeyi* (54–73), *T. raoi* (50–55), *T. ratnagiriensis* (40–44), and *T. yamagutii* (54–56). This new species has more proglottids (77–86) than *T. sephenis* (20–25), *T. simile* (75), *T. trygonis* (60), and *T. uarnak* (30–40). *Tetragonocephalum salarii* sp. n. differs from *T. bhagawatii* in the size of the acetabula (84–111×80–96 *vs* 56 dia.), testes (20–34×31–50 *vs* 18 dia.), and gravid proglottids (1,036–1,482×440–575 *vs* 860–920×300–360).

Discussion

The genus *Tetragonocephalum* possesses the following characteristics: unique bisaccate uterus, acraspedote strobila, testes distributed anterior to the cirrus sac, ovary C-shaped in cross-section, conspicuously enlarged genital atrium and pore (Ivanov and Campbell 2000, Jensen 2005, Jensen et al. 2016). The specimens described here as two new species of *Tetragonocephalum* from *H. randalli* from the Gulf of Oman are consistent with the current concept of the genus.

The genus *Tetragonocephalum* has a very controversial taxonomic history (Jensen 2005). After being erected (Shingley and Hornell 1905), between 1906 and 2000, the taxonomic status of this genus remained uncertain, accepted as valid by some authors, but only as a synonym of *Tylocephalum* Linton, 1890 by others (Ivanov and Campbell 2000, Jensen 2005). The validity of *Tetragonocephalum* was confirmed by Ivanov and Campbell (2000) in a revision of this genus and *Tylocephalum*. Further on, Jensen (2005) revised the order Lecanicephalidea, and Jensen et al. (2016) carried out a comprehensive molecular phylogenetic study of the order Lecanicephalidea. In the most recent revision of *Tetragonocephalum*, Jensen (2005) considered this genus to consist of 14 valid species.

The fourteen valid and the current two new species of *Tetragonocephalum* differ from each other based on the morphometric characteristics, including the number of testes, mature, and gravid proglottids and the size of the scolex, acetabula, testes, cirrus sac, ovary, eggs, mature and gravid proglottids.

The two new species were collected from different host individuals. One out of twenty-nine host specimens collected in May 2011 was parasitized by *T. sabae* sp. n. (n = 5) and one out of seven in October 2012 by *T. salarii* sp. n. (n = 4). These cestodes appear to be rare in the waters near Jod, Iran, Gulf of Oman. The occurrence of these species does not appear to be by chance, as collections were made over two years and at two different seasons. Furthermore, these host records do not appear to be aberrant or due to accidental infection because fish of the genus *Himantura* are known hosts for *Tetragonocephalum* spp. (Jensen 2005, Jensen et al. 2016), and many members of the Lecanicephalidea tend to have a high degree of host-specificity. This is often recorded for congeneric hosts; for example, *Tylocephalum* in the genus *Rhinoptera* Cuvier (see Ivanov and Campbell 2000) or *Hexacanalisis* Perrenoud, 1931 in the genus *Gymnura* van Hasselt (Cielocha and Jensen 2011).

In order to compare the new and valid species, the original descriptions of valid ones were used. However, there are some limitations and uncertainties which undermine comprehensive and detailed comparisons. Some original descriptions are incomplete and lacking important morphometric data. For example, there are no appropriate measurements and/or drawings of the internal organs in the original descriptions of *T. trygonis* and *T. uarnak* (Shipley and Hornell 1905, 1906); Pintner (1928) introduced *T. simile* as a new species without formal description and provided five drawings. Another problem in comparisons between different congeneric species of *Tetragonocephalum* is unreliable data provided in some original descriptions. Considering the descriptions of previous species, it seems that some measurements might be incorrect; for example, the maximum width of *T. trygonis* (30 at scolex [probably 300?]) (Shipley and Hornell 1905). As a result, such measurements should be treated with caution. Furthermore, it appears that some features that are common to different species of the genus *Tetragonocephalum* were described differently in various species, such as absence of cephalic peduncle, spatial pattern of testes, shape of ovary, and armature of cirrus. Also, there are some features, which have not been taken into account so far and might be important in species identification, such as the attachment pattern of apical organ to scolex proper modification and strobila to scolex proper, the degrees of overlap between fields of testes and anterolateral vitelline follicles, and the histological structure of testes.

In conclusion, the problematic taxonomic status of some previously described species, due to inappropriate and/or incomplete descriptions, and type materials which are either unspecified or missing, make it necessary to designate neotypes and re-describe all previously described species from the type hosts and localities, except for *T. passeyi* which is thoroughly described by Jensen (2005).

Acknowledgements

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A new species of *Melithaea* (Anthozoa, Octocorallia, Melithaeidae) from the Oman Sea, off Oman

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Abstract

A new species, *Melithaea davidi* **sp. n.**, is described from the eastern coast of Oman, Oman Sea, in the northwestern Indian Ocean, where it differs from its congeners in lacking capstans and having predominantly spindles in the coenenchyme. A molecular phylogenetic analysis of mtMutS and 28S rDNA genes suggests that it is genetically distinct from similar species in the Red Sea. Furthermore, a species previously reported as *Acabaria* sp. from the Arabian Sea is now identified as *Melithaea mabahissi* (Hickson, 1940).

Keywords

Persian Gulf, octocorals, Indian Ocean, Middle East, northwest Indian Ocean

Introduction

Melithaeidae, one of the 49 presently recognized families of octocorals (Ofwegen and McFadden 2010; McFadden and Ofwegen 2012, 2013; Breedy et al. 2012), has only two valid genera (Reijnen et al. 2014) and numerous species distributed worldwide. They are sessile, benthic, and colonial organisms that require a hard substrate for settlement and anchorage. The melithaeid species are widespread in marine environments, from shallow to deep sea, but are most abundant in warm and tropical waters (Fabricius and Alderslade 2001). Their geographical distribution ranges from East Africa and the Red Sea (Grasshoff 2000), Indian Ocean (Thomson 1916; Ofwegen 1987,

1989; Williams 1992), Indo-West Pacific (Ofwegen 1987; Grasshoff 1999; Ofwegen et al. 2000; Hoeksema and van Ofwegen 2004, 2008; Matsumoto and Ofwegen 2015) to Hawaii (Bayer 1956). In spite of this vast distribution range, only a few species of melithaeids have been reported from the northwestern Indian Ocean (the coasts of the Arabian Sea, the Red Sea, the Gulf of Aden, the Oman Sea, and the Persian Gulf). These include *Clathraria omanensis* off Oman in the Oman Sea, *Acabaria* spec. indet. 2 from West India in the Arabian Sea (Ofwegen 1987), *Acabaria* spec. indet. 1 from Kenya and Somalia, and *Acabaria mabahissi* from Somalia (Hickson 1940). Although no melithaeid species have so far been recorded and sampled from the shallow coastal waters of the Persian Gulf (Samimi-Namin and van Ofwegen 2009, 2012), several colonies have been distinguished in video footage and photographs taken from mesophotic depths (> 40 m) of the Strait of Hormuz (pers. obs.).

Melithaeidae is an uncommon family in the north-western Indian Ocean, and its maximum depth extends beyond that of conventional diving activities. This might explain the scarce records and rarity of these species within this region where they have not been documented in major coral studies (see Sheppard and Salm 1988; Sheppard and Sheppard 1999; Sheppard et al. 2000; Claereboudt 2006; Samimi-Namin and van Ofwegen 2016a, b). In contrast, this family is relatively common in the shallow warm waters of the central Indo-Pacific and the Coral Triangle (Fabricius and Alderslade 2001; Hoeksema and van Ofwegen 2004, 2008).

It is known that the Arabian Sea and Oman Sea have a complex hydrography, mainly caused by seasonal monsoons. The summer southwest monsoon generates one of the five largest upwelling areas of the world (Bakun et al. 1998), whereas the winter northeast monsoon reverses the circulation pattern and increases the biological production of the whole northern Indian Ocean (Burkill 1999, Wilson 2000). In spite of these hydrographical and geological complexities which might induce high endemism and diversity, there are still many species and habitats yet to be discovered and documented from shallow and deep waters of the north-western Indian Ocean. Traditionally six genera have been recognized in Melithaeidae based on the sclerite morphology of their coenenchyme: *Acabaria* Gray, 1859 and *Asperaxis* Alderslade, 2006, both with a variety of spindles and occasionally a few thorn-clubs; *Clathraria* Gray, 1859, with a complete layer of capstans, and double heads; *Melithaea* Linnaeus, 1758, with predominantly asymmetrical double-discs, a few leaf-clubs and thorn-clubs; *Mopsella* Gray, 1857, with leaf-clubs and thorn-clubs at the surface of the coenenchyme; and *Wrightella* Gray, 1870, with foliate spheroids (Hickson 1937; Ofwegen 1987, Grasshoff 2000). In the literature, numerous species have been described as being intermediate between these genera, with considerable variation and overlap in their sclerite morphology. Several researchers have pointed this out and have suggested a revised classification system for this family (Grasshoff 1999, 2000; Fabricius and Alderslade 2001; Reijnen et al. 2014). Although the latest study by Reijnen et al. (2014) considers all the genera in the classification proposed by Hickson (1937) as variations across geographical regions and suggests the merger of all these genera into one, i.e. *Melithaea*, the taxonomic situation of this group of octocorals has not been resolved satisfactorily and the family has to

be formally revised. Based on the previous taxonomic system, an undescribed species discovered in the Oman Sea falls into a group with the coenenchyme predominated by spindles and occasionally thorn-clubs (*Acabaria/Asperaxis*). However, to avoid further confusion we follow Reijnen et al. (2014) regarding generic classification, considering two valid genera in the Melithaeidae: *Melithaea*, and *Asperaxis*, with the latter genus only reported from Australia.

In this paper, we describe a new species of the genus *Melithaea* from approximately 80 m depth, off the coast of Oman.

Abbreviations

NBC	Naturalis Biodiversity Center, Leiden, The Netherlands; previously National Museum of Natural History (NNM); formerly Rijksmuseum van Natuurlijke Historie (RMNH)
RMNH	Rijksmuseum van Natuurlijke Historie, Leiden, currently NBC
UNESCO-IOC	United Nations Educational, Scientific and Cultural Organization-Intergovernmental Oceanographic Commission
ZMTAU	Zoological Museum, Tel Aviv University.

Material and methods

In situ observations and material collection was conducted in 2013, during a deep water dive in the Oman Sea (Fig. 1). *In situ* photographs were taken using a small compact underwater camera and the depth recorded using a dive computer. In total three colonies were collected and were preserved in ethanol. In order to identify the material, sclerites were obtained by dissolving the tissues in 10% sodium hypochlorite, followed by rinsing in fresh water. For scanning electron microscopy (SEM), the sclerites were carefully rinsed with double-distilled water, dried at room temperature, mounted on a stub with double-sided carbon tape, then coated with gold-palladium (AuPd), and examined using a Jeol 6480LV SEM operated at 10 kV.

All specimens are deposited at Naturalis Biodiversity Center, Leiden, the Netherlands (formerly Rijksmuseum van Natuurlijke Historie, Leiden, the Netherlands).

Molecular and phylogenetic analyses

DNA was extracted from specimen RMNH Coel. 42122 using the Qiagen DNEasy Blood & Tissue Kit. Published primers and protocols (Reijnen et al. 2014) were used to obtain DNA sequences for fragments of the mitochondrial mtMutS (486 nt) and nuclear 28S rDNA (670 nt) genes. The two genes were aligned with the concatenated mtMutS plus 28S sequences of Reijnen et al. (2014), and the dataset was then re-ana-

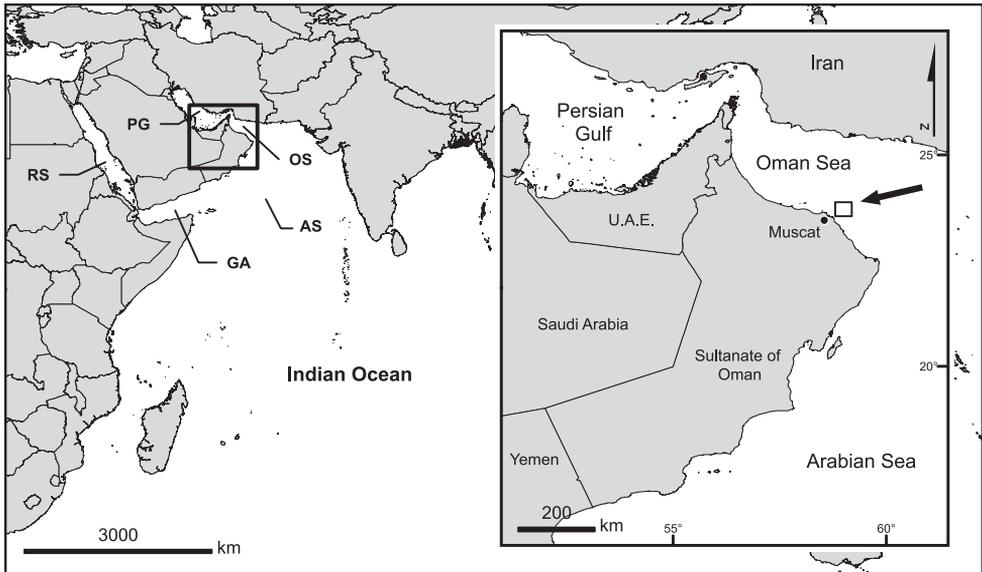


Figure 1. Type locality of *Melithaea davidi* sp. n. in the Oman Sea, off Muscat governorate. RS = Red Sea, PG = Persian Gulf, GA = Gulf of Aden, OS = Oman Sea, AS = Arabian Sea.

lysed using both maximum likelihood and Bayesian approaches. Maximum likelihood trees were constructed using PhyML (Guindon and Gascuel 2003) with the GTR+I+ Γ model of evolution and 100 bootstrap replicates. Bayesian analyses were run using MrBayes v. 3.2.1 (Ronquist et al. 2012) with that same model of evolution applied. Analyses were run for 2 million generations (until standard deviation of split partitions < 0.01) with a burn-in of 25% and default Metropolis coupling parameters. MEGA v.5 (Tamura et al. 2011) was used to calculate pairwise measures of genetic distance (Kimura 2-parameter) among sequences.

Molecular and phylogenetic results

Maximum likelihood and Bayesian analyses of mtMutS plus 28S rDNA recovered identical tree topologies (Fig. 2) and strong to moderate support for the same geographically structured clades found by Reijnen et al. (2014) in their analysis of a four-gene dataset. Specimen RMNH Coel. 42122 fell within a well-supported clade of species from the Red Sea including *M. erythraea* (Ehrenberg, 1834), *M. sinaica* (Grasshoff, 2000), and *M. rubrinodis* (Gray, 1859). It was well differentiated genetically from all three Red Sea species, differing from them by average genetic distance values (Kimura 2-parameter) ranging from 0.6–2.5% at mtMutS and 2.8–3.45% at 28S, values that are greater than those typically observed among conspecific octocorals (McFadden et al. 2014).

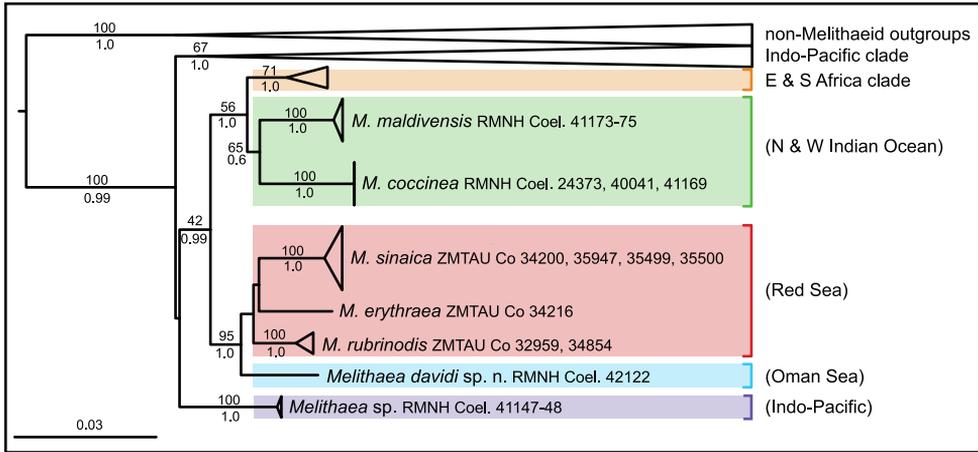


Figure 2. Maximum likelihood phylogeny of family Melithaeidae based on concatenated nucleotide sequences for mtMutS (486 nt) and 28S rDNA (670 nt) (sequence data for all but RMNH Coel. 42122 are from Reijnen et al. 2014). Well-supported clades of species from the Indo-Pacific, east and south Africa, and outgroup taxa (*Annella*, *Chironophthya*, *Euplexaura*, *Siphonogorgia*, *Solenocaulon*) have been collapsed to improve readability. Numbers above branches are maximum likelihood bootstrap percentages; numbers below branches are Bayesian posterior probabilities. Generic assignments follow the recommendations of Reijnen et al. (2014).

Morphological descriptions and systematic account

Class Anthozoa Ehrenberg, 1831

Subclass Octocorallia Haeckel, 1866

Order Alcyonacea Lamouroux, 1812

Family Melithaeidae Gray, 1870

Subfamily Melithaeinae Alderslade, 2006

Genus *Melithaea* Milne Edwards, 1857

Diagnosis. Colonies with segmented axis, and swollen nodes and straight internodes containing cigar-shaped sclerites. Densely branched in one or more planes, forming large fans or forming bushes. Sclerites of coenenchymal surface are spindles, thorn-clubs, double discs, leaf clubs, and foliate spheroids. Polyps monomorphic, small and retractile. Calyces can be low or tall. Polyps contain spindle-like and club-like forms arranged as collaret and points, with dragon wing sclerites (flattened, more or less twisted, boomerang-shaped platelets commonly with the convex edge serrated near the wider end; present in the proximal part of tentacles/see Grasshoff 1999, 2000) in the tentacles. The colonies can be yellow, orange, red, dark purple, pink, and white. Axes are usually coloured, often red. Azooxanthellate.

***Melithaea davidi* sp. n.**

<http://zoobank.org/62E50344-EFEF-4F94-8007-346D0AE2A9EF>

Figures 3–7

Material examined. *Holotype*: RMNH Coel. 42122, Oman, Oman Sea, 23.654267°N 58.629567°E, 79 m deep on a ship wreck, Robert's barge, coll. David Mothershaw and Robin Norman, 19 July 2013. *Paratypes*: RMNH Coel. 42123, RMNH Coel. 42124, same data as holotype.

Description. The holotype is branching dichotomously in several parallel planes, forming a network with many anastomoses. It is 12 cm high and 9 cm wide (Figure 3). The nodes are larger and more swollen in the basal parts of the colony. Many branches are covered with tiny white ophiuroids.

Polyp mounds and calyces are up to 1 mm in diameter. Calyces are projecting above the coenenchyme and are mostly situated along the sides of the branches. Polyps are situated 1–1.5 mm apart from each other (Figure 3). The coenenchyme has spindles, up to 0.35 mm long, with irregular simple tuberculation (Figure 4A). Additionally, the calyces have clubs, also up to 0.35 mm long, with simple tubercles and leaf-like projections at the distal end (Figure 4B). The calyx sclerites are mostly arranged en chevron. Some of the coenenchymal spindles have leaf-like or spinose side projections (Figure 5). In addition, there are some irregularly shaped sclerites present, up to 0.15 mm long, with or without leaf-like and spinose projections. Capstans are not present in the coenenchyme.

Polyps have two rows of collaret spindles and four spindles per point. The collaret spindles are up to 0.40 mm long, with more tuberculation on the middle of the convex side, and less tuberculation at the distal ends (Figure 6A).

The point sclerites are up to 0.25 mm long, with simple tubercles and projecting spines at the distal end (Figure 6B).

The tentacles contain flattened, dragon-wing shaped sclerites up to 0.15 mm long (Figure 6C).

The pharynx and introvert have small spiny sclerites that are up to 0.05 mm long (Figure 6D).

The nodes and internodes have internal rods and cigar-shaped sclerites up to 0.12 mm long, with or without median whorl of projections (Figure 6E).

Etymology. The species is named after David Mothershaw who collected the specimens.

Colour. The holotype is orange-red (Figure 3A). The colour of the nodes in younger parts of the colony is the same as the colony colour but in the older basal parts of the colony, they are brownish. All sclerites are reddish. The live colony had the same colour as the preserved one, with whitish translucent polyps (Figure 7).

Morphological variation. One paratype (RMNH Coel. 42124) is light pink (Figures 3C, 7B).

Remarks. The species resembles *Melithaea biserialis* (Kükenthal, 1908) and *M. sinaica* Grasshoff, 2000, both described from the nearby Red Sea. *M. biserialis* and *M.*



Figure 3. Colonies of *Melithaea davidi* sp. n.; **A** holotype, RMNH Coel. 42122 **B** paratype, RMNH Coel. 42123 **C** paratype, RMNH Coel. 42124.

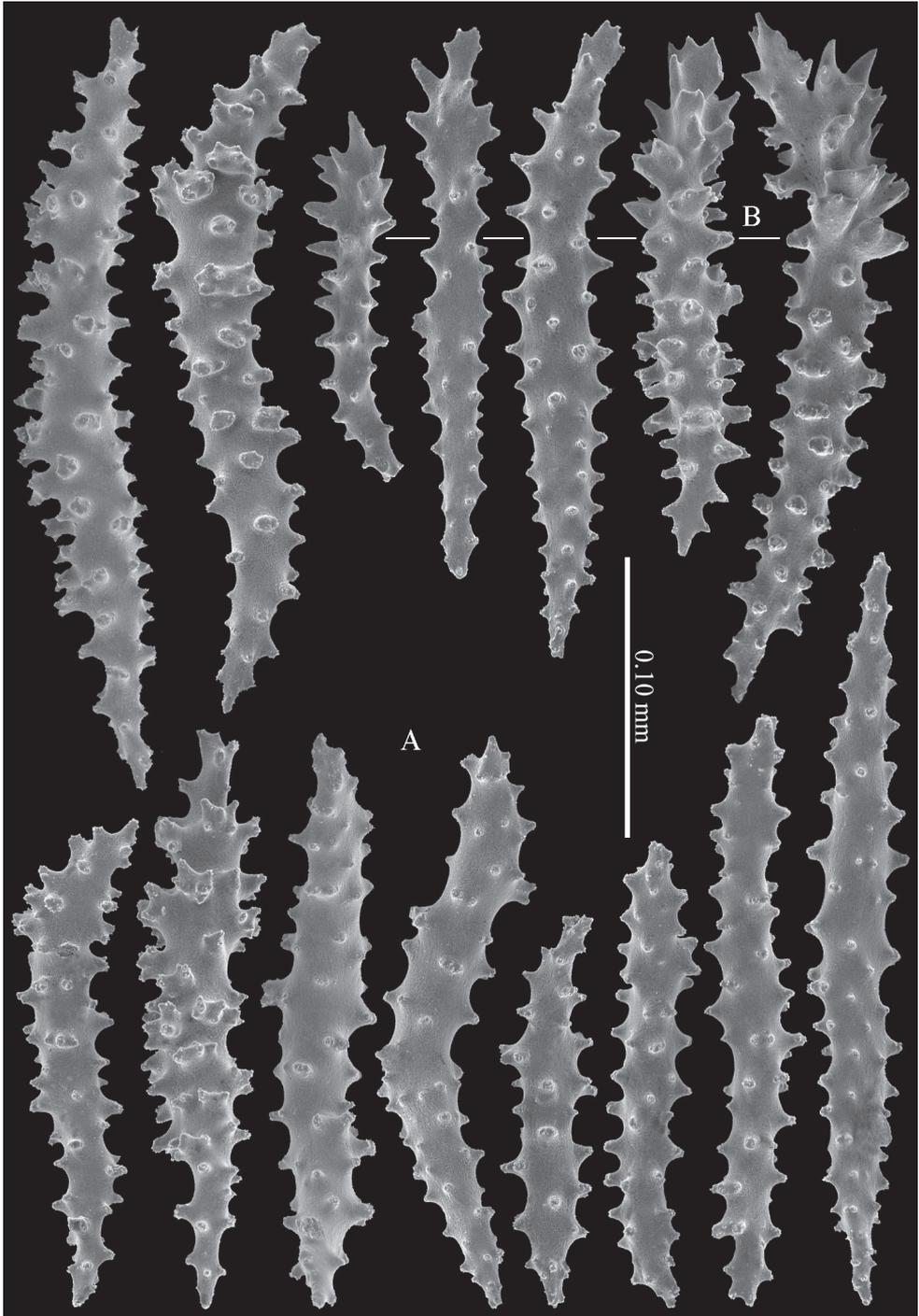


Figure 4. Coenenchymal sclerites of *Melithaea davidi* sp. n., holotype, RMNH Coel. 42122; **A** spindles
B clubs.

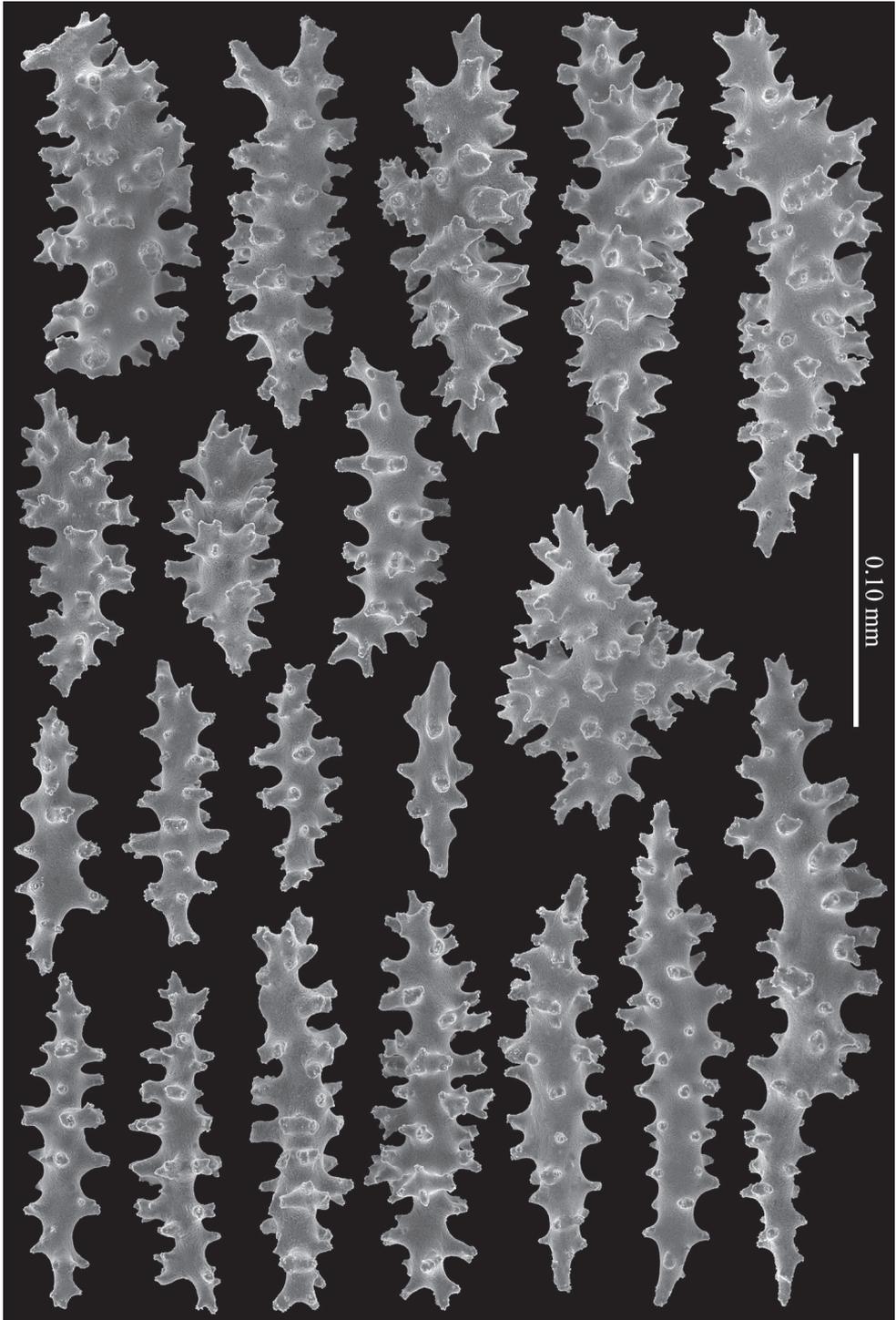


Figure 5. Coenenchymal sclerites of *Melithaea davidi* sp. n., holotype, RMNH Coel. 42122.

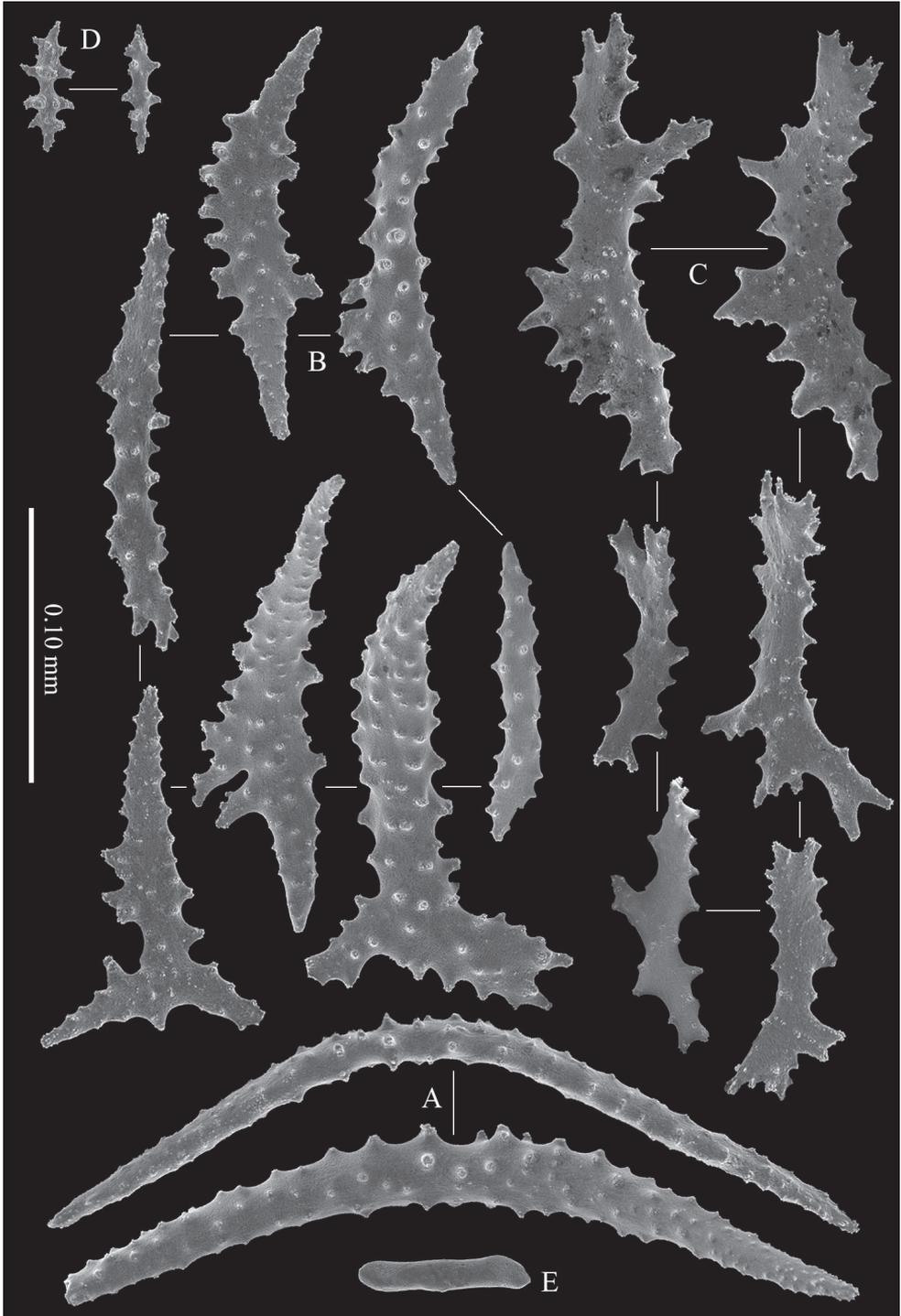


Figure 6. Polyp sclerites of *Melithaea davidi* sp. n., holotype, RMNH Coel. 42122; **A** collaret sclerites **B** point sclerites **C** tentacle sclerites **D** Pharynx sclerites **E** node sclerite.

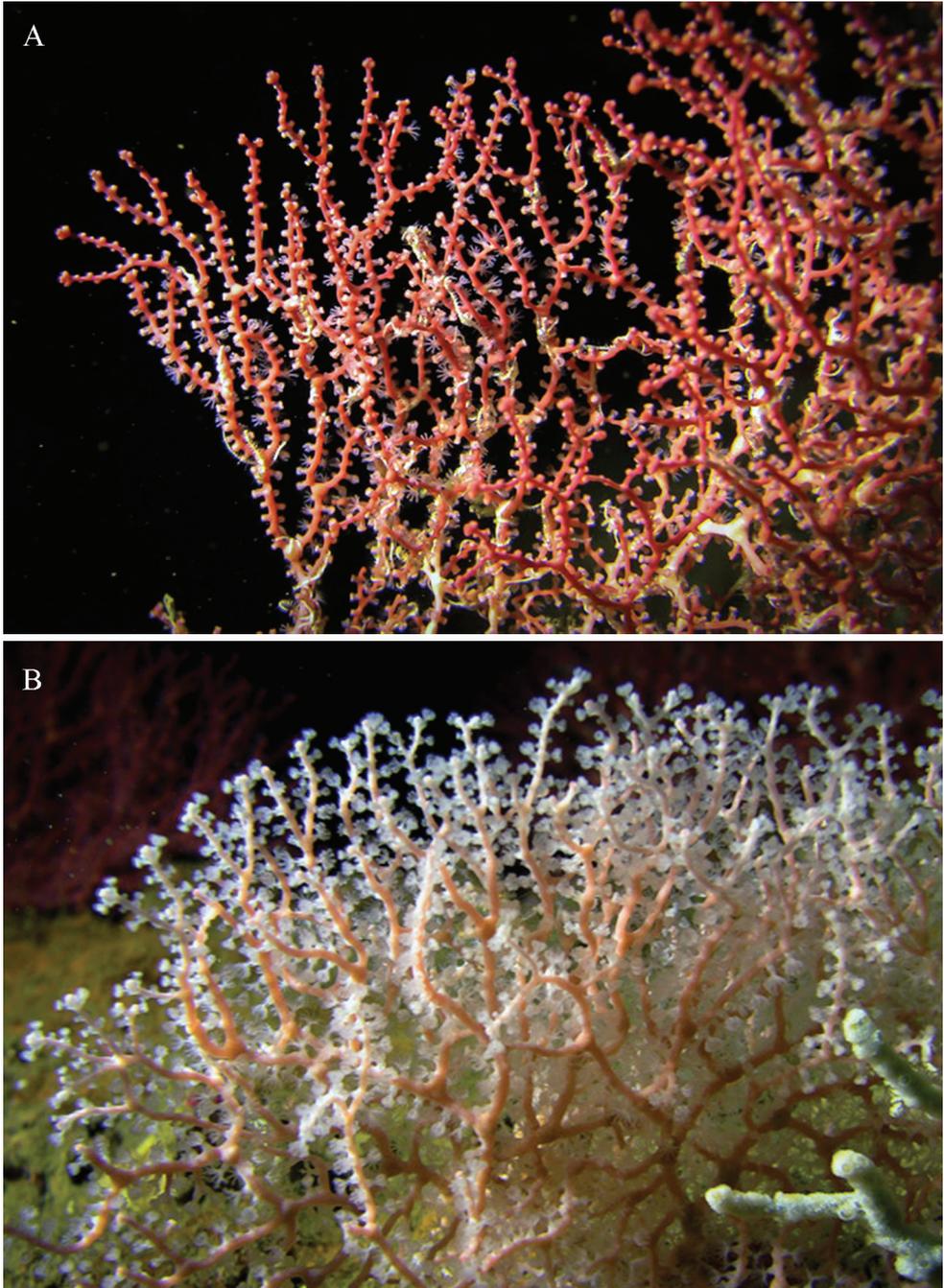


Figure 7. Underwater photographs of *Melithaea davidi* sp. n. at 79 m depth. **A** Holotype, RMNH Coel. 42122 **B** paratype, RMNH Coel. 42124.

sinaica both have more tuberculate sclerites and, additionally, capstans that are not present at all in *M. davidi*. The species also resembles *Acabaria* spec. indet. 2 Ofwegen (1987) from West India. However, that species also has capstans that are absent in *M. davidi*.

Acabaria indet. 2 Ofwegen (1987) might represent a new species, however, the material is not sufficient for describing a new species.

Acabaria mabahissi Hickson, 1940, off Cape Guardafui, Gulf of Aden, and the Arabian Sea is the same as *Acabaria* spec. indet. 1 (Ofwegen 1987) from Somalia and Kenya.

Distribution. Known only from the type locality.

Discussion

Reijnen et al. (2014) observed that melithaeid species appear to be grouped phylogenetically by geographical region, suggesting high regional endemism in this family. Our reanalysis of their mtMutS and 28S sequence data reflects this pattern, with species from the western Indo-Pacific (Indonesia, Malaysia, Japan, Palau, etc.), east and south African coasts (Tanzania, South Africa), northern and western Indian Oceans (Seychelles, Maldives), and the Red Sea separated into distinct well-supported clades (Figure 2). Therefore, the likelihood of species having wide geographical ranges is low, and consequently we did not compare the new species with similar-looking species occurring in other geographical regions. The molecular phylogenetic analysis suggests that *M. davidi* is closely related to but distinct from several other species found in the Red Sea region for which we had sequence data for comparison. Although we did not have sequence data for *Melithaea biserialis* or *Acabaria* spec. indet. 2 reported by Ofwegen (1987), morphological differences support the distinction of those species from *M. davidi*.

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A new species of pea crab of the genus *Serenotheres* Ah Yong & Ng, 2005 (Crustacea, Brachyura, Pinnotheridae) from the date mussel *Leiosolenus* Carpenter, 1857 (Mollusca, Bivalvia, Mytilidae, Lithophaginae) from the Solomon Islands

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Abstract

The pea crab genus *Serenotheres* Ah Yong & Ng, 2005 (Pinnotheridae) is currently only represented by one species, *S. besutensis* (Serène, 1967). A new species is now assigned to this genus, described from a date mussel *Leiosolenus obesus* Carpenter, 1857 (Mollusca: Bivalvia: Mytilidae: Lithophaginae) collected in the Solomon Islands. *Serenotheres janus* **sp. n.** differs from *S. besutensis* in possessing a conspicuously broader carapace, with the lateral margins of the dorsal lamellum distinctly produced and the posterolateral part deeply concave, the dorsal lamellum being highest at the median cleft, the rostrum is relatively more prominent, the surfaces of the anterolateral margin and hepatic region are less prominently pitted and eroded, the ischiomerus of the third maxilliped is relatively more rectangular, and the P2 merus is proportionately longer.

Keywords

Pinnotheridae, taxonomy, symbiotic crab, new species, symbiotic crab, *Serenotheres janus*, Solomon Islands

Introduction

Ahyong and Ng (2005) revised the species in the Indo-West Pacific pinnotherid genera *Durckheimia* De Man, 1889 (type species *Durckheimia carinipes* De Man, 1889) and *Xanthasia* White, 1846 (type species *Xanthasia murigera* White, 1846), and established two new genera, namely *Serenotheres* Ahyong & Ng, 2005, for *Durckheimia besutensis* Serène, 1967; and *Tridacnatheres* Ahyong & Ng, 2005, for *Xanthasia whitei* De Man, 1888. They commented that *Serenotheres* differed from all pinnotherid genera not only by the unusual carapace which has an additional large plate above its normal carapace surface (a dorsal lamellum) which overhangs the frontal margin, but also by possessing a two-segmented third maxilliped palp (Ahyong and Ng 2005: 121). It is also the only known pinnotherid associated with the rock-boring bivalves of the mytilid subfamily Lithophagidae (see Schmitt et al. 1973).

In this paper, a new species of *Serenotheres* is described from the Solomon Islands.

Material and methods

The specimen examined is deposited in the U.S. National Museum for Natural History (USNM), Smithsonian Institution, Washington D.C.

The following abbreviations are used: MXP3 = third maxilliped; P2–P5 = pereopods 2–5 (first to fourth ambulatory legs), respectively. Measurements (in millimetres) are of the carapace width and length, respectively. The terminology used essentially follows that in Manning (1993) and Ahyong and Ng (2005).

Molecular data

A mtDNA COI barcode was generated from this individual following standard Sanger sequencing protocols as outlined in Meyer (2003). PCR primers jgLCO1490 and jgHCO2198 (Geller et al. 2013) were used. The resulting sequence is ACCCTTATATTTTATCTTCGGAGCTTGGGCAGGTATAGTAGGAACCTCTT-TAAGTTTAATAATTCGAGCTGAACTTAGACAACCAGGCAGACTTATTG-GAAATGACCAAATTTATAATGTAATAGTTACAGCCCATGCTTTTGTT-ATAATTTTCTTTATAGTTATAACCAATTATAATCGGAGGCTTCGGAACT-GATTAGTTCCTTTAATACTTGGGGCCCCAGATATAGCATTC CCTCG-TATAACAATATAAGATTTTGACTCTTACCTCCATCTTTTATCACTCT-TACTTACAAGAAGAATAGTTGAAAGTGGAGTAGGAACAGGATGAACT-GTTTATCCTCCTCTAGCTTCAGCTATTGCCCATGCTGGAGCTTCTG-TAGATTTAGGAATTTTCTCGCTTCATTTGGCCGGTGTATCGTCAATCT-TAGGAGCAGTAAATTTTATTACTACTGTAATTAATATACGATCATATG-GAATAATGATAGACCAAATACCACTATTTGTCTGATCAGTATTTATCAC-CGCAATCCTCCTACTTCTATCCCTACCGGTTCTAGCAGGAGCTATTAC-

CATACTATTAACAGATCGTAATCTAAATACCTCATTCTTTGACCCAGCCG-GTGGTGGAGATCCTGTTCTCTATCAACATTATTT. This record is deposited in Genbank under submission number KX949585.

Systematics

Family Pinnotheridae De Haan, 1833

Genus *Serenotheres* Ah Yong & Ng, 2005

Serenotheres janus sp. n.

<http://zoobank.org/FD849337-EB57-46F6-965D-8CECBADECD5F>

Figs 1–6

Type material. Holotype ♀ (8.9 × 7.9 mm) (USNM 1421642), in *Leiosolenus obesus* (Philippi, 1847) (Mollusca: Bivalvia: Mytilidae: Lithophaginae), from Njari Island, New Georgia, Solomon Islands, station SOLOM_026; 8.01374°S, 156.75649°E, coll. C. Meyer, 9 October, 2014.

Diagnosis. Carapace distinctly pentagonal; lateral margins of dorsal carapace lamellum distinctly produced with posterolateral part deeply concave, highest at median cleft with 2 halves sloping gently outwards in direct frontal view; rostrum distinct with surface above antennular fossa prominently concave; surfaces of anterolateral margin and hepatic region less prominently pitted, eroded; MXP3 ischiomerus relatively more rectangular; P2 merus relatively long.

Description. Carapace deep, prismatic, pentagonal in dorsal view, distinctly broader than long; anterior, lateral, and dorsal surfaces pitted (Figs 1B, 2, 3, 5A). Posterior carapace margin wide, with median part gently concave (Figs 1B, 2A, B, 5A). Frontal and antero-dorsal part of carapace raised to form distinct dorsal lamellum (Figs 1, 2, 5A). Dorsal lamellum projecting anteriorly, forming eave which overhangs true frontal margin and orbits; margins thin, glabrous; surface gently concave, regions not clearly defined, gastro-cervical grooves just visible; anterior margin acute, forming a false front, separated into 2 rounded lobes by shallow median cleft; highest at edges of median cleft, with 2 lateral halves sloping gently outwards in direct frontal view (Figs 1B, 2, 3, 4A, B). Dorsal lamellum connected to rostrum by distinct broad, longitudinal median ridge; lateral junction between dorsal lamellum and true anterolateral margin marked by distinct concavity (Figs 3A, B). Surface between dorsal lamellum and true frontal and anterolateral margins deeply concave (Figs 3, 4A, B). True anterolateral margins subcristate, convex, lined with dense, short clavate setae which obscures margins (Figs 2C, 3A, 4B). True frontal margin triangular, separated into 2 low lobes by shallow concavity, lined with dense short clavate setae which completely obscures margin (Figs 3, 5B). Ventral surface of front with 2 pronounced depressions, above antennular fossae, not setose (Figs 3, 5B). Antennular fossae round, margins lined with dense short clavate setae which obscures margins; antennules folding obliquely (Figs 3B, C, 5B).

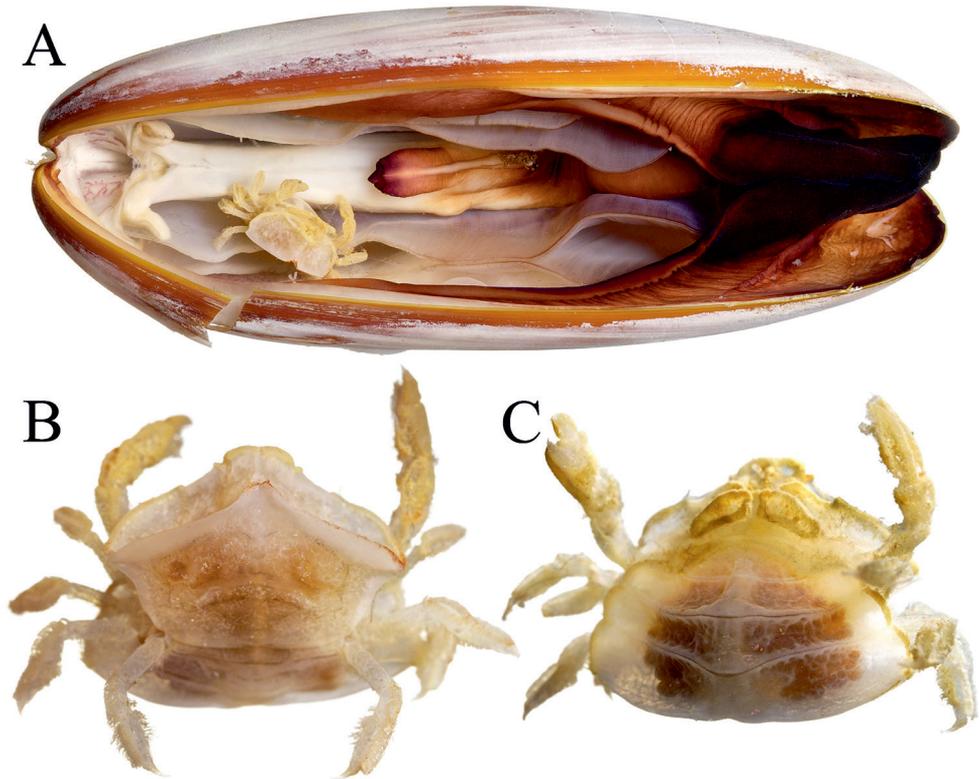


Figure 1. Colour in life. *Serenotheres janus* sp. n., holotype ♀ (8.9 × 7.9 mm) (USNM). **A** in situ in host date mussel, *Leiosolenus obesus* **B** dorsal view **C** ventral view. Photographs courtesy of Zachariah Kobrinsky and David Liittschwager.

Antenna with proximal 2 articles fused, immovably lodged in epistome; basal article (article 2) large, subrectangular; articles 3–5 increasingly smaller, with flagellum very short, not extending beyond orbit (Fig. 5B). Orbit small, not visible from dorsal view; cornea small, pigmented black, peduncle short; eyes just visible in frontal view (Figs 3B, C, 5B). Subhepatic region with deep, broad, slightly oblique groove, cristae delimiting groove lined with dense, short clavate setae (Figs 3, 5B). Pterygostomial region gently concave, forming shallow oblique groove (Figs 3, 5B). Epistome transversely subrectangular; median part of posterior margin gently concave (Figs 3B, 5B). Buccal cavity wide, margins lined with dense short setae (Fig. 3B, C).

MXP3 inserted obliquely, completely filling buccal cavity; outer surface and margins covered with short clavate setae which obscures structure, setae on longitudinal median part of ischiomerus distinctly less dense (Fig. 3B, C); ischium and merus completely fused without trace of suture; ischiomerus broadly subovate, longer than broad, outer surface with broad, shallow longitudinal sulcus, distal margins convex; palp 2-segmented, inserted on inner surface of ischiomerus, just below distal margin, carpus globose, dactylus conical, subspatulate, longer than carpus, extending beyond

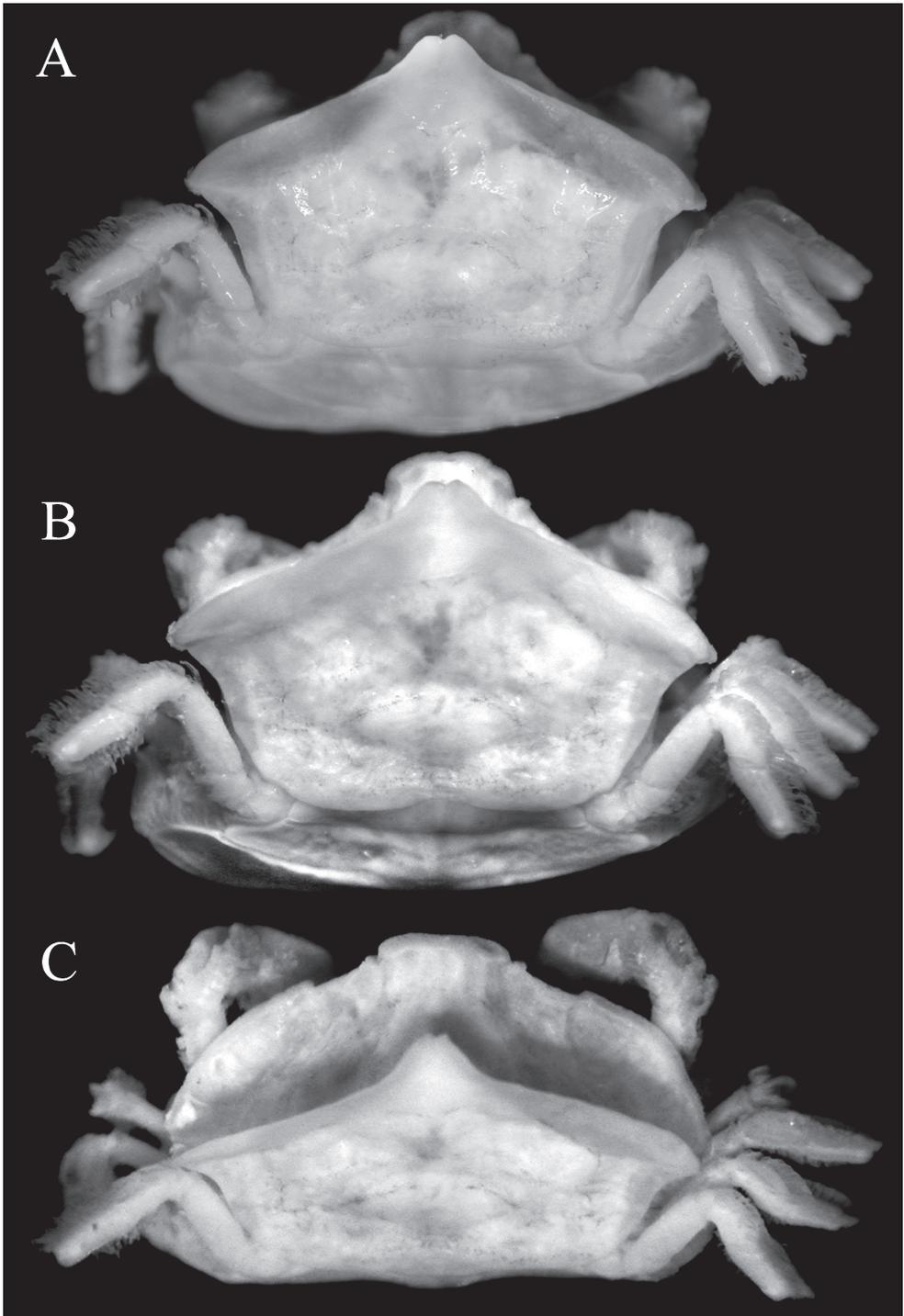


Figure 2. *Serenotheres janus* sp. n., holotype ♀ (8.9 × 7.9 mm) (USNM). Dorsal views of cephalothorax.

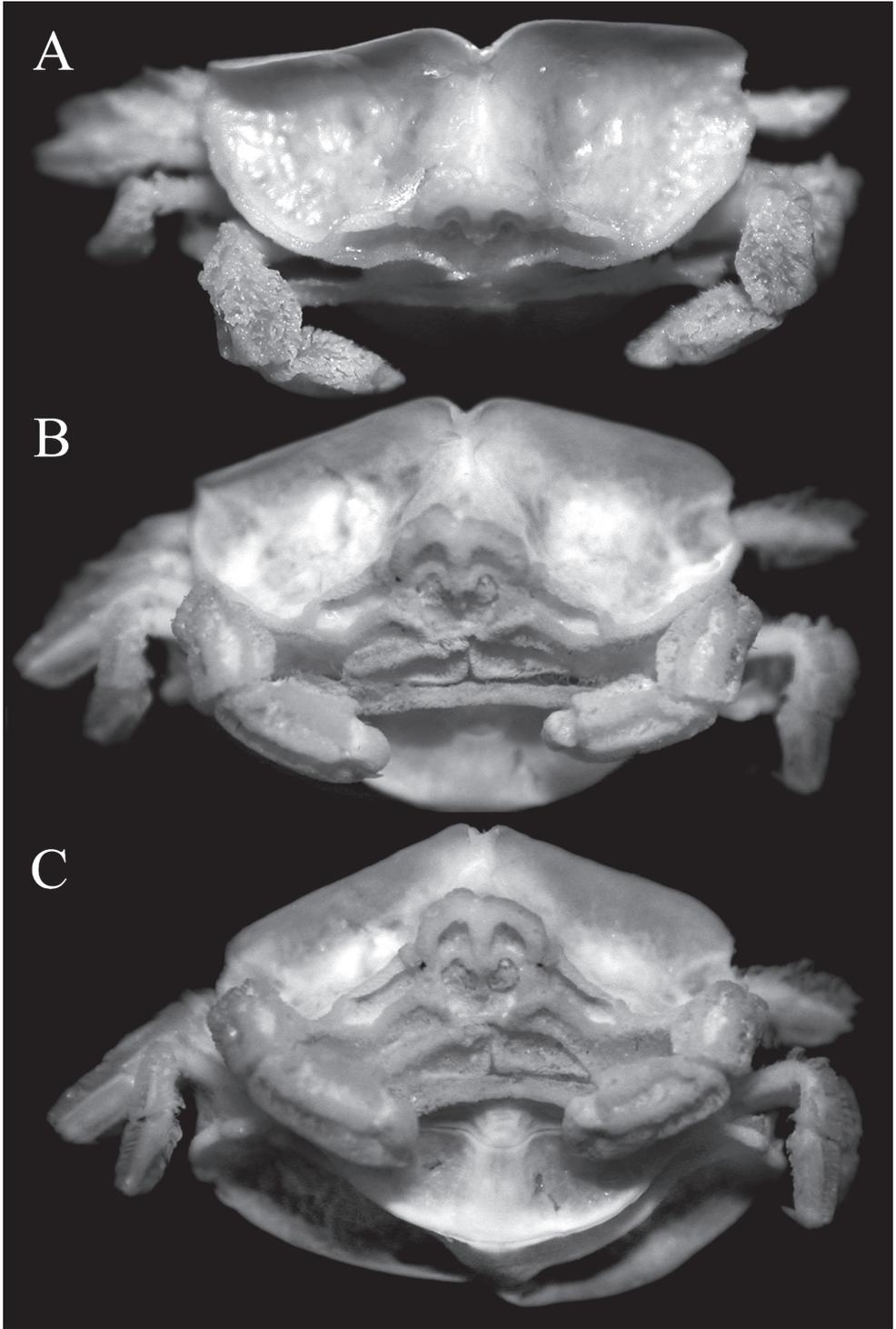


Figure 3. *Serenotheres janus* sp. n., holotype ♀ (8.9 × 7.9 mm) (USNM). Frontal views of cephalothorax.

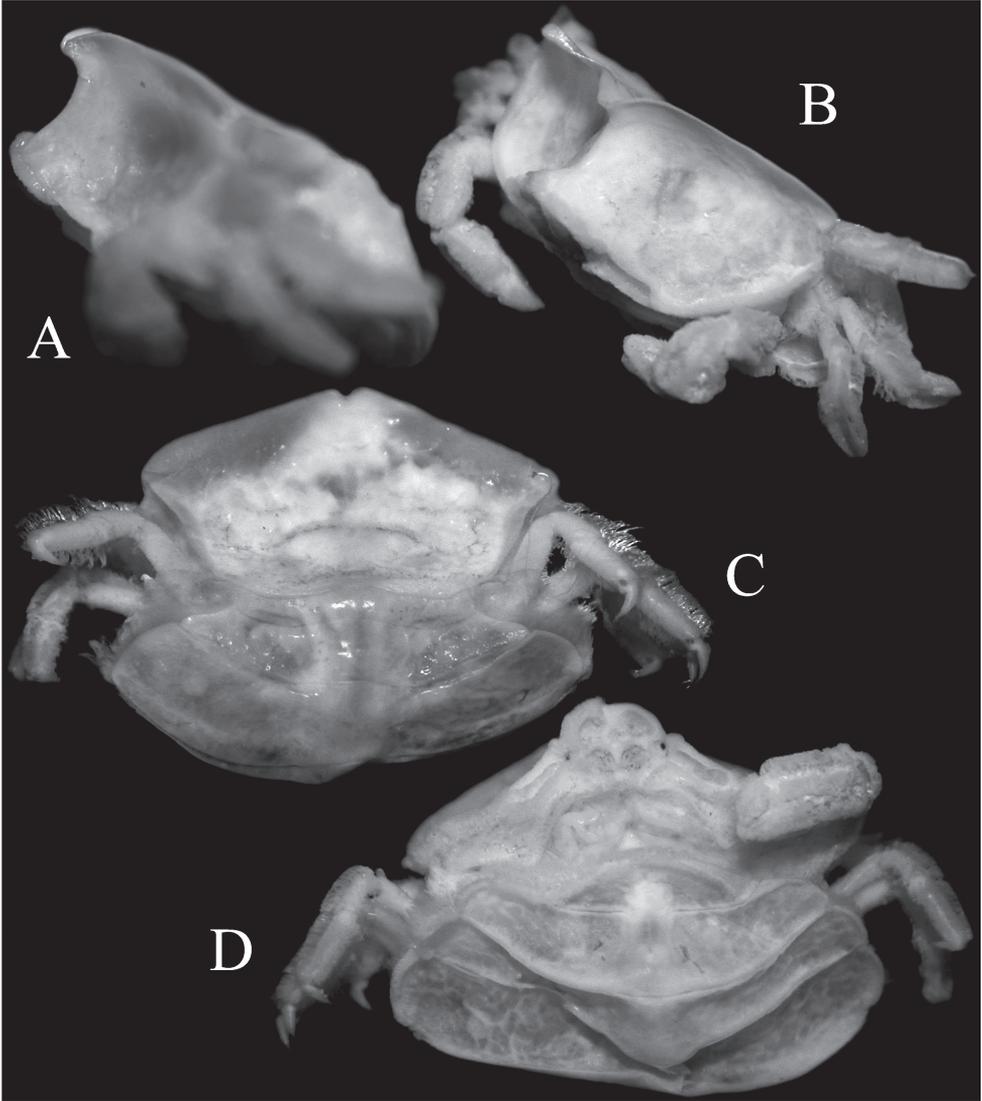


Figure 4. *Serenotheres janus* sp. n., holotype ♀ (8.9 × 7.9 mm) (USNM). **A** angled view of cephalothorax **B** lateral view of cephalothorax **C** posterior part of dorsal lamellum of carapace and abdominal somites 1–3 **D** abdominal somites 5, 6 and telson.

inner edge of ischiomerus; exopod elongate with basal part dilated, outer margin distinctly concave, with single elongate segment (Fig. 5C, D).

Chelipeds symmetrical, short, relatively stout, densely covered with dense short clavate setae (Figs 1B, 2C, 3); setae longer, denser on dorsal margin and along 2 longitudinal rows on outer surface of palm and carpus, forming 3 pseudo-ridges (Figs 3B, C, 4D); when denuded, surfaces of carpus and palm smooth (Fig. 6A, B). Dactylus and pollex covered with dense setae, giving fingers short, stout appearance (Figs 3B, C, 4D);

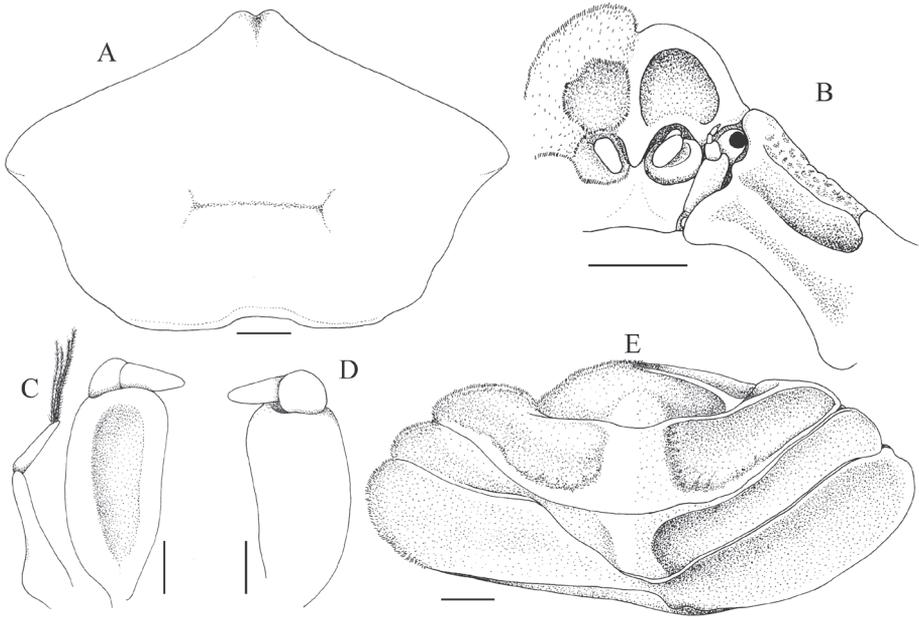


Figure 5. *Serenotheres janus* sp. n., holotype ♀ (8.9 × 7.9 mm) (USNM). **A** overall carapace dorsal lamellum **B** frontal view of cephalothorax (left side denuded) **C** outer view of right MXP3 and exopod (denuded) **D** inner view of right MXP3 (denuded) **E** abdominal somites 4–6 and telson (pits and eroded areas not drawn, left side denuded). Scales: **A, B, E** = 1.0 mm; **C, D** = 0.5 mm.

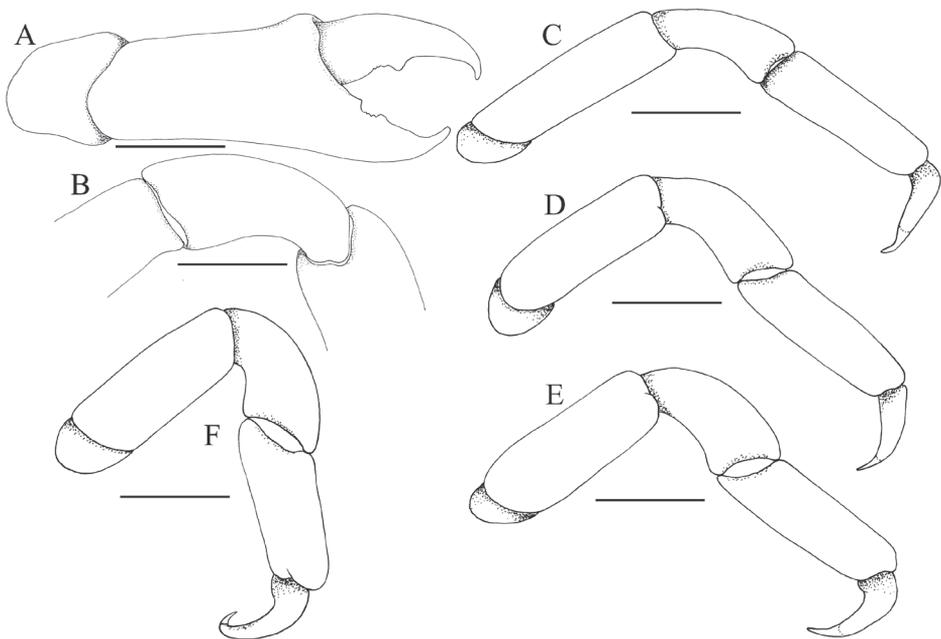


Figure 6. *Serenotheres janus* sp. n., holotype ♀ (8.9 × 7.9 mm) (USNM). **A** outer view of right chela **B** dorsal view of carpus of right cheliped **C–F** right P2–P5, respectively. All structures denuded. Scales = 1.0 mm.

when denuded, structures gently curved, tips sharp, crossing distally; cutting edge of dactylus with 1 submedian tooth and small posterior denticles; cutting edge of pollex with small tooth on proximal third, with smaller teeth and posterior denticles (Fig. 6A).

P2–5 (ambulatory legs) symmetrical from left to right, generally similar in form; margins of carpus and propodus lined with dense, long setae, setation on merus less distinct and more spare; dactylus covered with sparse long setae (Figs 1B, 2, 4C, D); relative lengths: $P2 > P3 = P4 > P5$ (Fig. 6C–F). Outer surface of merus, carpus and propodus smooth when denuded; merus subovate in cross-section, unarmed (Fig. 6C–F). P2–P4 dactylus falcate, long, distinctly shorter than propodus, tip gently curved (Fig. 6C–E); P5 dactylus with distal part prominently curved, with tip hooked obliquely inwards (Fig. 6F).

Abdomen very broad, extending beyond margins of thoracic sternum, partially covering bases of P1–P4, reaching bases of MXP3 (Figs 1C, 4D); somites 1–6 and telson free; somite 4 broadest; telson broadly triangular with rounded tip (Figs 4C, D, 5E); margins lined with dense short setae which obscures margins; surface pitted, appears eroded; surface of somites 4–6 and telson concave, pits and depression on surface more prominent, with margins and edges of sutures of these somites cristate (Figs 4C, D, 5E).

Colour. In life, the species is cream-yellow overall (Fig. 1).

Etymology. The species is named after Janus, the ancient two-faced Roman god, alluding to the unusual two parts of the carapace when viewed dorsally. The name is used as a noun in apposition.

Remarks. *Serenotheres janus* sp. n. can be separated from *S. besutensis* (Serène, 1967) in having the lateral margins of the dorsal carapace lamellum distinctly produced laterally to form a blunt angular lobe, with the posterolateral margin deeply concave (Figs 2, 5A) (vs. lateral margin not produced laterally and more rounded, with the posterolateral margin gently sinuous to almost straight in *S. besutensis*, cf. Serène 1967: pl. 2A; Ah Yong and Ng 2005: fig. 5A); the dorsal carapace lamellum is highest at the median cleft, with the two halves sloping gently outwards in direct frontal view (albeit with the margins of the cleft curving downwards) (Fig. 3) (vs. dorsal carapace lamellum is lowest at the median cleft, with the two halves sloping gently inwards in direct frontal view in *S. besutensis*, cf. Serène 1967: pl. 2B; Ah Yong and Ng 2005: fig. 5E); the rostrum is more prominent with the surface above the antennular fossa prominently concave (Figs 3B, C, 5B) (vs. rostrum relatively shorter with the region above the antennular fossa more shallow in *S. besutensis*, cf. Ah Yong and Ng 2005: fig. 5A, C, D, E); the surface of the actual anterolateral margin and hepatic region is less prominently pitted and eroded (Figs 3, 5B) (vs. prominently pitted and eroded in *S. besutensis*, cf. Serène, 1967: pl. 2B; Ah Yong and Ng 2005: fig. 5C, D); the MXP3 ischiomerus is relatively more rectangular in form (Fig. 5C, D) (vs. longitudinally ovate in *S. besutensis*, cf. Serène 1967: fig. 5; Ah Yong and Ng 2005: fig. 5K); and the P2 merus is relatively longer (Fig. 6C) (vs. relatively shorter in *S. besutensis*, cf. Ah Yong and Ng 2005: fig. 5G).

The type of *S. besutensis* (9.0 × 7.0 mm) (cf. Ng and Ah Yong 2005) is similar to that of the holotype of *S. janus* sp. n. (8.9 × 7.9 mm), so the differences observed cannot be explained by size.

The DNA barcode sequence data of *S. janus* sp. n. indicates a novel lineage among available Pinnotheridae sequences. The closest matches are 86–85% in sequence similarity to a handful of other pinnotherid genera including *Zaops*, *Calyptraeothers*, *Austinothers*, and *Pinnixa* (see Palacios-Theil et al. 2009; Palacios Theil et al. 2016). To date, no other closely related species has been sequenced.

Lithophagine mussels bore into coral rock and until recently, only one species of pinnotherid crab has been reported: *S. besutensis* from an unidentified species of *Lithophaga* collected in live coral from an island off the northeast coast of Peninsular Malaysia (Serène 1967). *Serenothers janus* sp. n. was collected from inside a large specimen of *Leiosolenus obesus* (Philippi, 1847) (Fig. 1A). The function of the unusual plates and lamellum is not known.

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Proneotermes macondianus, a new drywood termite from Colombia and expanded distribution of *Proneotermes* in the Neotropics (Isoptera, Kalotermitidae)

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Abstract

After more than one hundred years, a new drywood termite of the genus *Proneotermes* is described from the tropical dry forest in the Caribbean coast of Colombia. Morphological and genetic analyses are given for *Proneotermes macondianus* sp. n. This termite occurs in tropical dry forests in small colonies inside thin branches of dry wood. The soldier of *P. macondianus* is smaller and the genal horns are angled outward compared to the other two described *Proneotermes* species. The imago wings are unusually short and wide. Genetic analyses for COII, 12S, and 16S genes show less than three percent difference between sample localities of *P. macondianus*. Intergeneric comparison with selected kalotermitid genera indicates that *Bifiditermes* is the most closely related genus of those sequenced. New morphological descriptions and morphometric measurements of *Proneotermes latifrons* based on the soldier caste are also included. Neotropical locality records for *P. latifrons* and *Proneotermes perezii* are provided.

Keywords

Northern Colombian coast, *Proneotermes* keys, *Proneotermes macondianus* sp. n., tropical dry forest

Introduction

Colombia's diverse ecoregions harbour high termite diversity (Morrone 2006, Robledo et al. 2014). Vargas-Niño et al. (2005) and Krishna et al. (2013) together report 29 genera of higher termites (Termitidae). Ten additional generic termitid records, mostly Apicotermitinae, have been collected in Colombia (Scheffrahn unpubl. data). Among the Kalotermitidae, six genera (*Calcaritermes*, *Comatermes*, *Cryptotermes*, *Glyptotermes*, *Incisitermes*, and *Neotermes*) are known from Colombia (Krishna et al. 2013, Rodríguez et al. 2012). The tropical dry forest of Colombia's Caribbean coast has recently revealed a new species and two new records of *Cryptotermes* (Casalla et al. 2016).

For more than a century, the genus *Proneotermes* was represented by two species, *Proneotermes latifrons* (Silvestri, 1901) from Venezuela and *Proneotermes perezii* (Holmgren 1911) from Costa Rica (Krishna et al. 2013). DNA barcoding is a molecular tool used to identify and to track the evolutionary biology of species (Thompson et al. 2000, Inward et al. 2007, Hausberger et al. 2011, Bourguignon et al. 2014). Evolutionary analyses within the Kalotermitidae are incomplete and limited to some genera (Legendre et al. 2008). Hence a comparative genetic analysis can help to determine relationships for *Proneotermes*.

In this paper, a new species of *Proneotermes* is described, *P. macondianus*. In addition, new morphological descriptions are included for the soldier of *P. latifrons* and new soldier measurements provided for *P. perezii* as well as new locality records for *Proneotermes* in the Neotropics.

Methods

Study sites and sampling

Three study sites in a tropical dry forest near Colombia's Caribbean coast were selected and surveyed during July 2014 and August 2015 (Fig. 1). The area of "Los Primates" in the mountains of municipality of Colosó, Sucre and the "El Ceibal" in Santa Catalina Bolívar, are part of the system of protected areas, while the "El Parque Tayrona" is a Natural National Park of Colombia in Santa Marta, Magdalena. These forests are one of the best preserved areas of tropical dry forest in the Colombian Caribbean coast (Instituto de Investigación Alexander von Humboldt 2014). Samples of a new *Proneotermes* were collected in those places using a standardized sampling protocol (Jones and Eggleton 2000, Hausberger and Korb 2015) that included collecting small dry branches and dry wood on the ground. Specimens were preserved in 100% ethanol for DNA analysis and 80% ethanol for museum curation.

Identification

Morphometrics for *P. latifrons* and *P. perezii* were obtained from specimens from the University of Florida Termite Collection, Davie, Florida. Specimens of *P. macondianus*

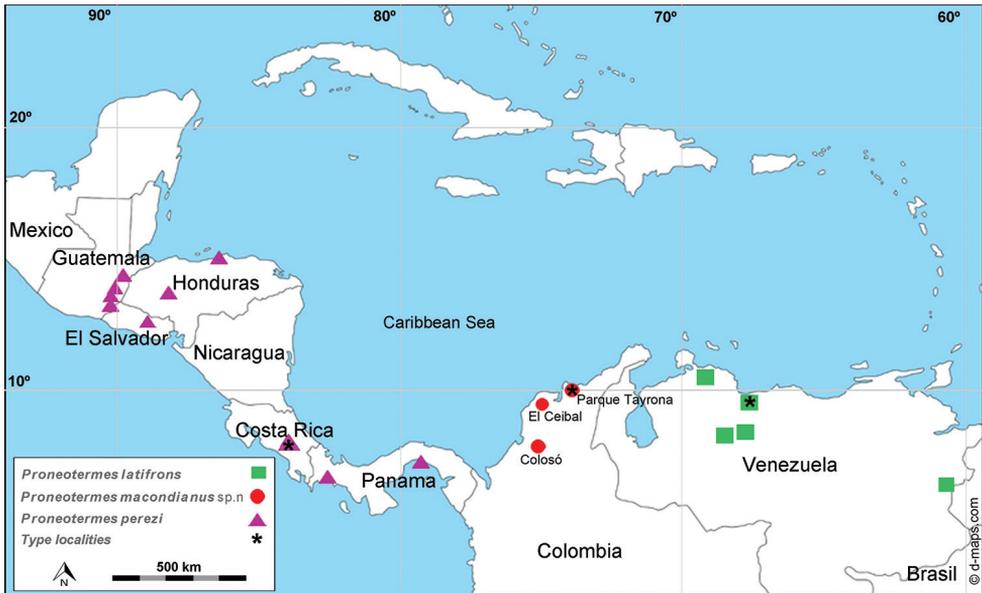


Figure 1. Sampling localities for *Proneotermes macondianus* sp. n in Colombia and new records from University of Florida collection for *P. latifrons* and *P. perezii*.

sp. n. were also sequenced for genetic comparisons. Total DNA was extracted from pseudergates and alate imagoes heads using the CTAB protocol (Doyle and Doyle 1987). PCRs and sequencing were performed for mitochondrial gene fragments from cytochrome oxidase II (COII) (~740 bp), 12S rDNA (~385 bp), and 16S rDNA (~480 bp) as described in Hausberger et al. (2011).

For the three different haplotypes of *P. macondianus* from the northern Colombian coast (separated ~200 km from each other), we used the combined COII, 12S, and 16S nucleotide sequences to calculate the *p*-distance (3000th Bootstrap replications, Gamma Distributed and Transitions + Transversions).

Due to limited availability of mitochondrial gene sequences for Kalotermitidae in National Center for Biotechnology Information (NCBI), we restricted our phylogenetic analysis to only the COII fragment. Twelve genera of Kalotermitidae and *Cryptocercus punctulatus* as the outgroup were used (Table 1). Sequences were aligned with MUSCLE alignment algorithm as implemented in MEGA 7.0 with default settings (Kumar et al. 2016). A phylogenetic tree was inferred based in a Bayesian approach using MrBayes 3.2.1. (Ronquist and Huelsenbeck and 2003) (10⁷ generations with every 1000th tree sampled, using the default of four chains). After checking for convergence, we discarded 50% as burn-in. The resultant tree was visualized using FigTree 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). Additionally, MEGA 7.0 was also employed (Kumar et al. 2016) to calculate *p*-distances (as described above) between all species using the COII fragment. All positions containing gaps and missing data were eliminated.

Table 1. GenBank accession numbers for COII, 12S and 16S sequences.

Species	Accession COII	GenBank Accession 12S	Accession 16S
<i>Cryptocercus punctulatus</i>	DQ007637.1	–	–
<i>Bifiditermes improbus</i>	AF189079.1	–	–
<i>Bifiditermes improbus</i>	AF189080.1	–	–
<i>Calcaritermes temnocephalus</i>	EU253877.1	–	–
<i>Comatermes perfectus</i>	EU253878.1	–	–
<i>Cryptotermes cavifrons</i>	FN377810.1	–	–
<i>Cryptotermes longicollis</i>	FN377806.1	–	–
<i>Epicalotermes mkuzii</i>	DQ442125.1	–	–
<i>Glyptotermes brevicornis</i>	AF189096.1	–	–
<i>Glyptotermes iridipenis</i>	AF189096.2	–	–
<i>Glyptotermes satsumensis</i>	KP026257.1	–	–
<i>Incisitermes immigrans</i>	AB109542.1	–	–
<i>Incisitermes tabogae</i>	EU253880.1	–	–
<i>Kalotermes flavicollis</i>	DQ442147.1	–	–
<i>Marginitermes</i> sp.	KJ907844.1	–	–
<i>Neotermes castaneus</i>	HQ215844.1	–	–
<i>Neotermes holmgreni</i>	EU253882.1	–	–
<i>Neotermes insularis</i>	AF189105.1	–	–
<i>Postelectrotermes amplus</i>	DQ442147.1	–	–
<i>Postelectrotermes howa</i>	EU253883.1	–	–
<i>Procryptotermes leewardensis</i>	EU253884.1	–	–
<i>Proneotermes macondianus</i> CE*	KX267096	KX267094	KX267091
<i>Proneotermes macondianus</i> CO**	KX267097	KX267093	KX267090
<i>Proneotermes macondianus</i> PT***	KX267098	KX267095	KX267092

Samples localities: CE* = El Ceibal (Santa Catalina, Bolívar), CO** = Los Primates (Colosó, Sucre), PT*** = Parque Tayrona (Santa Marta, Magdalena)

Imaging and measurements

Specimens were suspended in Hand Sanitizer and images were taken with a Leica MC205 C stereomicroscope coupled to a Leica MC190 HD digital camera. The software Helicon Focus was used to stack pictures. Measurements were done following Roonwal (1969). Wings and mandibles were detached and mounted onto slides and edited with Photoshop CS5 V12.0.

Deposit

Voucher specimens are held at the University of Freiburg, Germany. The holotype soldier and paratypes of *Proneotermes macondianus* will be deposited at the Natural History Museum of the Alexander von Humboldt Institute of Bogotá (MIAvH) and a paratype soldier at the collection of the American Museum of Natural History, New

York. De-alates (wings detached) and pseudergates of *P. macondianus* will be part of the collection of the Department of Chemistry and Biology at the Universidad del Norte, Barranquilla, Colombia.

Results

Family Kalotermitidae Froggatt, 1897

Genus *Proneotermes* Holmgren, 1911

Proneotermes macondianus sp. n.

<http://zoobank.org/AB0F7282-534A-448D-AC80-86E697A18E9E>

Diagnosis. The *Proneotermes macondianus* soldier is smaller and the head capsule lighter than those of *P. latifrons* and *P. perezii*. In *P. macondianus*, the lateral margins of the genal horns angle outward from the sides of the head capsule while, in the other two species, the lateral margins of the genal horns remain in line with the head capsule. The mandibular humps of *P. macondianus* are more pronounced and rounded than in *P. latifrons* and *P. perezii*. Both *P. latifrons* and *P. perezii* have more robust rugosity on the frons than *P. macondianus*. The imago of *P. macondianus* is smaller and has much shorter, wider, darker, and more punctate wings than that of *P. perezii*.

Description. Imago (Figs 2, 3A, Table 2). Head dorsal view: yellowish weakly trapezoidal, eyes moderately protruding and small, diameter 0.30 mm (Fig. 2A–B). Ocellus oval and almost touching eye (Fig. 2B). Antenna with 15 articles. Pronotum broader than head (Fig. 2A). Forewing with all major veins running parallel; subcosta running from suture to costal margin about 1/5 length of wing, radius to 1/3 wing length, radial sector with 4–6 branches, media less sclerotized than anterior veins, and cubitus unsclerotized. Wings brownish, especially near scale suture, membrane nodular; unusually wide and relatively short. Fore wing with a very long suture line margin; scale much darker than body pigmentation (Fig. 3A). Measurements are reported in Table 2.

Soldier (Figs 3B, 4, Table 3). Head in dorsal view with postclypeus almost black, grading to ferruginous orange near frontal flange, and yellow at occiput (Fig. 4A). Dorsal view with head elongate and sides parallel, frons wide and shallow and faint frontal flange (Fig. 4B). Eye spots distinct, unpigmented. Mandibles completely black (Fig. 3B, 4A–C). Pronotum yellowish with anterior borders brown. Frons angles below vertex approx. 43°. Rugosity vestigial on the frons or vertex regions of the head. Frontal horns robust and project towards the front (Fig. 4A). Genal horns prominent in dorsal view, angled antero-laterally about 45°. Mandible tips bend about 60–65° from longitudinal axis of mandibular blade, prominent dentition, with rounded and pronounced mandibular humps: left hump larger than right (Figs 3B, 4A). Postmentum somewhat constricted in middle, as cup-shaped (Fig. 4C). Third antennal article enlarged and sclerotized, formula 2<3>4=5=6 and 11 articles. Pronotum as broad as

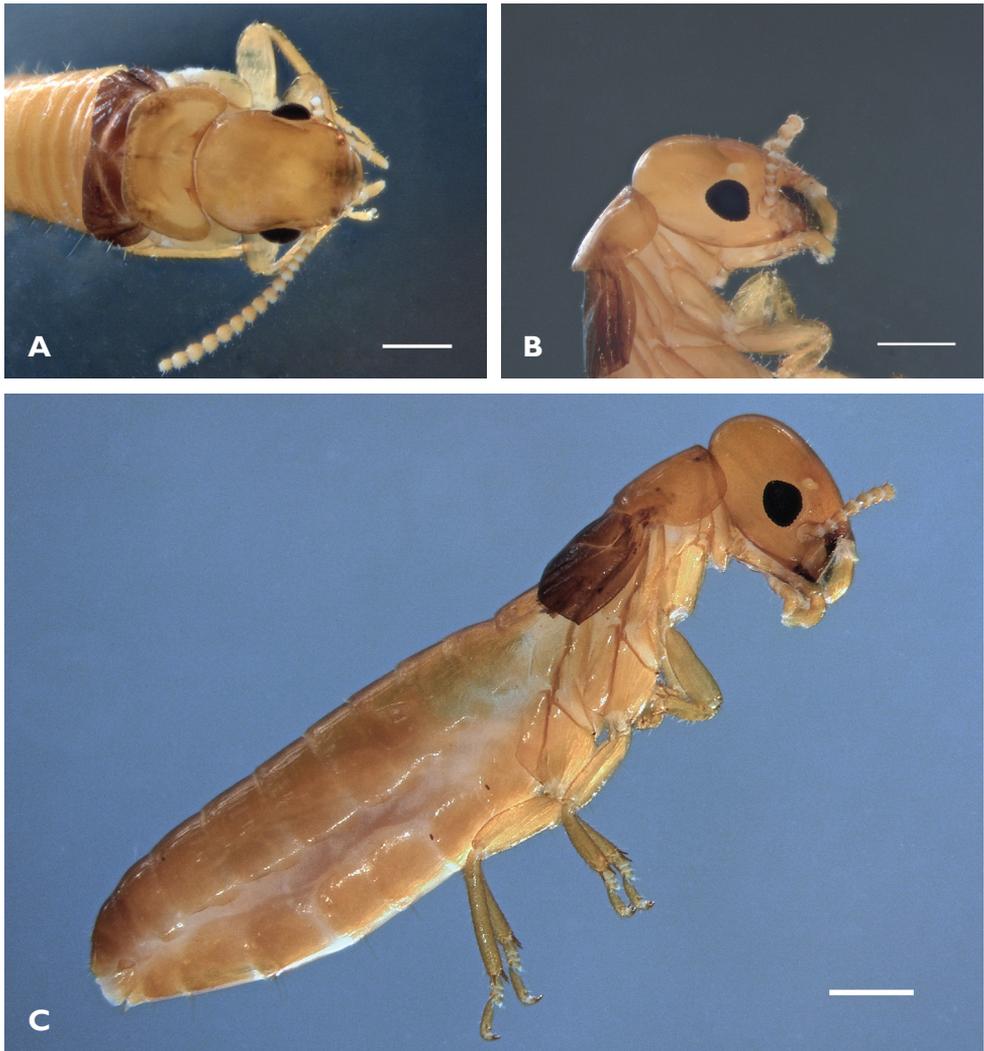


Figure 2. *Proneotermes macondianus* sp. n. imago: **A** Head in dorsal **B** lateral, and **C** whole body in lateral. Scale bars: 0.5 mm.

head; anterior emarginate. Measurements are reported in Table 3. The soldiers from the Tayrona National Park (Santa Marta, Magdalena) showed slightly darker coloration than those from the samples sites at El Ceibal (Santa Catalina, Bolívar) and Colosó (Colosó, Sucre).

Genetic analysis. The COII, 12S, and 16S sequences obtained in this study are deposited in GenBank under accession numbers KX267090-KX267098 (Table 1). The combined COII, 12S, and 16S nucleotide data of three different haplotypes of *P. macondianus* from the northern Colombian coast (separated ~200 km from each other) revealed genetic distances of about 2.5% (p -distance, SE 0.004; 38bp / 1488bp)

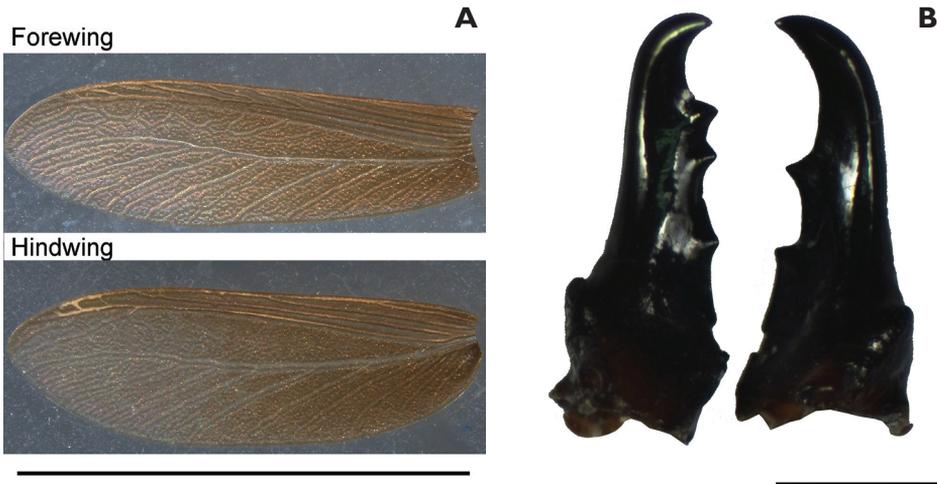


Figure 3. *Proneotermes macondianus* sp. n. Alate: **A** fore- and hindwing, scale bar: 5 mm. Soldier: **B** mandibles, scale bar: 1 mm.

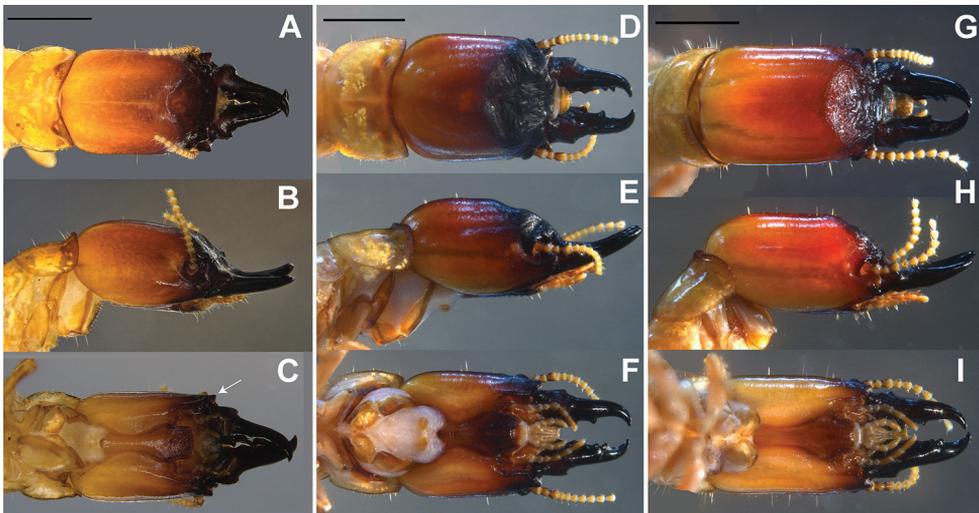
Table 2. Morphometrical measurements for *P. macondianus* sp. n. imagoes.

No.	Measurements in mm (n =10).	Mean	SD	Range
1	Head length with labrum	1.28	0.06	1.18–1.35
2	Head length to postclypeus	1.14	0.04	1.06–1.19
3	Head width, maximum at eyes	0.95	0.04	0.88–1.01
4	Eye diameter, maximum	0.30	0.02	0.26–0.31
5	Eye to head base, minimum	0.14	0.01	0.12–0.16
6	Ocellus diameter	0.11	0.02	0.09–0.13
7	Pronotum maximum width	1.05	0.05	0.94–1.13
8	Pronotum maximum length	0.66	0.02	0.63–0.69
9	Total length without wings	5.45	0.33	5.04–5.87
10	Total length with wings (n = 1)	7.52	-	-
11	Fore wing length to suture (n = 1)	5.24	-	-
12	Fore wing maximum width (n = 1)	1.63	-	-
13	No. antennal articulations	9	2.4	7–15

(Table 4). Our Bayesian phylogenetic tree shows more than 74% Bayesian Posterior Probability (BPP) support for all nodes (Fig. 5). The COII based tree suggests that *Proneotermes* is the sister taxon to a cluster consisting of [(*Marginitermes* + *Epicalotermes*) + *Bifiditermes*] + [(*Cryptotermes* + *Procryptotermes*) + *Incisitermes*]. Since Kalotermitid sequences for 12S and 16S were very sparse in public databases such as The Barcode of Life Data Systems (BOLD) or NCBI, we only used COII to estimate genetic distances between species and for phylogenetic tree inference. Inter-generic COII *p*-distances for available genera (Table 5) showed that *Bifiditermes* is closest to *Proneotermes* (0.153 SE 0.015).

Table 3. Morphometrical measurements for *P. macondianus* sp. n. soldiers.

No.	Measurements in mm (n =11).	Mean	SD	Range
1	Head length to tip of mandibles	2.75	0.11	2.50–2.95
2	Head length to frontal horns	1.72	0.10	1.54–1.90
3	Frontal flange width	1.03	0.04	0.92–1.08
4	Genal horns outside span	1.12	0.05	1.02–1.19
5	Head width max.	1.23	0.06	1.14–1.32
6	Head height excluding postmentum	0.99	0.06	0.85–1.08
7	Pronotum max. width	1.19	0.07	1.05–1.27
8	Pronotum max. length	0.82	0.05	0.73–0.87
9	Left mandible length, tip to ventral condyle (n = 1)	1.10	-	-
10	Total length	6.57	0.44	5.43–6.98
11	No. antennal articulations	11	0.7	10–12

**Figure 4.** Soldier heads of *Proneotermes macondianus* sp. n. (A–C), *Proneotermes latifrons* (D–F), *Proneotermes perezii* (G–I). Head in dorsal (A, D, G), lateral (B, E, H) and ventral position (C, F, I). Arrow in 4C denote genal horn projected prominently in *Proneotermes macondianus* sp. n. Scale bars: 1 mm.

Ecological notes. *Proneotermes macondianus* sp. n. was found in tropical dry forests of the Colombian Caribbean near to coastal areas up to 25 km inland (Fig. 1, Appendix 1 - Figure S1). Encounters of *Proneotermes* were scarce. In line transects that covered a total area of 1500m × 2m, only 0.82% of all termite samples (n = 1102) were *P. macondianus* (n = 9). All samples were from thin pieces of drywood branches: less than 2 cm diameter on the ground, with a maximum of 20 individuals per branch. Pellets were hexagonal in shape, beige in colour and had a length of 0.92 +/- 0.04 mm (Appendix 1: Figure S1, S2, Table S1). It was impossible to identify the plant species from the small dry branches where *P. macondianus* sp. n. was found.

Table 4. Nucleotide distances for combined analysis of COII, 12S rDNA and 16S rDNA genes between localities of *P. macondianus* sp. n. (*p*-distance). Standard error estimates are shown above the diagonal.

No.	Species (n = number of sequenced specimens)	Localities	CE	CO	PT
1	<i>Proneotermes macondianus</i> n = 1	CE		0.002	0.004
2	<i>Proneotermes macondianus</i> n = 4	CO	0.004		0.004
3	<i>Proneotermes macondianus</i> n = 1	PT	0.024	0.025	

Samples localites: CE = El Ceibal (Santa Catalina, Bolívar), CO = Los Primate (Colosó, Sucre), PT = Parque Tayrona (Santa Marta, Magdalena)

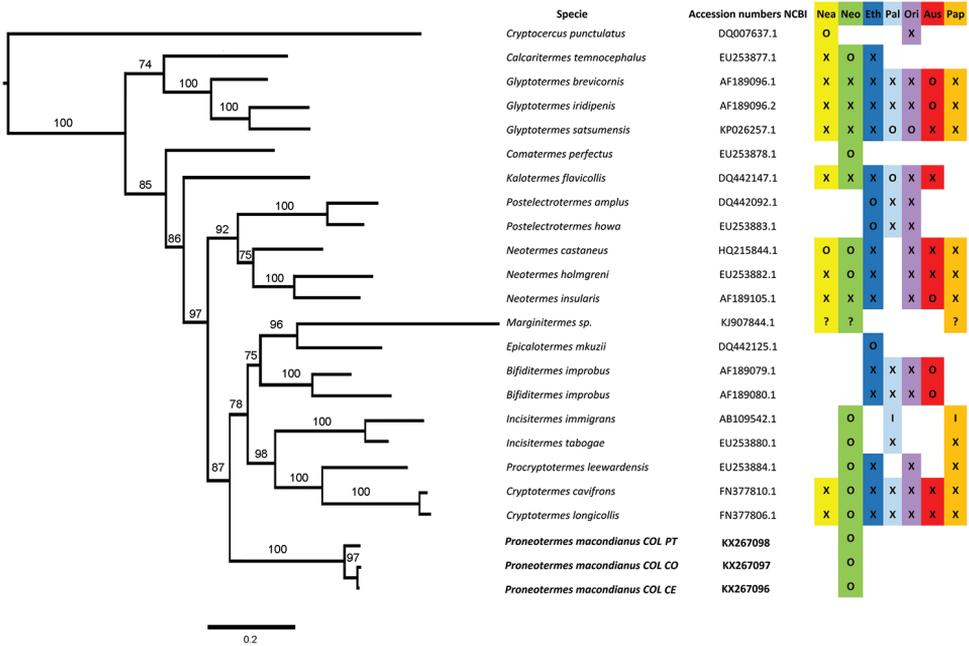


Figure 5. Bayesian inference tree, inferred with MRBAYES from COII sequence data (nodes show posterior probability support). Kalotermitidae distribution (X), Species distribution known (O), unknown (?) and established introductions from other regions (I). Neartic = Nea, Neotropic = Neo, Ethiopian = Eth, Palearctic = Pal, Oriental = Ori, Australian = Aus, Papuan = Pap. Distribution based on Krishna et al. 2013.

Material examined. Holotype colony: **Colombia:** Municipality of Santa Marta, Magdalena. Tayrona National Natural Park, Gairaca Bay: 11.3152°N, 74.1032°W (Fig. 1), 6m, 27.VI.15 by R. Casalla. COLPT4K1-206. Holotype: Soldier, paratypes: 5 soldiers, 2 reproductives, and few pseudergates, two used for DNA analysis. Municipality of Santa Catalina, Bolívar. Protected area “El Ceibal”: 10.6336°N, 75.2517°W, 25m, 30.VIII.14 R. Casalla. COLCE3F5-155, COLCE3G5-158, COLCE3H2-160: Paratypes: 4 soldiers, 5 functional reproductives, and few pseudergates, two used for DNA analysis. Municipality of Colosó, Sucre. Serranía de Coraza y Montes de María.

Table 5. Nucleotide distances for COII sequences between sample localities *P. macondianus* sp. n, the cockroach *Cryptocercus punctulatus* and different Kalotermitidae (*p*-distance). Standard error estimates are shown above the diagonal.

No.	Specie	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Proneotermes macondianus CE* KX267097		0.003	0.008	0.015	0.016	0.016	0.016	0.017	0.017	0.016	0.017	0.016	0.017	0.018	0.017	0.022
2	Proneotermes macondianus CO** KX267098	0.007		0.008	0.015	0.016	0.017	0.016	0.017	0.017	0.016	0.016	0.016	0.017	0.017	0.017	0.022
3	Proneotermes macondianus PT*** KX267096	0.040	0.044		0.016	0.016	0.016	0.016	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.021
4	Bifiditermes improbus AF189080.1	0.153	0.157	0.164		0.016	0.015	0.017	0.015	0.016	0.016	0.017	0.016	0.017	0.017	0.016	0.020
5	Postelectrotermes howa EU253883.1	0.176	0.176	0.174	0.159		0.016	0.018	0.015	0.016	0.016	0.019	0.016	0.017	0.018	0.017	0.021
6	Neotermes castaneus HQ215844.1	0.184	0.179	0.177	0.165	0.157		0.017	0.016	0.017	0.017	0.018	0.016	0.018	0.017	0.016	0.022
7	Incisitermes immigrans AB109542.1	0.175	0.179	0.172	0.188	0.205	0.191		0.018	0.017	0.018	0.017	0.017	0.018	0.019	0.018	0.023
8	Epicalotermes mkuzii DQ442125.1	0.182	0.185	0.189	0.160	0.166	0.176	0.189		0.017	0.016	0.018	0.016	0.017	0.017	0.016	0.022
9	Kalotermes flavicollis DQ442147.1	0.185	0.186	0.188	0.192	0.183	0.192	0.200	0.188		0.017	0.018	0.016	0.018	0.017	0.017	0.022
10	Glyptotermes brevicornis AF189096.1	0.187	0.187	0.197	0.185	0.184	0.197	0.214	0.196	0.200		0.018	0.017	0.018	0.016	0.018	0.022
11	Cryptotermes cavifrons FN377810.1	0.186	0.189	0.186	0.182	0.213	0.202	0.190	0.187	0.211	0.224		0.018	0.016	0.018	0.018	0.023
12	Comatermes perfectus EU253878.1	0.196	0.192	0.209	0.176	0.175	0.181	0.212	0.189	0.202	0.191	0.215		0.017	0.017	0.017	0.022
13	Procryptotermes leewardensis EU253884.1	0.199	0.199	0.189	0.194	0.176	0.187	0.189	0.194	0.202	0.219	0.160	0.204		0.018	0.019	0.022
14	Calcaritermes temnocephalus EU253877.1	0.206	0.209	0.196	0.199	0.213	0.192	0.214	0.194	0.203	0.169	0.212	0.199	0.218		0.017	0.023
15	Marginitermes sp. 9MH1 KJ907844.1	0.209	0.211	0.211	0.193	0.185	0.196	0.231	0.185	0.221	0.230	0.219	0.211	0.225	0.216		0.021
16	Cryptocercus punctulatus DQ007637.1	0.282	0.282	0.279	0.254	0.255	0.281	0.291	0.261	0.266	0.267	0.280	0.266	0.279	0.290	0.257	

Samples localities: CE* = El Ceibal (Santa Catalina, Bolívar), CO** = Los Primates (Colosó, Sucre), PT*** = Parque Tayrona (Santa Marta, Magdalena)

Protected área “Los Primates”: 9.5332°N, 75.3479°W, 223m, 27.VII.14 R. Casalla. COLCO4F4-226: Paratypes: 1 soldier, 1 winged imago, 2 dealated imagoes, one used for DNA analysis. Measurements for holotype, paratype soldiers and imagoes are reported in Table 2 and 3. The holotype and clearly colored paratype soldiers from COLCE3F5-155 will be deposited in the Arthropod Collection of the Natural History Museum of the Alexander von Humboldt Institute of Bogotá, Colombia (MIAvH). A paratype soldier from holotype colony, will be deposited in the American Museum of Natural History, New York, United States. Morphotype imagoes, paratype soldiers and pseudergates will be part of the collection of the Department of Chemistry and Biology at the University del Norte, Barranquilla, Colombia.

Etymology. *Macondianus*: In honour of Nobel laureate Gabriel García Marquez and the fictional town “Macondo” in his novel “One hundred years of solitude”. “Macondiano/a” is also a Spanish word used in Colombia to describe an incredible, rare or surprising event that could only be compared with the fictional universe and magical realism of this novel.

Redescription of *Proneotermes latifrons*

Silvestri’s (1901, 1903) descriptions of the *P. latifrons* soldier are incomplete. Some characters such as frons angle, horns, and postmentum morphology were not included. Also morphometrical measurements are incomplete. Herein, we included morphometrical measurements for the soldier caste. The imago caste is unknown.

Proneotermes latifrons (Silvestri, 1901)

Material examined. Venezuela: Bolivar State, El Pauji: 4.4675°N, 61.5947°W, 600m, 25.VII.2003, J Perozo, University of Florida no. SA336: 2 soldiers and pseudergates. Falcon State, La Chapa: 11.2657°N, 69.6022°W, 703m, 27.V.2008, Scheffrahn et al., VZ833, 2 soldiers, pseudergates. Lara State, Copeyal: 10.4409°N, 69.4402°W, 590m, 28.V.2008, Scheffrahn et al., VZ1014, 10 soldiers, pseudergates. Yaracuy State, Licua: 10.3377°N, 69.1344°W, 650m, 30.V.2008, Scheffrahn et al., VZ1180-11183, 4 colonies, many soldiers, pseudergates.

Soldier (Fig. 4D–F, Table 6). Head in dorsal view with frons dark glossy until faint bridge, grading from ferruginous orange to orange-yellow toward vertex. Postclypeus whitish at borders. Mandibles black anteriorly and reddish brown at hump. Head in lateral view with dark ferruginous orange, then turns orange to genal region. Head in ventral view with postmentum chestnut-dark brown and whitish at anterior border and genal margin pale orange. Eye spots distinct, unpigmented. Pronotum hyaline with sclerotized borders. First three antennal segments darker.

Head subsquare with sides slightly convergent, posteriorly and rounded to vertex. Frontal area wide and long, occupies ca 2/5 of head length to postclypeus; narrowly depressed

Table 6. Morphometrical measurements for soldiers of *Proneotermes latifrons*.

No.	Measurements in mm (n = 11).	Mean	SD	Range
1	Head length to tip of mandibles	2.96	0.19	2.65–3.35
2	Head length to frontal horns	1.82	0.31	1.10–2.25
3	Frontal flange width	1.06	0.12	0.90–1.25
4	Genal horns, outside span	1.33	0.12	1.20–1.55
5	Head width max.	1.46	0.12	1.15–1.60
6	Head height excluding postmentum	1.17	0.14	1.00–1.50
7	Pronotum max. width	1.49	0.12	1.20–1.65
8	Pronotum max. length	0.82	0.09	0.70–0.95
9	Left mandible length, tip to ventral condyle	1.06	0.03	1.00–1.10
10	Total length	7.38	0.61	6.80–8.80
11	No. antennal articulations	11	0.7	10–12

in center, and laterally view with faintly convex and few undulations, sloping angle ca. 50° near to postclypeus. Labrum short and sub-squared. Antennal socket protruded with third antennal segment longer and sclerotized, formula 2<3>4=5=6 and 11 articulations. Postmentum very broad in front. Pronotum as broad as head with anterior emarginated. Mandibles strong and curved inward ca. 45–50°. Measurements are reported in Table 6.

Comparisons. Soldiers of *P. latifrons* are separated from congeners in having a wide and darker convex frons with narrow undulations dorso-laterally. Postclypeus whitish at border, labrum wider than long and darker postmentum. *P. latifrons* is distributed in Venezuela, while *P. perezii* is widely distributed in Central America, from Guatemala to Panama (Fig. 1).

Additional descriptions for *Proneotermes perezii*

Material examined. Guatemala: San Jose La Arada: 14.6965°N, 89.6255°W, 992m, 3.VI.2006, Scheffrahn et al., GUA768-769, 2 colonies many soldiers, pseudergates 3 alates. Ipala: 14.5992°N, 89.6411°W, 873m, 3.VI.2006, Scheffrahn et al., GUA793, 5 pseudergates 6 km NW Jutiapa: 14.3307°N, 89.8622°W, 964m, 3.VI.2006, Scheffrahn et al., GUA822-824, 3 colonies 3 soldiers, many pseudergates, many alates. San José Acatempa: 14.2537°N, 90.1259°W, 1277m 3.VI.2006, Scheffrahn et al., GUA845-851, 7 colonies many soldiers, pseudergates, 4 alates. **Honduras:** P. N. Capiro summit: 15.8697°N, 85.9564°W, 942m, 29.V.2007, Scheffrahn et al., HN217, 5 soldiers, many pseudergates. Amarateca: 14.2247°N, 87.3765°W, 991m, 2.VI.2007, Scheffrahn et al., HN693, 1 soldier, many pseudergates. **Panama:** Gamboa: 9.12°N, 79.70°W, 9.VI.2005, W. Reeves, University of Florida no. CTA48, 2 alates after rain. Valle de las Minas: 8.6369°N, 82.2114°W, 1050m, Scheffrahn et al., 1.VI.2010, PN1166-1167, 2 colonies many soldiers, pseudergates.

Table 7. Morphometrical measurements for soldiers of *Proneotermes perezii*.

No.	Measurements in mm (n = 10).	Mean	SD	Range
1	Head length to tip of mandibles	3.36	0.23	3.05–3.80
2	Head length to frontal horns	2.23	0.09	2.10–2.35
3	Frontal flange width	0.93	0.11	0.75–1.10
4	Genal horns outside span	1.41	0.11	1.20–1.60
5	Head width max.	1.52	0.09	1.30–1.65
6	Head height excluding postmentum	1.24	0.08	1.10–1.40
7	Pronotum max. width	1.46	0.13	1.20–1.55
8	Pronotum max. length	0.90	0.08	0.80–1.00
9	Left mandible length, tip to ventral condyle	1.28	0.08	1.15–1.40
10	Total length	7.88	0.60	7.00–8.80
11	No. antennal articulations	12	0.9	11–14

***Proneotermes perezii* (Holmgren, 1911)**

Krishna (1961) described alates and soldiers of *P. perezii* but did not include measurements. Herein, we report measurements of the soldier, along with new locations and images (Figs 1, 4G–I, Table 7).

Genus *Proneotermes* Holmgren, 1911

Redescription. Soldiers with head robust, dark coloration in frons, nearly black, grading from ferruginous orange to orange-yellow toward vertex. Frons sloping between 43–50° to postclypeus, without a ridge and vestigial rugosity. Mandibles bended upwards, with a strong dentition and left mandible bigger than right. Mandibular humps pronounced. Eyes spot unpigmented. Third antennal article larger than second and fourth and sclerotized. Pronotum almost as broad as head width. Femur tick, short and strong. Tibal spurs 3:3:3.

Based on our measurements and morphological description, we developed an identification key for the three *Proneotermes* species

Key to the species *Proneotermes* based on soldier caste

- 1 Smaller species; maximum head width 1.14–1.32 mm (mean 1.23 SD 0.06 mm). Lateral margins of the genal horns angle outward from the sides of the head capsule (Fig. 4A, C)..... ***P. macondianus* sp. n**
- Larger species; maximum head width 1.15–1.65 mm (mean 1.49 SD 0.11 mm); lateral margins of the genal horns remain in line with the head capsule (Figs 4F, I)..... **2**

- 2 In lateral view; frons forms even curve below vertex and mandibles (Fig. 4E); postmentum about twice as long as wide. Posterior margin conical (Fig. 4F)*P. latifrons*
- In lateral view; frons forms rather straight angle from vertex to mandibles (Fig. 4H); postmentum about three fifths as long as wide. Posterior margin convex (Fig. 4I)*P. perezii*

Discussion

The phylogenetic relationships within the Kalotermitidae are not clearly resolved yet. Krishna (1961) hypothesized that, based on wing venation and morphology of imago mandibles, *Proneotermes* is sister group to a clade composed of *Tauritermes*, *Allotermes*, *Mariginitermes*, and *Incisitermes*. In contrast, our phylogenetic tree shows *Proneotermes*, a Neotropical group, is monophyletic (87% BPP), separated from ancestral line of those who originated *Marginitermes* and the Old World genus *Bifiditermes*, *Epicalotermes*, and New World *Incisitermes* and the Pantropical *Cryptotermes*. However, our results suggest that the genetic distances between *Proneotermes* and congeners are quite high (p -distance 0.153 – 0.211 for COII fragment) and the closest genus to *Proneotermes* is *Bifiditermes* (Table 4).

Using a single mitochondrial marker (COII) available for 12 Kalotermitidae genera, our results resemble those from Legendre et al. (2008), who included seven gene fragments in combination with morphological characters in their analyses. To fully resolve phylogenetic relationships within the cosmopolitan Kalotermitidae, a denser taxon sampling along with covering more genetic markers, ideally including nuclear loci and moreover morphological characters is needed.

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Appendix I



Figure S1. Macrophotography of *P. macondianus* sp. n. alive from National Park Tayrona. Santa Marta. Magdalena Colombia. Two soldiers (one partly hidden), some pseudergates (whitish, red arrow), two larva (yellow arrow), two eggs (green arrow), and some dry pellets.



Figure S2. Dry pellets of *P. macondianus* sp. n. Scale bar 1 mm.

Table S1. Size of pellet of *P. macondianus* sp. n.

Pellet	Length (mm)
1	0.92
2	0.86
3	0.92
4	0.91
5	0.96
6	0.96
7	0.91
8	0.97
9	0.95
10	0.87
11	0.9
Mean	0.92
SD	0.04

Review of *Parathoracaphis* Takahashi, 1958 with description of a new species from China (Hemiptera, Aphididae, Hormaphidinae)

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Abstract

The aphid genus *Parathoracaphis* Takahashi, 1958 is reviewed. *Parathoracaphis spinapilosa* sp. n., found on *Quercus* sp. and on an unidentified species of Fagaceae in China is described and illustrated. A generic diagnosis and a key to *Parathoracaphis* species are presented.

Keywords

aphid, key, morphology, new taxa, Nipponaphidini

Introduction

The aphid genus *Parathoracaphis* was erected by Takahashi (1958), with *Thoracaphis setigera* Takahashi, 1932 as the type species, based on the morphological characters that head and thorax are completely fused with abdominal segments I–VII, dorsum lacks pustules but bears spine-like submarginal setae, and siphunculi absent. *Thoracaphis elongata* Takahashi, 1941 and *Thoracaphis kayashimai* Takahashi, 1950 were also assigned to this genus (Takahashi 1958). Eastop and Hille Ris Lambers (1976) subsequently included two other species, *Thoracaphis cheni* Takahashi, 1936 and *Thoracaphis gooti* Takahashi, 1950, under *Parathoracaphis*. Ghosh (1988) considered *Hoplothoracaphis*

Pramanick, Samanta & Raychaudhuri, 1983 as a synonym of *Parathoracaphis*, and consequently referred *Hoplothoracaphis manipurensis* Pramanick, Samanta & Raychaudhuri, 1983 to *Parathoracaphis*.

Herein, a new species *Parathoracaphis spinapilosa* sp. n. is described, found on *Quercus* sp. and an unidentified species of Fagaceae in Fujian and Yunnan, China. Therefore, the genus *Parathoracaphis* now includes seven species: *P. cheni* (Takahashi), *P. elongata* (Takahashi), *P. gooti* (Takahashi), *P. kayashimai* (Takahashi), *P. manipurensis* (Pramanick, Samanta & Raychaudhuri), *P. setigera* (Takahashi), and *P. spinapilosa* sp. n.

Materials and methods

Morphological description. Aphid terminology in this paper generally follows Takahashi (1958) and Ghosh (1988). The unit of measurements is millimetres (mm). In Table 1, the following abbreviations are used: Ant.IIIBD, basal diameter of antennal segment III; URS, ultimate rostral segment; BW URS, basal width of ultimate rostral segment; 2HT, second hind tarsal segment; BW Cauda, basal width of cauda.

COI sequencing. COI barcode sequence was obtained for the new species with primers LepF and LepR (Footitt et al. 2008) and has been deposited in GenBank.

Specimen depositories. The holotype and some paratypes of the new species and all examined specimens of *P. manipurensis* and *P. setigera* are deposited in the National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing, China (NZMC). Four paratypes of the new species and the examined specimens of *P. cheni* are deposited in the Natural History Museum, London, UK (NHM).

Taxonomy

Parathoracaphis Takahashi, 1958

Parathoracaphis Takahashi, 1958: 13. Type species: *Thoracaphis setigera* Takahashi, 1932; by original designation.

Hoplothoracaphis Pramanick, Samanta & Raychaudhuri, 1983: 1. Type species: *Hoplothoracaphis manipurensis* Pramanick, Samanta & Raychaudhuri, 1983; by monotypy.

Parathoracaphis Takahashi: Ghosh and Raychaudhuri 1973: 486; Eastop and Hille Ris Lambers 1976: 336; Ghosh 1988: 194; Tao 1990: 71; Blackman and Eastop 1994: 799; Remaudière and Remaudière 1997: 188; Tao 1999: 22; Nieto Nafria et al. 2011: 305.

Generic diagnosis. In apterae, body elongate oval, oval, or subcircular, aleyrodiform, and strongly sclerotized. Prosoma consisting of fused head, thorax, and abdominal segments I–VII, abdominal segment VIII free. Dorsum of prosoma reticulated, corrugated, convoluted, or with wax pores. Submarginal setae on dorsal prosoma distinctly

spine-like, pointed or somewhat blunt at apices, sometimes arising from tuberculate bases. Dorsal spinal setae on prosoma minute, long and fine, or spine-like. Abdominal tergite VIII with 4 setae, similar to submarginal setae on dorsal prosoma. Eyes 3-faceted. Antennae concealed under head, 2–4-segmented. Legs short, concealed under body; tarsi small, unsegmented or 2-segmented; claws small, normal, or absent. Siphunculi absent. Cauda knobbed and constricted at base. Anal plate bilobed.

Distribution. China, India, Japan, Malaysia, and Thailand.

Host plants. Fagaceae (*Castanopsis*, *Cyclobalanopsis*, *Lithocarpus*, and *Quercus*) and Lauraceae (*Litsea*).

Comments. Only apterous viviparous females are known. The life cycles of most species are unknown. *Parathoracaphis* is related to *Neohormaphis* Noordam, 1991 in sharing the consolidated head, thorax, and abdominal segments I–VII and spine-like submarginal setae on prosoma.

Parathoracaphis cheni (Takahashi, 1936)

Thoracaphis cheni Takahashi, 1936: 21.

Parathoracaphis cheni: Eastop and Hille Ris Lambers 1976: 336; Blackman and Eastop 1994: 799; Remaudière and Remaudière 1997: 188; Tao 1999: 22.

Specimens examined. Twelve apterous viviparous females, **CHINA**: Zhejiang (Huangyan), Jan 1934, on *Myrica rubra*, coll. F.G. Chen (NHM).

Distribution. China (Zhejiang).

Host plant. The host plant is recorded as *Myrica rubra* with a question mark in the original description (Takahashi 1936). We think it may be an erroneous record.

Biology. Sitting tightly on the undersides of leaves of host plant (Takahashi 1936, Blackman and Eastop 1994). The life cycle is unknown.

Parathoracaphis elongata (Takahashi, 1941)

Thoracaphis elongata Takahashi, 1941: 22.

Parathoracaphis elongata: Takahashi 1958: 14; Ghosh and Raychaudhuri 1973: 486; Eastop and Hille Ris Lambers 1976: 336; Blackman and Eastop 1994: 799; Remaudière and Remaudière 1997: 188.

Distribution. Thailand.

Host plants. *Quercus* sp. and other unspecified Fagaceae species.

Biology. This species occurs in large numbers on the undersides of leaves of host plant (Takahashi 1941). The life cycle is unknown.

Comments. Known only from the original description. Takahashi (1941) mentioned that the dorsal prosoma of this species has 5 pairs of spine-like spinal setae, tarsi are unsegmented, and claws are absent.

***Parathoracaphis gooti* (Takahashi, 1950)**

Thoracaphis gooti Takahashi, 1950: 605.

Parathoracaphis gooti: Eastop and Hille Ris Lambers 1976: 336; Blackman and Eastop 1994: 799; Remaudière and Remaudière 1997: 188.

Distribution. Malaysia.

Host plant. *Quercus* sp.

Biology. Infesting the undersides of leaves of host plant (Takahashi 1950). The life cycle is unknown.

Comments. Known only from the original description. The figure in Takahashi (1950) indicates that the dorsal prosoma of this species has 16 pairs of spine-like submarginal setae and 5 pairs of long and fine spinal setae, a pair of submarginal setae on head dorsum located near the front end, and 2 pairs between the eyes. The original description mentioned that the antennae of *P. gooti* are 3- or 4-segmented, the tarsi are distinctly 2-segmented, and the claws are slender.

***Parathoracaphis kayashimai* (Takahashi, 1950)**

Thoracaphis kayashimai Takahashi, 1950: 602.

Parathoracaphis kayashimai: Takahashi 1958: 14; Ghosh and Raychaudhuri 1973: 486; Eastop and Hille Ris Lambers 1976: 336; Blackman and Eastop 1994: 799; Remaudière and Remaudière 1997: 188.

Distribution. Malaysia.

Host plant. *Quercus* sp.

Biology. Feeding on the undersides of leaves of host plant (Takahashi 1950). The life cycle is unknown.

Comments. Takahashi (1950) mentioned that *P. kayashimai* was closely related to *P. setigera*, differing in ornamentation of dorsum and leg measurements. The key to Nipponaphidini species on *Quercus* and the figure of *P. kayashimai* in Blackman and Eastop (1994) indicate that submarginal setae on abdominal tergite VI of this species are much smaller than setae on other tergites.

***Parathoracaphis manipurensis* (Pramanick, Samanta & Raychaudhuri, 1983)**

Figs 1–3

Hoplothoracaphis manipurensis Pramanick, Samanta & Raychaudhuri, 1983: 2.

Parathoracaphis manipurensis: Ghosh 1988: 196; Remaudière and Remaudière 1997: 188.



Figures 1–3. *Parathoracaphis manipurensis* (Pramanick, Samanta & Raychaudhuri). **1** Dorsal view of body of apterous viviparous female **2** a dense colony on underside of leaf of *Castanopsis* sp. **3** apterous adults in life, covered with much wax (**2, 3**: Mt. Zixi, Yunnan, China; 22 Oct 2010). Scale bar: 0.10 mm.

Specimens examined. One apterous viviparous female, **CHINA:** Yunnan (Chuxiong City, Mt. Zixi), 22 Oct 2010, No. 24888, on *Castanopsis* sp., coll. X.L. Huang (NZMC); 1 apterous viviparous female, **CHINA:** Yunnan (Chuxiong City, Mt. Zixi), 22 Oct 2010, No. 24894, on Fagaceae, coll. X.L. Huang (NZMC).

Distribution. China (Yunnan), India.

Host plants. *Castanopsis* sp. and *Litsea sebifera*.

Biology. Forming large colonies on the undersides of leaves of host plant, attended by ants sometimes (Fig. 2). Apterous adults bear much filiform and flocculent wax (Fig. 3). The life cycle is unknown.

Comments. Ghosh (1988) described an apterous morph on *Amaranthus* sp. (Amaranthaceae) as a fundatrix of *P. manipurensis*. However, the specimen was not collected at the type locality, no life cycle observations were conducted, and *Amaranthus* sp. is unlikely to be a primary host plant of Nipponaphidini species. Additionally, the presence of siphunculi suggests that it does not belong in *Parathoracaphis*.



Figures 4–7. *Parathoracaphis setigera* (Takahashi). Apterous viviparous female: **4** dorsal view of body **5** submarginal setae on head dorsum **6** branched linear markings on marginal area of prosoma dorsum **7** apterous adult on underside of leaf of *Quercus* sp., bearing wax filaments marginally (Kunming, Yunnan, China; 5 Dec 2009). Scale bars: 0.10 mm.

***Parathoracaphis setigera* (Takahashi, 1932)**

Figs 4–7, 11

Thoracaphis setigera Takahashi, 1932: 72.

Parathoracaphis setigera: Takahashi 1958: 14; Ghosh and Raychaudhuri 1973: 486; Eastop and Hille Ris Lambers 1976: 336; Tao 1990: 71; Blackman and Eastop 1994: 799; Remaudière and Remaudière 1997: 188; Tao 1999: 22.

Specimens examined. Two apterous viviparous females, **CHINA:** Yunnan (Kunming City, 25.1407°N, 102.7465°E, altitude 1910 m), 18 Nov 2009, No. 23861, on *Quercus* sp., coll. J. Chen and Z.H. Luo (NZMC); 16 apterous viviparous females, **CHINA:** Yunnan (Kunming City, 25.0600°N, 102.7726°E, altitude 2000 m), 5 Dec 2009, No. 24111, on *Quercus* sp., coll. J. Chen and Z.H. Luo (NZMC); 17 apterous

viviparous females, **CHINA:** Taiwan (Urai), 6 Sept 1931, No. Y7903, on *Quercus* sp., coll. R. Takahashi (NZMC).

Distribution. China (Sichuan, Taiwan, and Yunnan), Japan.

Host plants. *Cyclobalanopsis gilva*, *Lithocarpus* sp., *Quercus glauca*, and *Quercus myrsinaefolia*.

Biology. Apteræ are scattered on the undersides of leaves of host plant, with a circle of thin and curved wax filaments along the margin of body and two rather long wax filaments at the hind end of body (Fig. 7). In Japan, apteræ occur on undersides of leaves of *Quercus* throughout the year (Takahashi 1958).

***Parathoracaphis spinapilosa* sp. n.**

<http://zoobank.org/D5BEC449-00CA-4C14-83B6-5B3576BCC5E2>

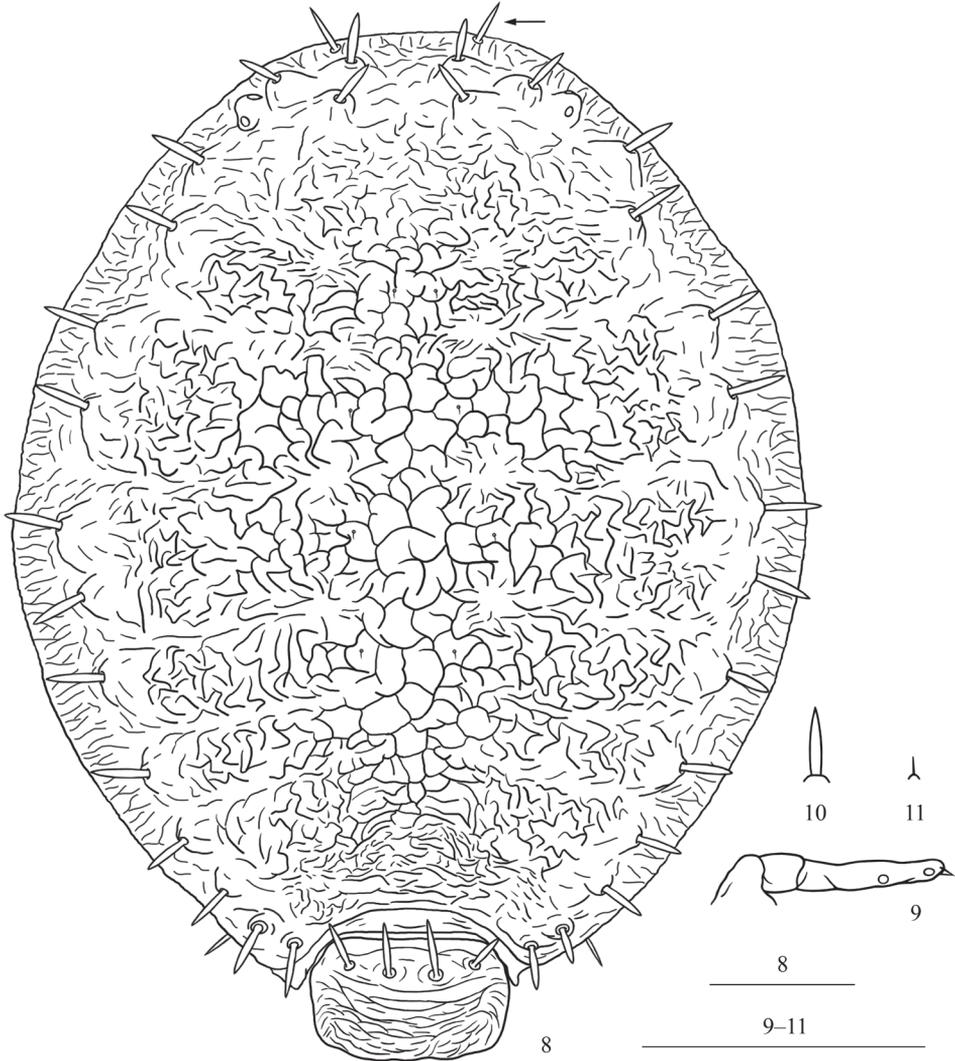
Figs 8–10, 12–25, Table 1

Etymology. The new species is named for a pair of spine-like, long, thick, and pointed frontal setae. “*Spina*” (Latin) means “thorn”, “*pilosa*” (Latin) means “hair”.

Diagnosis. Body small, aleyrodiform. Dorsum of prosoma densely covered with convoluted markings medially and short folded-line shaped sculptures pleuro-marginally. Head with a pair of spine-like frontal setae. Dorsum of prosoma with four pairs of minute spinal setae and 16 pairs of spine-like submarginal setae. Antennae 3- or 4-segmented. Tarsi 2-segmented. Claws normal.

Description. *Apterous viviparous females:* Body oval, aleyrodiform, and strongly sclerotized (Fig. 12). Black in life, with a fringe of long and curved wax filaments, the filaments sparse at the hind end of body (Fig. 25). For morphometric data see Table 1.

Mounted specimens. Body brown; cauda, anal plate, and genital plate pale in color. Prosoma consisting of fused head, thorax, and abdominal segments I–VII; abdominal segment VIII free (Figs 8, 12). Dorsum of prosoma densely covered with convoluted markings medially and short folded-line shaped sculptures pleuro-marginally (Figs 8, 12–14). The margin of dorsal prosoma with a short transversely striped band, band margin with small shallowly crenulated wax glands (Fig. 15). Between each segment of thoracic notum and abdominal tergites I–IV, shallow concave lines present at pleural and marginal area of prosoma; concave lines between abdominal tergites III and IV sometimes indistinct (Fig. 12). Abdominal tergite VIII with long and short ripples, distributed densely on posterior margin (Fig. 8). Head with a pair of frontal setae, spine-like, long, thick, and pointed (Figs 10, 16, indicated with an arrow in Fig. 8). Dorsum of prosoma with 16 pairs of long thick and spine-like submarginal setae, pointed or somewhat blunt at apices, arising from tuberculate bases (Fig. 8); head dorsum with two pairs anterior to eyes and a pair between eyes (Figs 8, 17), pro-, meso-, and meta-notum each with two pairs, abdominal tergites I–VII each with a pair (Fig. 8); submarginal setae on abdominal tergite V finer and shorter than setae on other tergites, pointed at apices, located near body margin (Fig. 18). Pro-, meso-, meta-notum,



Figures 8–11. 8–9 *Parathoracaphis spinapilosa* sp. n. Apterous viviparous female: **8** dorsal view of body **9** antenna **10–11** Frontal seta: **10** *P. spinapilosa* sp. n. **11** *P. setigera*. Scale bars: 0.10 mm.

and abdominal tergite I each with a pair of minute spinal setae. Abdominal tergite VIII with four setae, similar to submarginal setae on dorsal prosoma (Fig. 8). Frons not protuberant. Eyes 3-faceted. Antennae 3-segmented, rarely 4-segmented, concealed under head, with two apical setae (Figs 9, 19). Primary rhinaria small, rounded, and placed wide apart. Rostrum short, reaching to fore coxae. Ultimate rostral segment short, thick, and blunt, with two pairs of primary setae and a pair of secondary setae (Fig. 20). Legs short, smooth, concealed under body, trochanter and femur fused. Tarsi 2-segmented. First tarsal chaetotaxy: 2, 2, 2. Dorsoapical setae on second tarsal segment expanded at apex and longer than claws. Claws normal. Siphunculi absent. Cau-

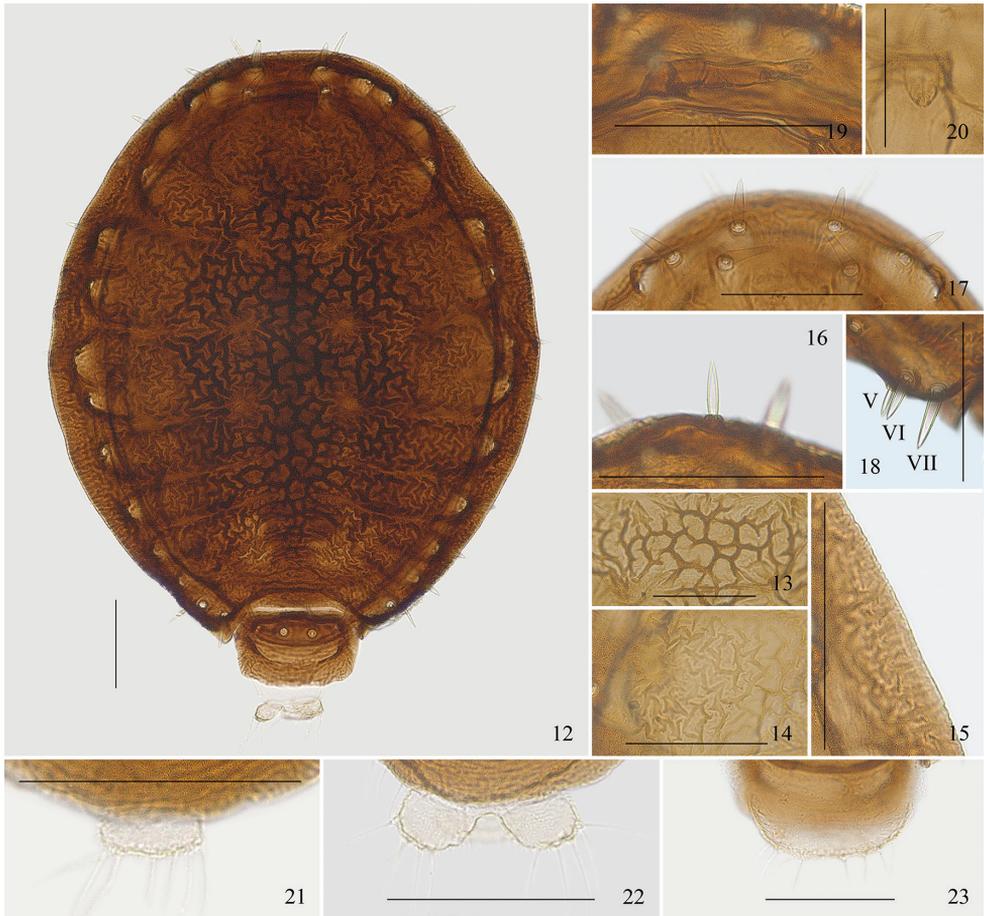
Table 1. Morphometric data of *Parathoracaphis spinapilosa* sp. n.

Parts (For abbreviations see Materials and methods)		Apterous viviparae (n = 25)		
		Mean	Range	Standard deviation
Length (mm)	Body length	0.710	0.624–0.778	0.038
	Body width	0.524	0.422–0.581	0.042
	Whole antenna	0.065	0.059–0.074	0.004
	URS	0.029	0.026–0.031	0.001
	Hind trochanter and femur	0.076	0.072–0.082	0.003
	Hind tibia	0.090	0.084–0.096	0.004
	2HT	0.030	0.026–0.033	0.002
	Cauda	0.016	0.012–0.017	0.002
	BW Cauda	0.026	0.022–0.030	0.003
	Ant.IIIBD	0.011	0.010–0.014	0.001
	Frontal setae	0.032	0.028–0.037	0.003
	Submarginal setae on Tergite I	0.037	0.032–0.041	0.002
	Spinal setae on Tergite VIII	0.042	0.034–0.048	0.005
	Ratio (times)	Whole antenna / Body	0.09	0.08–0.11
Hind tibia / Body		0.13	0.11–0.14	0.007
URS / BW URS		1.14	1.04–1.26	0.071
URS / 2HT		1.01	0.92–1.13	0.082
Cauda / BW Cauda		0.61	0.50–0.70	0.063
Frontal setae / Ant.IIIBD		2.93	2.33–3.75	0.380
Submarginal setae on Tergite I / Ant.IIIBD		3.39	2.58–4.25	0.446
Spinal setae on Tergite VIII / Ant.IIIBD	3.82	2.80–5.00	0.701	

da and anal plate with spinules, genital plate with spinulose transverse stripes. Cauda knobbed, constricted at base, with six or seven setae (Fig. 21). Anal plate bilobed, each lobe with 4–6 setae (Fig. 22). Genital plate broadly rounded, with two anterior setae and 6–8 setae along the posterior margin (Fig. 23).

Specimens examined. *Holotype*: apterous viviparous female, **CHINA**: Yunnan (Kunming City, Mt. Xishan), 24 Apr 1995, No. 13480-1-4-2, on Fagaceae, coll. G.X. Qiao (NZMC). *Paratypes*: 25 apterous viviparous females, with the same collection data as holotype (NZMC); 4 apterous viviparous females, No. 13480-1-5, with the same collection data as holotype (NHM); 9 apterous viviparous females (COI: KX709878), **CHINA**: Fujian (Jiangle County, Mt. Longqi, 26.5109°N, 117.2907°E, altitude 730 m), 17 Jun 2011, No. 26901, on *Quercus* sp., coll. J. Chen, Q.H. Liu, and X.T. Li (NZMC).

Taxonomic notes. The new species resembles the type species *P. setigera* (Takahashi), but differs from it as follows: dorsum of prosoma densely covered with convoluted markings medially and short folded-line shaped sculptures pleuro-marginally (Figs 8, 12–14) (the latter with convoluted markings in medial and pleural area, and marginal area covered with branched linear markings radiating outwards, Figs 4, 6); head with a pair of long thick and spine-like frontal setae (Fig. 10) (in the latter: these are much shorter and finer, Fig. 11); dorsum of prosoma with 16 pairs of submarginal setae (the latter: 15 pairs, the pair on abdominal tergite V absent); head dorsum with



Figures 12–23. *Parathoracaphis spinapilosa* sp. n. Apterous viviparous female: **12** dorsal view of body **13** convoluted markings on medial area of prosoma dorsum **14** short folded-line shaped sculptures on pleuro-marginal area of prosoma dorsum **15** short transversely striped band on margin of prosoma **16** spine-like frontal setae on head **17** 3 pairs of submarginal setae on head dorsum **18** submarginal setae on abdominal tergites V–VII **19** antenna **20** ultimate rostral segment **21** cauda **22** anal plate **23** genital plate. Scale bars: 0.10 mm.

two pairs of submarginal setae anterior to eyes, along the body margin, and a pair between eyes (Fig. 17) (the latter: all three pairs located along the body margin, Fig. 5); antennae 3- or 4-segmented (the latter: 2-segmented).

Distribution. China (Fujian and Yunnan).

Host plants. *Quercus* sp. and unidentified Fagaceae species.

Biology. Forming large colonies on the undersides of leaves of host plant (Fig. 24). The colony is attended by ants. Apteræ bear long and curved wax filaments around the body (Fig. 25). The life cycle is unknown.



Figures 24–25. *Parathoracaphis spinapilosa* sp. n. (Mt. Longqi, Fujian, China; 17 Jun 2011) **24** A colony on underside of leaf of *Quercus* sp. **25** apterous adults in life, bearing long and curved wax filaments around the body.

Key to species of *Parathoracaphis* (apterous viviparous females)

- 1 Dorsum of prosoma with 4 pairs of minute spinal setae (the pair on abdominal tergite II absent)..... **2**
- Dorsum of prosoma with 5 pairs of minute, long and fine, or spine-like spinal setae..... **4**
- 2 Head with a pair of long thick and spine-like frontal setae; dorsum of prosoma with 16 pairs of spine-like submarginal setae; antennae 3- or 4-segmented ***P. spinapilosa* sp. n.**
- Head with a pair of short, fine, and pointed frontal setae; dorsum of prosoma with 15 pairs of spine-like submarginal setae (the pair on abdominal tergite V absent); antennae 2-segmented **3**
- 3 Submarginal setae on abdominal tergite VI much smaller than setae on other tergites..... ***P. kayashimai* (Takahashi)**
- Submarginal setae on abdominal tergite VI thick spine-like, similarly sized with setae on other tergites ***P. setigera* (Takahashi)**
- 4 Dorsal spinal setae on prosoma spine-like, similar to submarginal setae; tarsi unsegmented, without claws ***P. elongata* (Takahashi)**
- Dorsal spinal setae on prosoma minute or long and fine; tarsi 2-segmented, with claws **5**
- 5 Dorsum of prosoma with 15 pairs of spine-like submarginal setae (the pair on abdominal tergite V absent); antennae 2-segmented ... ***P. cheni* (Takahashi)**
- Dorsum of prosoma with 16 pairs of spine-like submarginal setae; antennae 3- or 4-segmented..... **6**

- 6 Dorsum of prosoma without minute wax pores; head dorsum with a pair of submarginal setae near the front end and 2 pairs between eyes.....
..... *P. gooti* (Takahashi)
- Dorsum of prosoma densely covered with minute wax pores; head dorsum with 3 pairs of submarginal setae arranged in a row along the body margin
..... *P. manipurensis* (Pramanick, Samanta & Raychaudhuri)

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Hydraena Kugelann, 1794 (Coleoptera, Hydraenidae) from the Seychelles, Indian Ocean, with description of a new species

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Abstract

Hydraena matyoti sp. n. (Coleoptera, Hydraenidae) is described from the Seychelles, Indian Ocean. *Hydraena mahensis* Scott, 1913 is redescribed. The latter is here recorded from La Digue for the first time. A key to the species of the genus *Hydraena* Kugelann, 1794 of the Seychelles is presented.

Keywords

Coleoptera, Hydraenidae, *Hydraena*, new species, Seychelles, Mahé, La Digue, Silhouette, Indian Ocean

Introduction

So far, only one species of Hydraenidae, *Hydraena mahensis* Scott, 1913, has been recorded from the Seychelles (see Hansen 1998, Jäch and Madl 2009). However, when Jäch and Madl (2009) summarized the water beetle fauna of the Seychelles they were not aware that the species from Mahé, which they had photographed (Jäch and Madl 2009: Fig. 15), was actually an undescribed one. Only five years later, when Michael Madl rediscovered numerous specimens of the true *H. mahensis* on Mahé Island did they realize this error. In the present paper *H. mahensis* is redescribed, and the second species is described as new for science.

Material and methods

Line drawings were prepared with the aid of a *camera lucida* attached to a Nikon eclipse E600 microscope. Habitus photographs were taken with a Nikon DS-U2 unit Camera attached to a Leica MZ9S stereomicroscope. Images were stacked using CombineZP.

Abbreviations

BMNH	The Natural History Museum, London, UK
CDUM	Coll. J.A. Delgado, University of Murcia, Spain
IBE	Institute of Evolutionary Biology (Institut de Biologia Evolutiva), Barcelona, Spain
NMW	Naturhistorisches Museum Wien, Austria

Taxonomy

Hydraena (Hydraenopsis) mahensis Scott, 1913

Figs 1, 3–11

Hydraena (s.str.) *mahensis*: Scott 1913: 196; Knisch 1924: 39; Hansen 1998: 49.

Hydraena mahensis: Marlier 1979: 53.

Hydraena (Hydraenopsis) mahensis: Jäch et al. 2000: 80; Jäch and Madl 2009: 19 (*partim*).

Type localities. Marshes of coastal plain at Anse aux Pins and Anse Royale, Mahé, Seychelles.

Type material. Two syntypes. One of these syntypes is deposited in the BMNH (M. Barclay, email, 22.VII.2016). It is labelled as “Holotype” (red edged disc) and as ‘Type’ (blue rectangle). The second syntype (“Mahe 146” [handwritten on the rear edge of the card carrying the beetle], “Mahe 1908-09 Seychelles Exp.” [printed], “Hydraena mahensis H. Scott Paratype.” [handwritten]) is deposited in the Cambridge University Museum (W. Foster, email, 7.IX.2016).

Material examined. Mahé: 26 exs. (CDUM, NMW): Mahé (south), Petite Police Bay, swamp, 4°48.10'S 55°31.03'E, IX.2014, leg. M. Madl. La Digue: 1 ♂, 1 ♀ (NMW): La Réunion, ca. 5 m a.s.l., 7.IV.2007, leg. G. Wewalka (7).

The DNA of one female (voucher number IBE-AN186) was non-destructively extracted using the DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany) in the IBE. Two fragments of the cytochrome C oxydase subunit (COI) were sequenced, the 5' end (the barcode fragment, primers LCO1490 and HCO2198, Folmer et al. 1994) and the 3' end (primers Jerry-M202 and Pat-M70, Simon et al. 1994), and submitted to GenBank with accession numbers LT593860 and LT593861 respectively. The extracted specimen and DNA are deposited in the IBE.



Figure 1. Habitus of *Hydraena mahensis* Scott, 1913, male. Scale bar: 0.5 mm.

Redescription. Habitus as in Fig. 1. Body length (without abdomen): 1.20–1.40 mm. Dorsum brown, frons dark brown to black, posterior and lateral sides of pronotum paler yellowish brown; maxillary palpi and legs yellowish to yellowish brown.

Labrum deeply excised anteriorly; lobes rounded anteriorly. Middle of clypeus very sparsely punctate and glabrous, lateral parts densely micropunctate and mat. Frontoclypeal suture more or less straight, slightly impressed. Frons moderately densely punctate laterally, sparsely punctate medially, interstices shining; interocular grooves obsolete. Eyes large, protruding, more than 30 facets visible in dorsal view.

Pronotum wider than long, widest near middle; anterior margin weakly concave; anterior angles rounded; lateral rim denticulate; surface moderately densely punctate, but disc sometimes more sparsely punctate; discal foveae more or less obsolete.

Elytra elongately oval; with about nine rows of punctures between suture and shoulder; punctures small, not deeply impressed, arranged in almost regular, usually not impressed lines; intervals and interstices flat and glabrous; explanate margin of elytra only moderately wide, not strongly serrate posteriorly. Elytral apices usually separately rounded.

Foretibia very slightly curved in both sexes.

Mesoventral process parallel-sided, apically truncate, width sexually dimorphic. Metaventrite moderately deeply impressed in the middle; metaventral plaques rather indistinct, sometimes obscured by dense punctures.

Male terminal sternite and spiculum (Fig. 7): Sternite firmly connected with spiculum, subrectangular, almost twice as long as wide, almost symmetrical, with small subapical cavity; base with very small lateral projections.

Aedeagus (Figs 3–6): Total length: 190 μm . Main piece short, almost straight, with two moderately long setae, and a few very short ones on left side near base of distal lobe; phallobase slightly asymmetrical, closed proximally. Distal lobe quite large, about as long as main piece, forming a distinct angle with main piece (in lateral view), apically furcate. Right paramere wide, elongate, about half as long as main piece, articulately connected with main piece, inserted near apex of main piece; with four long apical setae and four moderately long setae on ventral face along right margin. Left paramere similar to right one, but slightly shorter, firmly connected with main piece, inserted on left side of apex.

Gonocoxite (Fig. 8): Subpentagonal, transverse; lateral margins curved; basal apophyses short; inner plate not projecting.

Female tergite X (Fig. 9): Subtrapezoidal, transverse; disc sparsely covered with trichoid setae; subapical setae vermiform; apical margin excised medially.

Spermatheca (Figs 10–11): Proximal portion crescentic; distal portion cup-shaped; ductus apically wrinkled.

Secondary sexual characters: Male mesoventral process more slender; in male more or less as wide as mesotibia, in female slightly wider than mesotibia.

Habitat. Swamps and ditches in coastal plain. The swamp at Petite Police Bay (Fig. 20) dries out periodically.

Distribution. This species is so far known only from Mahé (Anse aux Pins, Anse Royale, Petite Police Bay) and La Digue (La Réunion). It is here recorded from La Digue for the first time.

***Hydraena (Hydraenopsis) matyoti* Jäch & Delgado, sp. n.**

<http://zoobank.org/510E6CEA-96B4-484F-939C-ED99D955C58E>

Figs 2, 12–19

Hydraena (Hydraenopsis) mahensis: Jäch & Madl 2009: 19 (*partim*), 29.

Type locality. Small puddle on Sans Souci Hiking Trail, northern Mahé, Seychelles.

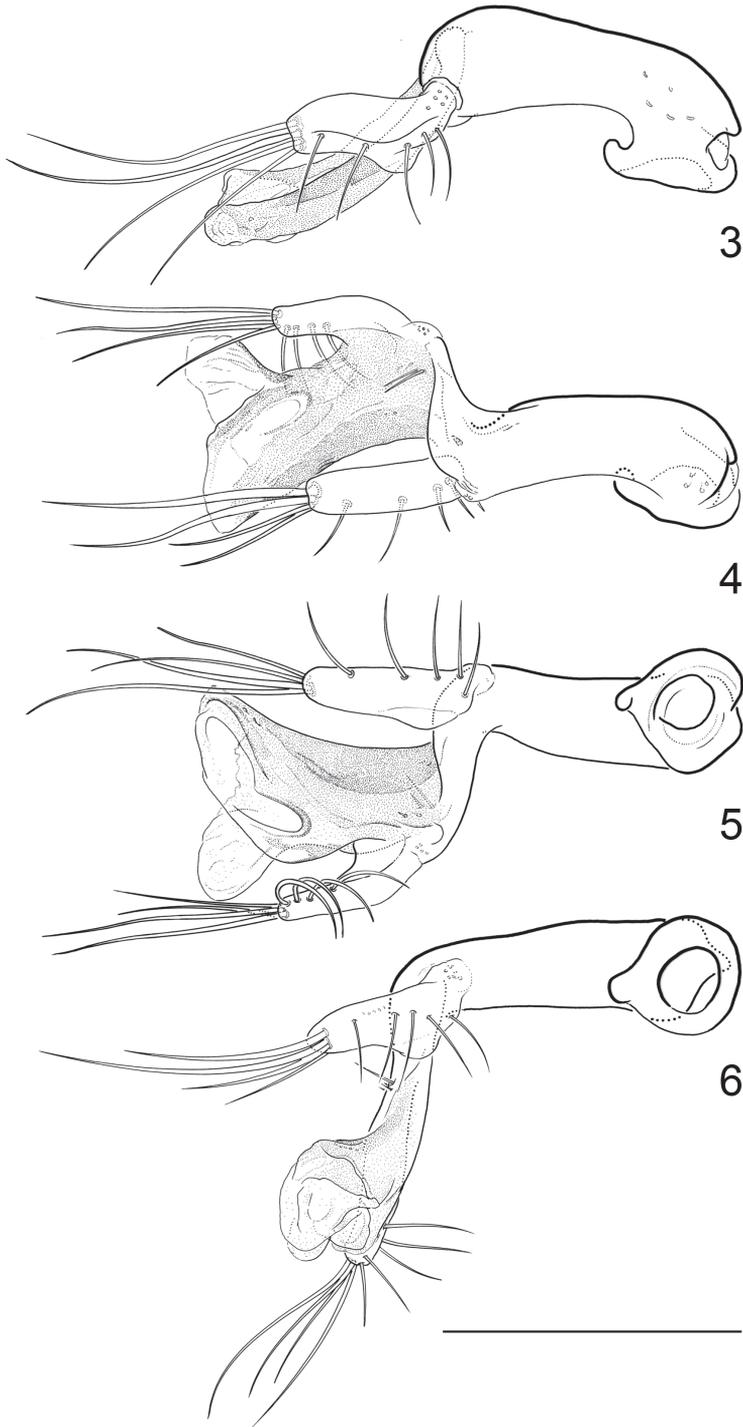


Figure 2. Habitus of *Hydraena matyoti* Jäch & Delgado, sp. n., holotype, male. Scale bar: 0.5 mm.

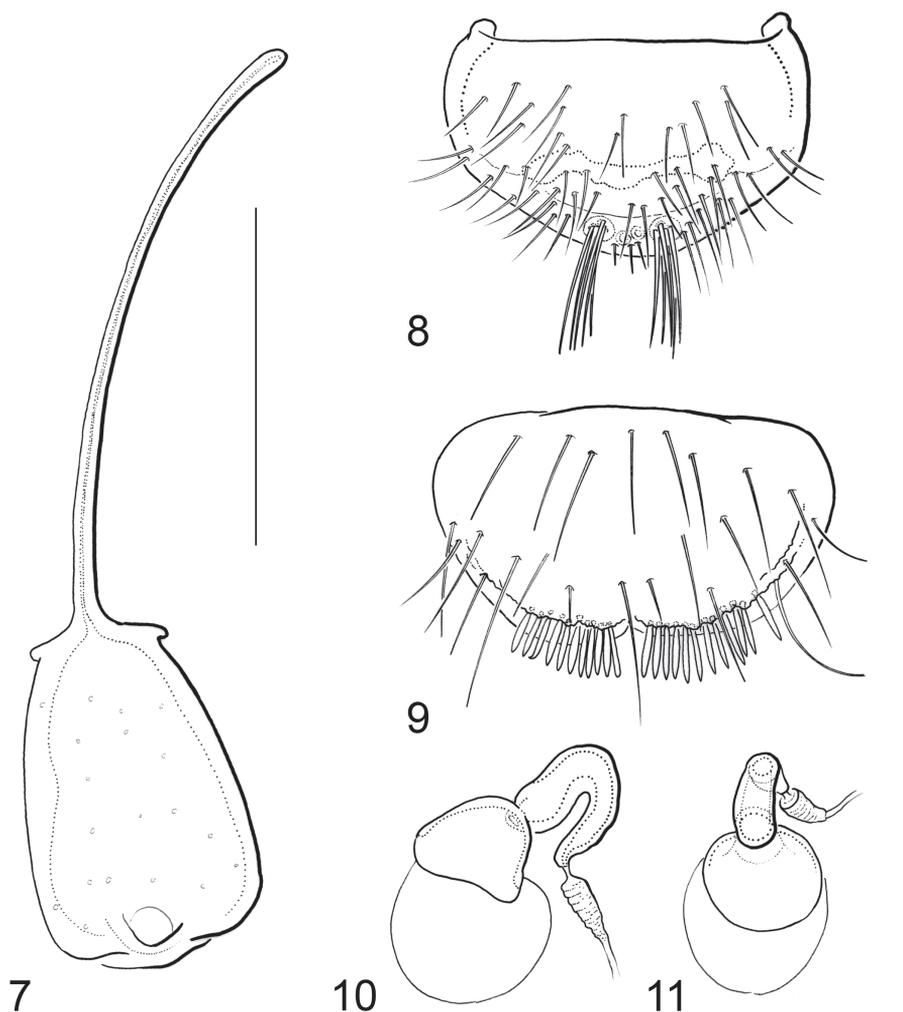
Type material. Holotype ♂ (NMW), glued on pinned card, genitalia extracted and glued on same card. Label data: “SEYCHELLES: Mahé Sans Souci, XI. 1994, leg. E. Heiss”. **Paratypes:** 1 ♀ (NMW): same label data as holotype; 1 ♂ (NMW): “SEYCHELLES: Mahé Morne Seychellois NP Casse Dent, trail 25.03.2011 leg. M. Madl”; 2 ♀♀ (NMW): “SEYCHELLEN, Mahé 1996 Morne Seychellois NP Congo Rouge 600–800m 25. V. leg. Schödl (12c)”; 1 ♀ (NMW): “Seychelles, Silhouette, Jardin Marron [field name], 400m, 12. 4. 2007, leg. Wewalka (12)”.

Description. Habitus as in Fig. 2. Body length (without abdomen): 1.56–1.70 mm. Dorsum reddish brown, lateral parts of frons (near eyes) more or less black; maxillary palpi yellowish, apical tip pale.

Labrum deeply excised anteriorly; anterior angles rounded. Middle of clypeus sparsely punctate, usually glabrous, lateral parts usually micropunctate and mat. Fronto-clypeal suture straight or feebly arcuate, slightly impressed. Frons moderately



Figures 3–6. *Hydraena mahensis* Scott, 1913, aedeagus: **3** strictly lateral view **4** dorsal view **5** strictly ventral view **6** ventral view, slightly rotated to left side. Scale bar: 0.1 mm.

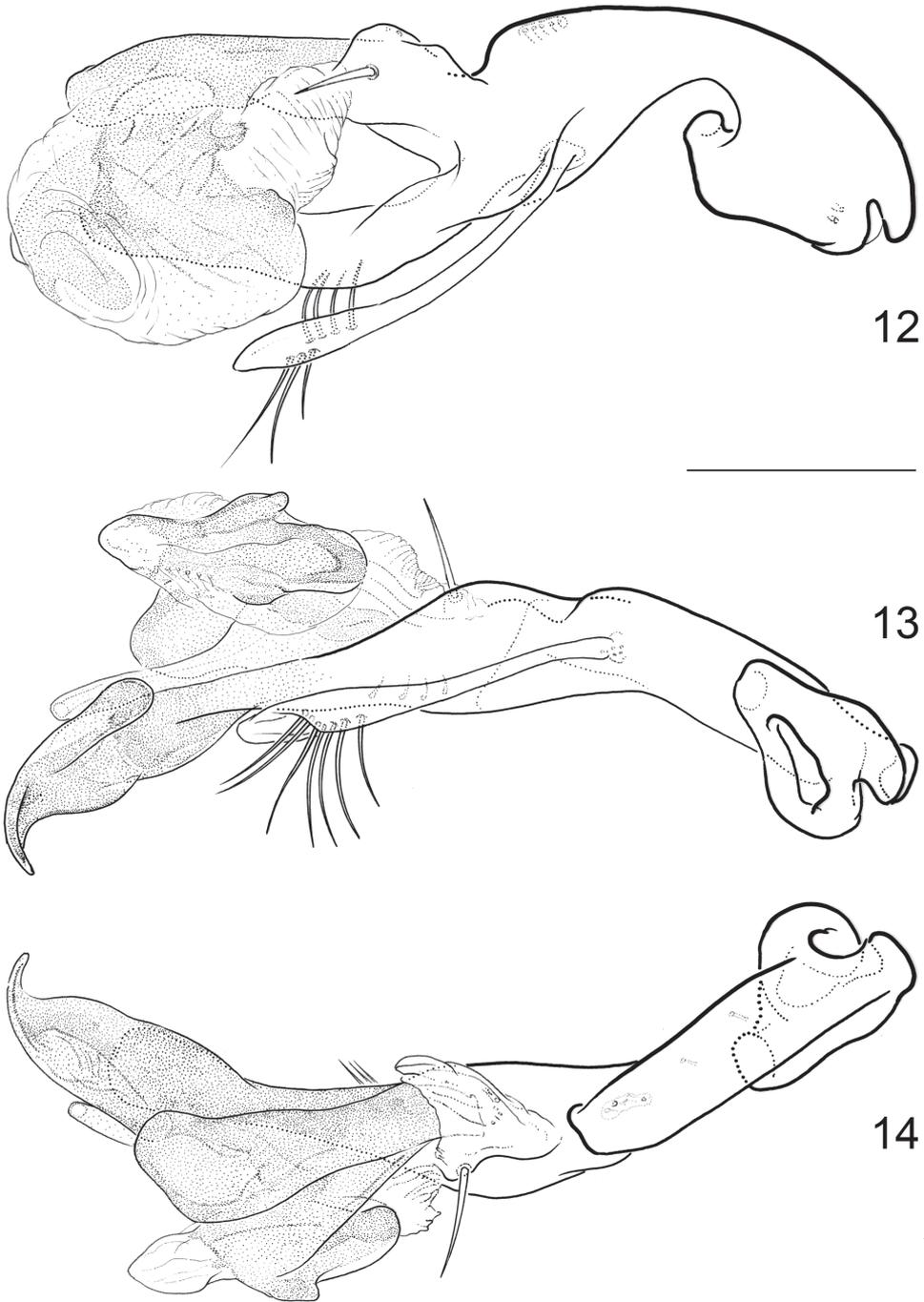


Figures 7–11. *Hydraena mahensis* Scott, 1913: **7** male terminal sternite and spiculum **8** gonocoxite **9** female tergite X **10–11** spermatheca, in different views. Scale bar: 0.1 mm.

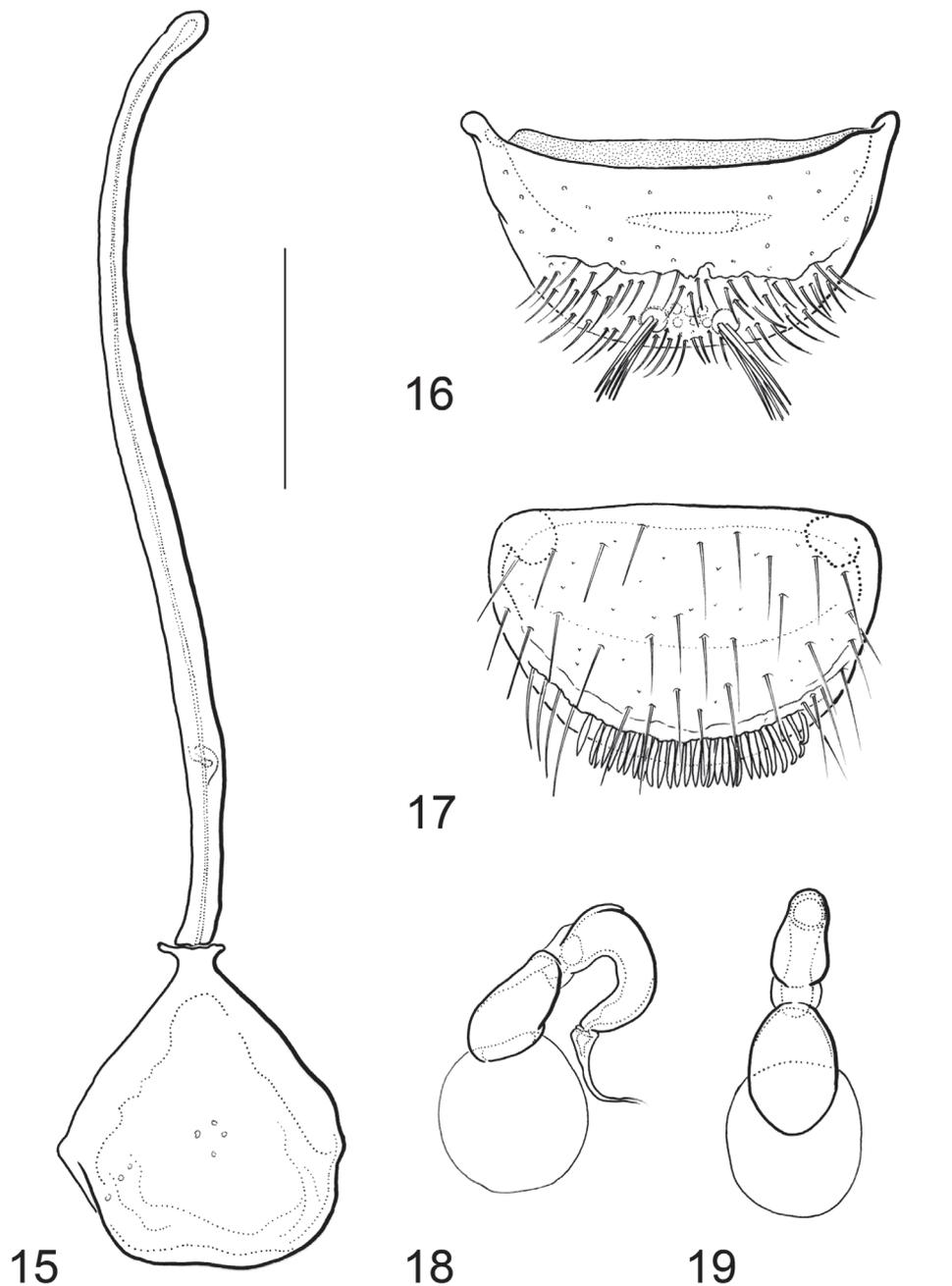
densely (middle) or more densely and sometimes even rugosely (laterally) punctate, interstices shining; interocular grooves shallow. Eyes large, protruding, more than 30 facets visible in dorsal view.

Pronotum distinctly wider than long, widest near middle; anterior margin concave; anterior angles rounded; lateral margin very slightly concave in anterior and posterior half; lateral rim denticulate; surface moderately densely to densely punctate, but disc sometimes only sparsely punctate; anterior discal foveae obsolete, posterior discal foveae hardly noticeable.

Elytra elongately oval; with about nine rows of punctures between suture and shoulder; punctures small, but rather deeply impressed, usually arranged in regular,



Figures 12–14. *Hydraena matyoti* Jäch & Delgado, sp. n., aedeagus: **12** lateral view **13** ventral view **14** dorsal view. Scale bar: 0.1 mm.



Figures 15–19. *Hydraena matyoti* Jäch & Delgado, sp. n.: **15** male terminal sternite and spiculum **16** gonocoxite **17** female tergite X **18–19** spermatheca, in different views. Scale bar: 0.1 mm.



Figure 20. Habitat of *Hydraena mahensis* Scott, 1913. Swamp at Petite Police Bay, Mahé.

usually not impressed lines; intervals sometimes convex, glabrous; explanate margin of elytra comparably wide, abruptly attenuate subapically, slightly to distinctly serrate posteriorly. Elytral apices usually separately rounded.

Foretibia and metatibia sexually dimorphic.

Mesoventral process parallel-sided, apically truncate, width sexually dimorphic. Metaventrite moderately deeply impressed between glabrous metaventral plaques, the latter reduced to very thin glabrous elevated short, widely separated streaks.

Male terminal sternite and spiculum (Fig. 15): Sternite not firmly connected with spiculum, subtrapezoidal, approximately as wide as long, slightly asymmetrical; base with very small lateral projections.

Aedeagus (Figs 12–14): Total length: 400 μm . Main piece elongate, in apical half divided into a ventral and a dorsal branch, ventral branch with characteristic claw-like apex; single dorsal seta inserted on dorsal branch near base of distal lobe; phallobase strongly asymmetrical, closed proximally. Distal lobe inserted on dorsal branch of main piece; moderately large, amorphic, partly distinctly hyaline. Right paramere long and slender, with rows of subapical setae; articulately connected with main piece. Left paramere absent. The aedeagus can be distinguished from the aedeagi of *H. borbonica* Fairmaire, 1898 (from La Réunion) and *H. ofella* Balfour-Browne, 1958 (from the Comoros) by the wider and less regular shape of the ventral branch of the main piece.



Figure 21. Habitat of *Hydraena matyoti* Jäch & Delgado, sp. n. Small stream, Morne Seychellois National Park, Mahé.

Gonocoxite (Fig. 16): Subtrapezoidal, strongly transverse; basal part without setae, distal part strongly setose; basal apophyses small; inner plate slightly projecting.

Female tergite X (Fig. 17): Subsemicircular, transverse; disc sparsely covered with trichoid setae; subapical setae vermiform; apical margin without excision.

Spermatheca (Figs 18–19): Proximal portion crescentic; distal portion elongately cup-shaped.

Secondary sexual characters: Foretibia and metatibia slightly curved in male. Male mesoventral process more slender; in male more or less as wide as mesotibia, in female slightly wider than mesotibia.

Habitat. On Mahé this species was collected in a small puddle on a forest trail (leg. E. Heiss), and in small mountain streams at more than 600 m a.s.l. (leg. M. Madl and S. Schödl) – the single specimen collected by M. Madl was found on a small piece of wood lying in a very small stream (Fig. 21). A single female was collected on Silhouette, Jardin Marron, near a hiking trail, ca. 400 m a.s.l., approx. 4°29.16'S 55°14.16'E, on a hygropetric rockface (leg. G. Wewalka).

Distribution. Endemic to the Inner Seychelles. So far known only from Mahé and Silhouette.

Etymology. This species is named for Pat Matyot, a Seychellois naturalist with a special interest in entomology. Pat Matyot is employed by the Seychelles Broadcasting Corporation and has produced many television features on the country's fauna and flora. He has served on the boards and science committees of a number of conservation organisations in Seychelles and is at present a board member of the Island Conservation Society (ICS) and the Silhouette Foundation. The epithet is a noun in the genitive case.

Key to the species of *Hydraena* of the Seychelles

- 1 Body length (without abdomen): 1.20–1.40 mm. Frons without interocular depressions (Fig. 1). Foretibia of female slightly curved. Metatibia of male straight (Fig. 1). Explanate margin of elytra narrow (Fig. 1). Male terminal sternite firmly connected with spiculum, subrectangular (Fig. 7). Aedeagus (Figs 3–6) very small (190 µm long), more or less y-shaped in ventral/dorsal view, with two parameres. Female tergite X (Fig. 9) excised apically *mahensis*
- Body length (without abdomen): 1.56–1.70 mm. Frons with shallow interocular depressions (Fig. 2). Foretibia of female straight. Metatibia of male slightly curved (Fig. 2). Explanate margin of elytra comparably wide, abruptly attenuate subapically (Fig. 2). Male terminal sternite not firmly connected with spiculum, subtrapezoidal (Fig. 15). Aedeagus (Figs 12–14) distinctly larger (400 µm long), not y-shaped in ventral/dorsal view, with one slender paramere. Female tergite X (Fig. 17) not excised apically *matyoti*

Discussion

The two *Hydraena* species of the Seychelles obviously live in different habitats. While *H. mahensis* is known only from lowland stagnant water, i.e., coastal swamps near the

sea, the new species, *H. matyoti*, was collected only at higher elevations in the mountainous interior of the Seychelles Islands, i.e., in a small puddle, mountain streams, and in a seepage on a cliff. In suitable habitats, *H. mahensis* can be found in abundance, while *H. matyoti* seems to be generally very rare. In total, only six specimens were collected between 1994 and 2011 by four Austrian entomologists.

Although both species belong to the same subgenus they are not closely related and in fact they represent different species groups. *Hydraena mahensis* is very closely related to *H. erythraea* Régimbart, 1905 (*H. erythraea* group; “*erythraea*-phylum” sensu Balfour-Browne 1950: 11), described from Eritrea. The aedeagi of these two species are characterized by the very small size, the angulate form, as well as the position and shape of the parameres. The right paramere of *H. erythraea* is distinctly smaller than in *H. mahensis*. The *H. erythraea* group is wide-spread in East Africa. *Hydraena matyoti* is probably related to *H. borbonica* and *H. ofella* (*H. borbonica* group). The aedeagi of these three species possess a deeply furcate main piece with a single dorsal seta inserted on dorsal branch near base of distal lobe, and with a single elongate and slender paramere on the right side. Possibly, *H. balfourbrownei* Bameul, 1986 and *H. legorskyi* Jäch & Brojer, 2012 also belong to this group. Although the aedeagi of these two species possess a very long and slender left paramere, the deeply furcate main piece and the shape and position of the right paramere suggest a close relationship.

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A key to *Grouvellinus* Champion, 1923 from mainland China with descriptions of two new species (Coleoptera, Elmidae)

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Abstract

A key to *Grouvellinus* Champion from mainland China is provided. Two new species of *Grouvellinus* are described from Jiangxi, China, *Grouvellinus orbiculatus* **sp. n.** and *Grouvellinus sagittatus* **sp. n.** Descriptions, diagnoses, and illustrations of the new species are given. Habitus photos of the nine known species are provided including the four species from Taiwan.

Keywords

China, Elmidae, *Grouvellinus*, new species, taxonomy

Introduction

The generic name *Grouvellinus* was proposed by Champion, 1923 and is widely distributed in Oriental and Palearctic regions. Jäch (1984) made a revision of this genus based on material occurring in Himalaya and southeast Asia, re-describing seven species and reporting eight new species. Jäch and Kodada (1995) provided a check list of the Elmidae of China: four *Grouvellinus* species were mentioned as occurring in China, and five species were reported from bordering villages (Myanmar, Nepal). Subsequently, Jeng and Yang (1998) made a revision of *Grouvellinus* occurring in Taiwan

and Japan, and three new species were reported from Taiwan. A total of 36 species of *Grouvellinus* are known so far (Brown 1981, Jäch et al. 2016, Jung et al. 2014).

According to Jäch et al. (2016), six species were known from mainland, China, and four species were reported from Taiwan. Jeng and Yang (1998) had already provided a key to *Grouvellinus* from Taiwan and Japan.

In this paper, a key for male to *Grouvellinus* from mainland China is provided and two new species of this genus are reported from Jiangxi, China. The genus *Grouvellinus* is reported from Jiangxi for the first time. Type material of the new species were deposited in Institute of Applied Ecology, Shenyang, Chinese Academy of Sciences (IAECAS). Type materials of the known species were from Natural History Museum, Vienna (NHMW). Habitus photos of *Grouvellinus carus* Hinton, 1941 were provided by Dr. Maxwell V. L. Barclay (London).

Material and methods

Specimens were examined with a Leica M205c stereomicroscope. Further details were studied under an Olympus BX51 compound microscope. Body length is the sum of pronotal and elytral lengths, body width means the broadest width of the elytra. The first strial interval means the sutural interval. Genitalia illustrations were drawn with the aid of a drawing tube. Male genitalia were placed in concentrated lactic acid in a cavity slide for at least several hours before they were examined. Photos were made with KEYENCE VHX-2000 – Super Resolution Digital Microscope System. Label data for holotypes, paratypes, and paralectotypes has been recorded verbatim, with lines on the same label separated by “/” and labels separated by “;”.

Key to species reported from mainland China (male)

- 1 Metasternum with two small pits on median suture..... 2
- Metasternum without two small pits on median suture 3
- 2 Punctures on pronotum finer and sparser. Penis end up with an arrow, parameres gradually narrowed to apex (Figs 26–28) *G. sagittatus* sp. n.
- Punctures on pronotum bigger and denser. Penis end up with expanded rounded apex, and parameres distinctly narrowed at basal 1/3(Figs 23–25)
..... *G. orbiculatus* sp. n.
- 3 Body length no less than 2.5 mm..... 4
- Body length less than 2.5 mm..... 5
- 4 Disc of metasternum with a pair of protuberances on each side of median suture at base (Fig. 18); penis sharply narrowed from base toward apex
..... *G. hercules*
- Disc of metasternum with a pair short carinae from basal 0.2 to 0.6 on each side of median suture (Fig. 20); penis sharply narrowed in distal 0.2 *G. tibetanus*

- 5 Pronotum with a shallow oval impression medially on disc (Fig. 21)... *G. carus*
- Pronotum without impression medially on disc..... **6**
- 6 Pronotum with one median carina extending from base to basal 0.6; each elytral interval with a carina, carinae on intervals 3, 5, 7, 8 more developed (Fig. 11)..... *G. nepalensis*
- Pronotum with a pair of short median carinae at base; elytra with striae intervals 7, 8 carinate (Fig.15)..... *G. sinensis*

Classification

***Grouvellinus* Champion, 1923**

Grouvellinus Champion, 1923: 168. Type species: *Macronychus caucasicus* Victor, 1839.

***Grouvellinus orbiculatus* sp. n.**

<http://zoobank.org/ED10C0B1-C00D-4FD2-AC10-D397E7D578D9>

Figures 1–2, 23–25

Type material. Holotype: male, “CHINA: Jiangxi / Ji’ an City, Suichuan/ County, Duiqian Town; 26°20’N 114°17’E, 228 / m, 2009.10.02, leg. / Bian & Tong (loc. 10)” (white label); “HOLOTYPE / *Grouvellinus* / *orbiculatus* sp. n.” (red label). Paratypes: 3 males, 4 females, the same data as holotype. 4 males, 2 females: “CHINA: Jiangxi / Ji’ an City, Suichuan / County, Caolin Town; 26°16’N 114°22’E, 228 / m, 2009.10.03, leg./ Bian & Tong (loc. 11)” (white label); “PARATYPE / *Grouvellinus* / *orbiculatus* sp. n.” (red label). 1 male: “CHINA: Jiangxi / Ganzhou City, Shangyou / County, Wuzhifeng Town; 25°57’N 114°05’E, 554 / m, 2009.10.05., leg. / Bian & Tong (loc. 12)”(white label); “PARATYPE / *Grouvellinus* / *orbiculatus* sp. n.” (red label).

Diagnosis. This species is characterized by its small size (<2 mm), dense punctures on pronotum, sparse pubescence on the elytra, and the metasternum with two small pits on median suture; the penis end has a rounded expanded apex, and the parameres are distinctly narrowed at basal 1/3 in dorsal view.

Description. *Body* length 1.70 mm, body width 0.80 mm. Body shape elongate obovate, subparallel (Figs 1–2). Head black, pronotum and elytra brown, femora and tibiae dark brown. Antennae and tarsi yellowish brown.

Head smooth, covered with long pubescence; surface with small circular punctures sparsely and superficially impressed. Clypeus surface smooth, weakly punctate, lateral sides sparsely covered with long pubescence. Labrum smooth, weakly punctate and densely pubescent laterally.

Pronotum 1.2 times as broad as long, broadest at basal 2/5, surface smooth and shining, covered with adpressed pubescence; small circular punctures densely impressed. Sub-lateral carinae present on basal 2/5, feebly raised and feebly converging



Figures 1–4. Habitus. **1–2** *Grouvellinus orbiculatus* sp. n., holotype **3–4** *G. sagittatus* sp. n., holotype **1, 3** dorsal view **2, 4** ventral view. Scale bar: 1 mm. Photograph by Dongju Bian.

anteriorly; an oblique impression on each side extending from the end of carina to anterior angle. Base with two elongated oval impressions in front of anterior angles of scutellum.

Elytra 1.5 times as long as broad, 1.4 times as broad as pronotum; sides subparallel in basal 2/3, and then gradually narrowed to separated rounded apex. Strial punctures larger and deeper in basal half separated by 0.5–1.0 diameter; punctures becoming smaller and widely separated (2–5 times diameters); intervals slightly convex, and each interval with one or two rows of small punctures and pubescence; intervals 2–4 slightly elevated at base; interval 8 carinate.

Process of *prothoracic ventrite* approximately 1/3 as broad as pronotum, distinctly rimmed; basal 2/3 disc smooth and shining, slightly elevated, apex somewhat truncate (Fig. 2). Disc of metasternum broadly impressed, surface smooth and shining, weakly punctate, and two small pits present on median suture; sub-lateral area of metasternum densely pubescent; metasternum with two rows of punctures on each side, one is oblique consisting of very large punctures which begin at anterior margin laterally and end up in front of metacoxae, the other one consisting of moderate-sized punctures is in front of posterior margin. Middle regions of ventrites I–IV smooth and shining, with small punctures sparsely distributed, and sub-lateral regions densely pubescent; middle region of ventrite V at basal 1/3 is similar to ventrites I–IV, elsewhere on ventrite V densely pubescent and sparsely granulate.

Male genitalia (Figs 23–25) long and slender, about 690 μm in length. Penis distinctly surpasses parameres, gradually narrowed from base to distal portion, apex expanded ending up with broadly rounded apex; ventral sac with lateral sides weakly sclerotized, surpassing the parameres. Parameres robust at base, distinctly narrowed at basal 1/3 in dorsal view.

Female similar to male, but disc of metasternum not broadly impressed, slightly elevated.

Distribution. China: Jiangxi.

Etymology. The specific name comes from the Latin word “orbiculatus” and refers to the rounded apical portion of penis.

Remarks. *Grouvellinus orbiculatus* sp. n. is similar to *G. sagittatus* sp. n. in body size, habitus, interval 8 on elytra with a carina, and two small pits on median suture of metasternum, but can be distinguished from the latter by its denser punctures on pronotum, penis ends with expanded rounded apex, and parameres distinctly narrowed at basal 1/3.

***Grouvellinus sagittatus* sp. n.**

<http://zoobank.org/609F02D9-A6C8-4F29-B2DF-A8EEBB5903F9>

Figures 3–4, 26–28

Type materials. Holotype, male: “China: Jiangxi / Ganzhou, Longnan / County, Jiulianshan; 24°37'N 114°32'E, 560 / m 2009.10.10, Leg. Bian / & Tong (16)” (white label); “HOLOTYPE / *Grouvellinus* / *sagittatus* sp. n.” (red label). Paratypes: 2 males, 2 females: “CHINA: Jiangxi, / Ganzhou City, Longnan / County, Jiulianshan; Downstream of loc. 16; / 2009.10.10; leg. Bian & / Tong (loc. 17)” (white label); “PARATYPE / *Grouvellinus* / *sagittatus* sp. n.” (red label).

Diagnosis. This species is characterized by its small size (<2 mm), dorsum sparsely pubescent, and metasternum with two small pits on the median suture; penis ends with an arrow, and parameres gradually narrowed from base towards apex.

Description. *Body* length 1.75 mm, body width 0.80 mm. Body shape elongate obovate, subparallel (Figs 3–4). Dorsal surface brown to dark brown, with strong bronze luster; elytra paler than pronotum. Ventral surface brown to dark brown, femora and tibiae brown, antennae, mouth parts, and tarsi yellowish-brown.

Head smooth and shining, covered with long pubescence; small circular punctures sparsely impressed. Clypeus surface smooth, sparsely punctate and pubescent; labrum smooth, surface of disc weakly punctate and pubescent, and densely pubescent laterally.

Pronotum 1.2 times as broad as long; broadest at basal 2/5. Surface smooth and shining, accompanied with long adpressed pubescence; small circular punctures sparsely and superficially impressed. Basal sub-lateral carinae present on basal 2/5, but only slightly raised, feebly converging anteriorly; an oblique impression on each side extending from apical end of carina to anterior angle; base with two elongate oval impressions in front of anterior angles of scutellum.

Elytra 1.6 times as long as broad, 1.4 times as broad as pronotum; sides subparallel in basal 2/3, and then tapering to separated rounded apex. Strial punctures larger and deeper on disc, but becoming finer and shallower on apical declivity; intervals convex, smooth and shining, each interval with one or two rows of small punctures and pubescence; intervals 2–4 slightly elevated at base; interval 8 carinate.

Process of *prothoracic ventrite* approximately 1/3 as broad as pronotum, subquadrate, strongly rimmed, with transverse, smooth elevation at base; surface almost without punctures and pubescent on disc (Fig. 4). Disc of metasternum flat, smooth and shining, sparsely punctate, with two small pits on median suture, one is longitudinal which on the middle of metasternum, and the other one is transverse which is in front of the posterior margin; sub-lateral region densely pubescent, with two rows of coarse punctures on each side, one is oblique with very large punctures which begin at anterior margin laterally and end up in front of the metacoxae, the other one consisting of moderate-sized punctures is in front of posterior margin. Middle regions of ventrites I–IV smooth and shining, with few small punctures; sub-lateral regions densely pubescent; distal 2/3 of ventrite V granulate.

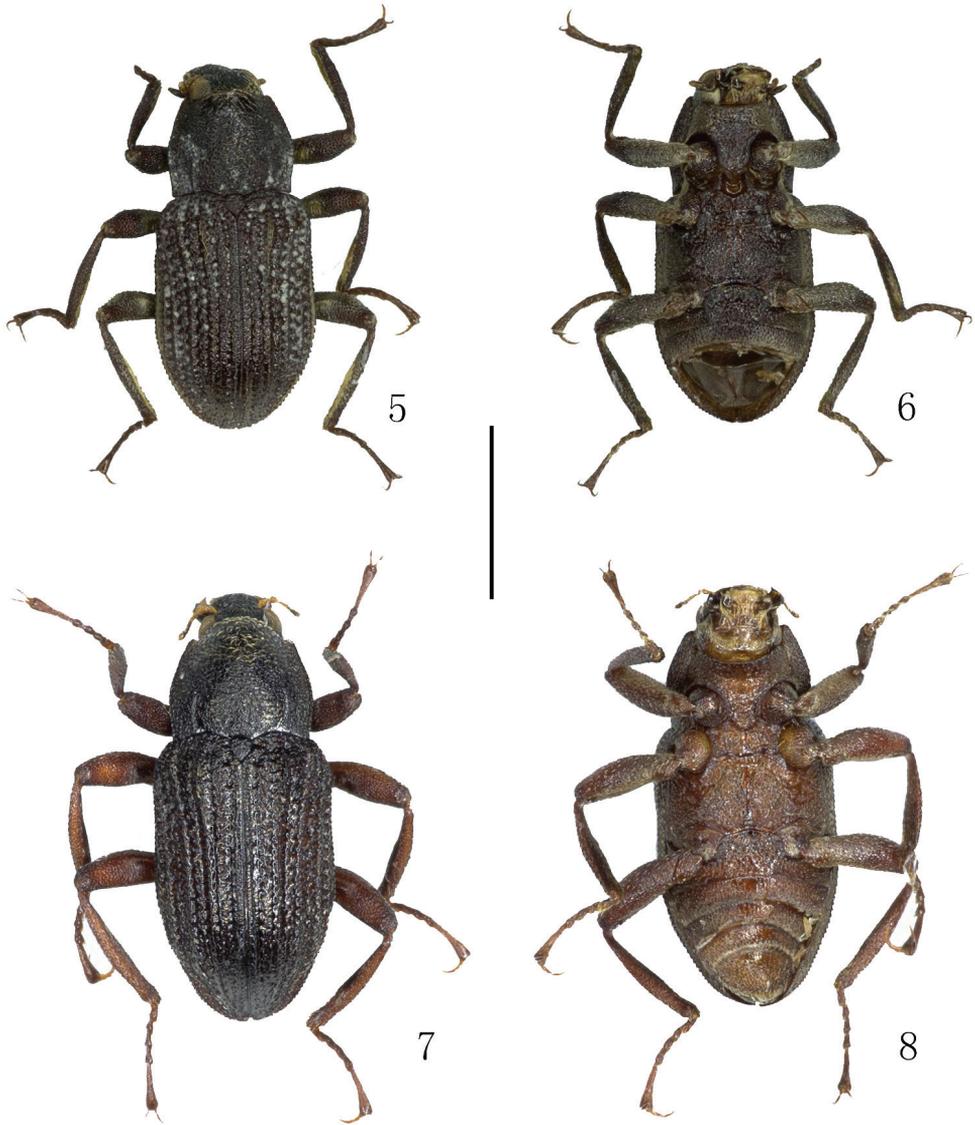
Male genitalia (Figs 26–28): long and slender, 605 μm in length; penis extends beyond parameres, distinctly narrowed from base toward apex, arrowed at the tip; ventral sac developed, longer than parameres. Parameres gradually narrowed from base to apex in dorsal view.

Females similar to males, but disc of metasternum not flat, distinctly elevated.

Distribution. China: Jiangxi.

Etymology. The specific name comes from the Latin word “sagittatus” meaning “arrow-shaped” and refers to the inflated apical portion of penis.

Remarks. *Grouvellinus sagittatus* sp. n. is similar to *G. orbiculatus* sp. n. in body size, habitus, interval 8 with a carina, and two small pits on median suture, but can be distinguished from the latter by sparser punctures on pronotum, penis ends with an arrow, and parameres gradually narrowed from base to apex in dorsal view.



Figures 5–8. Habitus. **5–6** *G. babai babai* Nomura, 1963 **7–8** *G. hydropetricus* Jeng & Yang, 1998, paratype **5, 7** dorsal view **6, 8** ventral view. Scale bar: 1 mm. Photograph by Dongju Bian.

***Grouvellinus babai babai* Nomura, 1963**

Figures 5–6

Grouvellinus babai babai Nomura, 1963: 55. TL: China, Taiwan.

Distribution. China: Taiwan.

Material examined. 1 male: “Taiwan, Taipei / Wanli 13. 8. 90 / M.L. Jeng (111)”.

***Grouvellinus chinensis* Mařan, 1939**

Grouvellinus chinensis Mařan, 1939: 42. TL: China, Sichuan.

Distribution. China: Sichuan.

Remarks. This species was described by Mařan in 1939 based on a female specimen. The type specimen was deposited in National Museum, Prague, Czech Republic (NMPC). Dr. Martin Fikáček was contacted, but he did not find the type specimen in NMPC, and also did not find any record of rent loans. According to the original description, this species was 3.0 mm long, 1.3 mm wide, strial intervals transversely wrinkled on elytra, and intervals 3, 5, 7, 8 carinate, while the two new species were less than 2.0 mm long, strial intervals smooth and shining on elytra, and interval 8 carinate. This species was also not included in the key because the key was only for males.

***Grouvellinus hydropetricus* Jeng & Yang, 1998**

Figures 7–8

Grouvellinus hydropetricus Jeng & Yang, 1998: 533. TL: China, Taiwan.

Distribution. China: Taiwan.

Material examined. Paratype: 1 male: “Taiwan 8.IX. 93 / Taipei, Wulei / leg. M. L. Jeng” (white label); “PARATYPUS / *Grouvellinus* / *hydropetricus* n. sp. / des Jeng & Yang, 1993” (red label).

***Grouvellinus montanus* Jeng & Yang, 1998**

Figures 9–10

Grouvellinus montanus Jeng & Yang, 1998: 530. TL: China, Taiwan.

Distribution. China: Taiwan.

Material examined. Paratype: 1 male: “Ilan Taiwan / Chiduan / 29 III. 1990 / Jeng M. L. leg.” (white label); “PARATYPUS / *Grouvellinus* / *montanus* / JENG & YANG / des. Jeng & Yang” (red label).

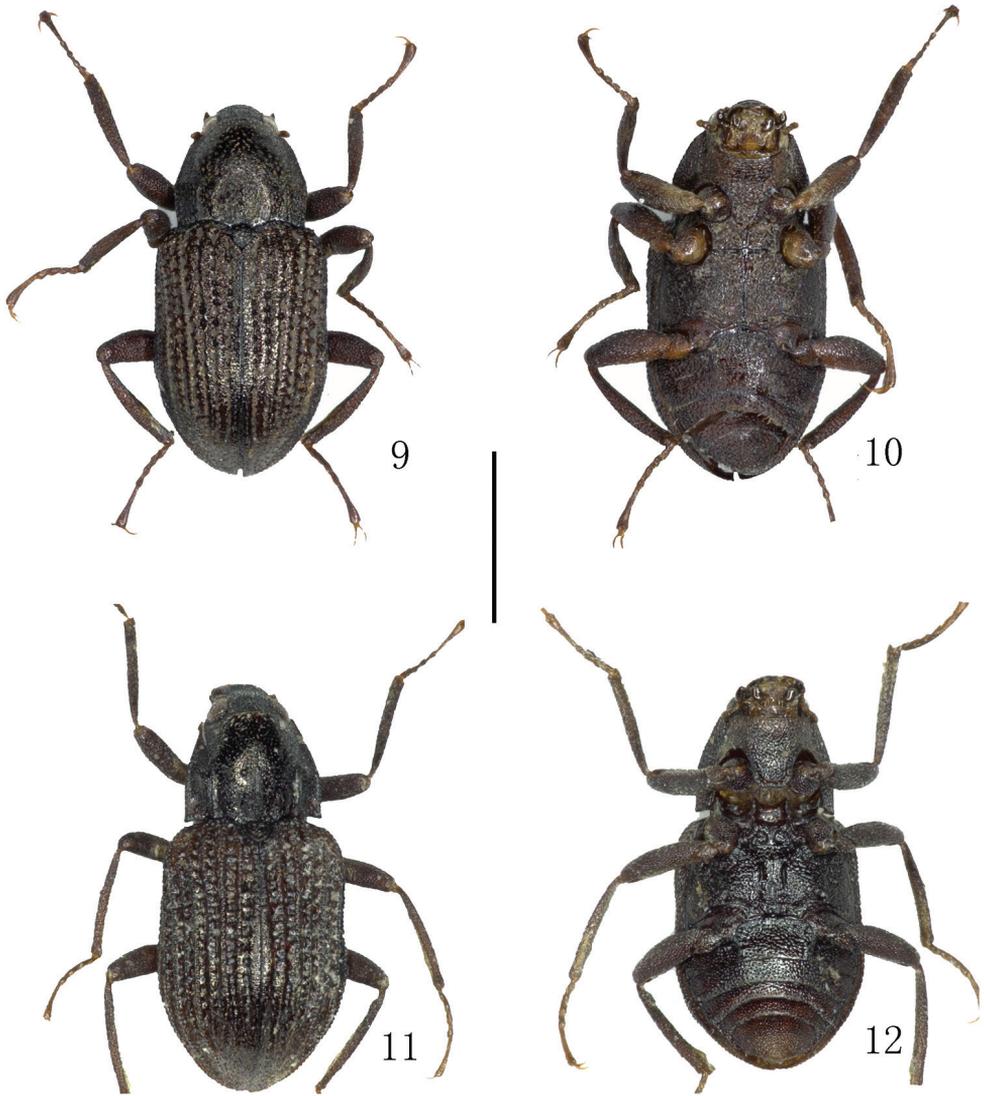
***Grouvellinus nepalensis* Delève, 1970**

Figures 11–12

Grouvellinus nepalensis Delève, 1970: 321. TL: China, Nepal.

Distribution. China: Xizang; Nepal.

Material examined. 1 male: “Nepal, 28.2.31 / Tibetan. Grenze / leg. M. Jäch”.



Figures 9–12. Habitus. **9–10** *G. montanus* Jeng & Yang, 1998, paratype **11–12** *G. nepalensis* Delève, 1970 **9, 11** dorsal view **10, 12** ventral view. Scale bar: 1 mm. Photograph by Dongju Bian.

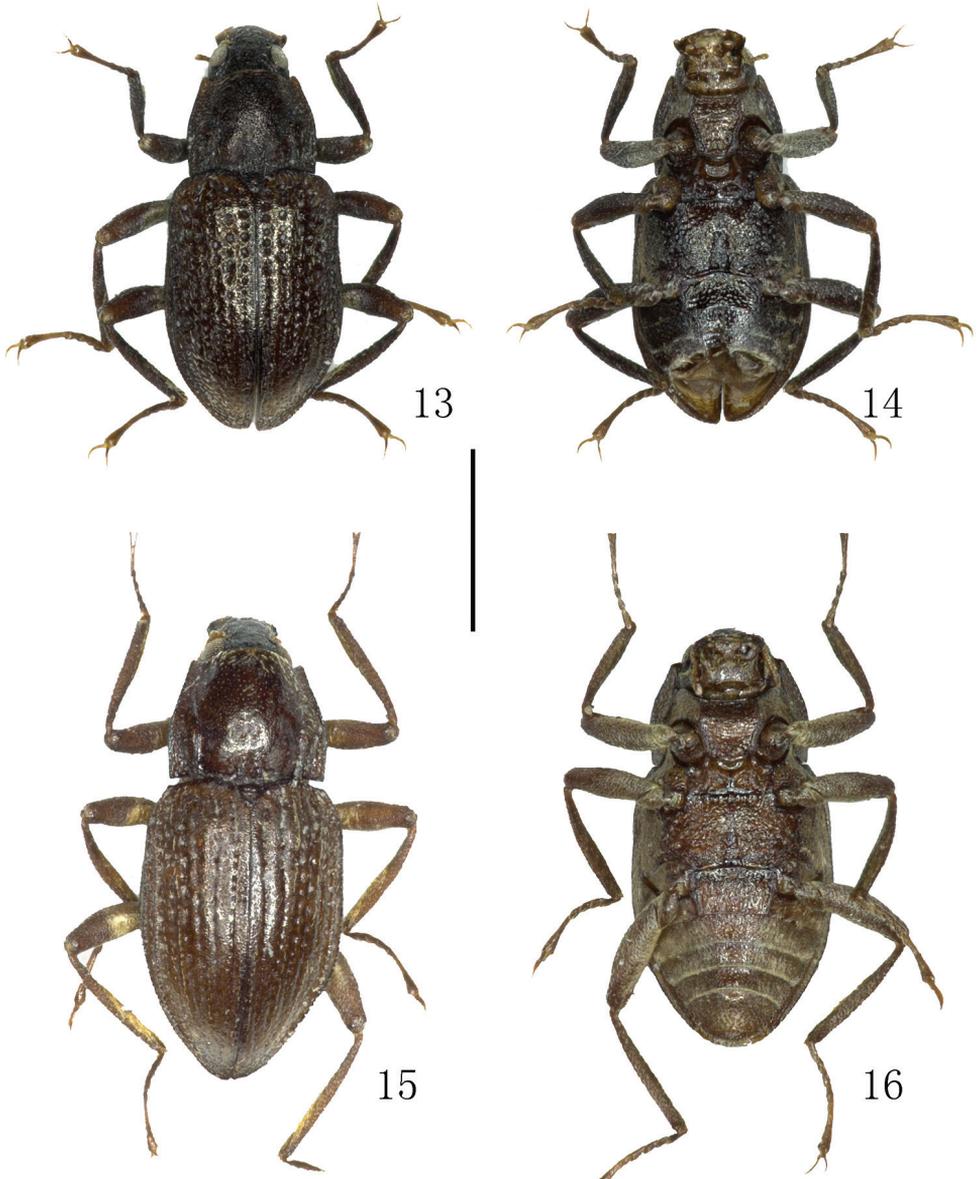
***Grouvellinus pilosus* Jeng & Yang, 1998**

Figures 13–14

Grouvellinus pilosus Jeng & Yang, 1998: 527. TL: China, Taiwan.

Distribution. China: Taiwan.

Material examined. Holotype: 1 male: “Taiwan 16. VIII. 90 / Taipei / Sanshah: Yomushi / leg. M. L. Jeng”; “HOLOTYUS / *Grouvellinus* / *pilosus* sp.n. / des. Jeng & Yang 1993” (red label).



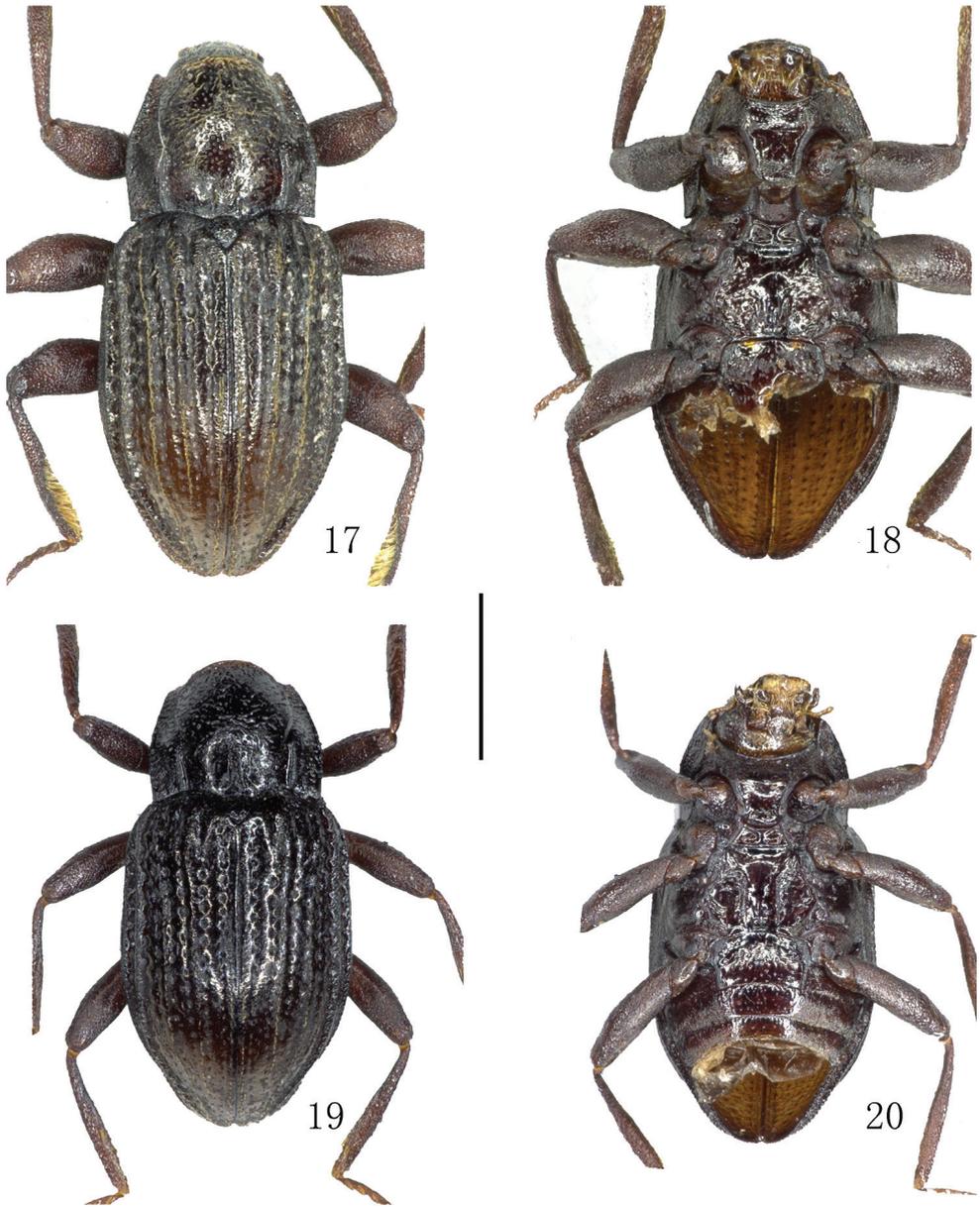
Figures 13–16. Habitus. **13–14** *G. pilosus* Jeng & Yang, 1998, holotype **15–16** *G. sinensis* Grouvelle, 1906, paralectotype **13, 15** dorsal view **14, 16** ventral view. Scale bar: 1 mm. Photograph by Dongju Bian.

***Grouvellinus sinensis* Grouvelle, 1906**

Figures 15–16

Grouvellinus sinensis Grouvelle, 1906: 126. TL: China, Yunnan.

Distribution. China: Yunnan.



Figures 17–20. Habitus. **17–18** *G. hercules* Jäch, 1984 **19–20** *G. tibetanus* Jäch, 1984, paratype **17, 19** dorsal view **18, 20** ventral view. Scale bar: 1 mm. Photograph by Dongju Bian.

Material examined. Paralectotypes: 1 male, 1 female: “Yun-nan, Chine / Grouvelle / 1906” (white label); “PARALECTOTYPUS / Microdes / sinensis Grouvelle / des. M. A. Jäch” (red label).

***Grouvellinus hercules* Jäch, 1984**

Figures 17–18

Grouvellinus hercules Jäch, 1984: 113. TL: Nepal.**Distribution.** China: Xizang; Nepal.**Material examined.** 1 male: “Nepal, 35 Km NW Pokhara / Ulleri 2000m / leg. Wewalka 5.5. 1984 (N4)”.***Grouvellinus tibetanus* Jäch, 1984**

Figures 19–20

Grouvellinus tibetanus Jäch, 1984 : 114. TL: Nepal.**Distribution.** China: Xizang; Nepal.**Material examined.** Paratype: 1 male: “Nepal, 1.3.81. / Tibetan. Gregze /leg. M. A. Jäch, N 31/ Tatopani” (white label); “Para / TYPUS” (red label).***Grouvellinus carus* Hinton, 1941**

Figures 21–22

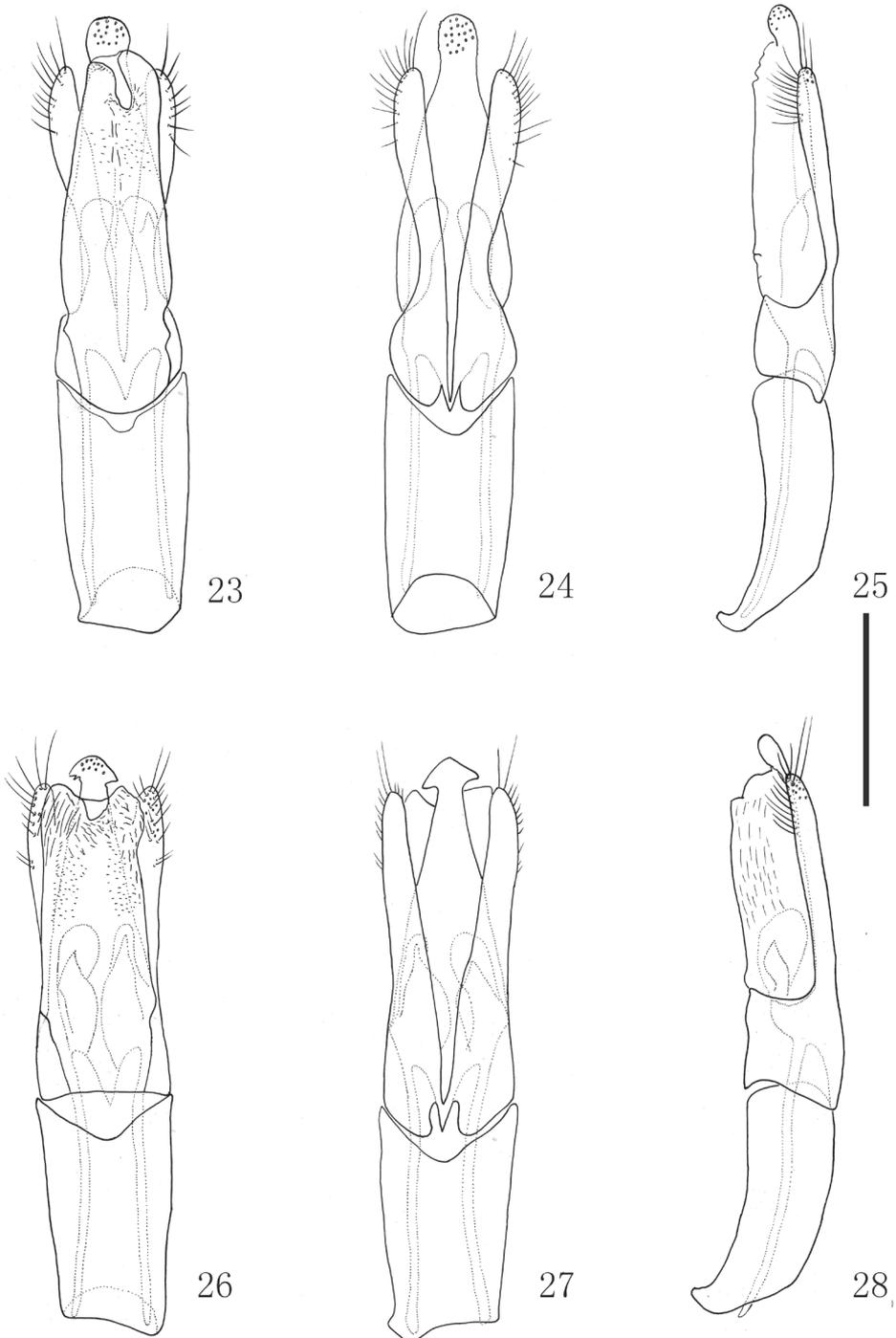
Grouvellinus carus Hinton, 1941: 71. TL: China, Fujian.**Distribution.** China: Fujian.**Remarks.** This species was described by Hinton in 1941, and the type specimens were deposited in Natural History Museum, London. Dr. Maxwell V. L. Barclay (London) provided the habitus photos of the holotype which were taken by Keita Matsumoto (London). According to the photos and the original description, the following differences between the *G. carus* and the two new species are noted: *G. carus* has an oval impression medially on pronotum, surface of head with a few granules, metasternum without two small pits on median suture, and all of ventrites II to V densely pubescent and sparsely granulate, while the two new species are without impressions medially on pronotum, head smooth and shining, without granules, metasternum with two small pits on median suture, and middle regions of ventrites I to IV smooth and shining, without granules, not densely pubescent.**Concluding remarks**

The focus of this study, is on the species of *Grouvellinus* which occur in mainland China and Taiwan. Ten species including the two new species were examined. Some spe-



Figures 21–22. *G. carus* Hinton, 1941, holotype. **21** habitus, dorsal view **22** lateral view. Photograph by Keita Matsumoto (London).

cies occurring in neighboring countries such as *G. brevior* (Nepal), *G. nitidus* Nomura (Japan), and *G. subopacus* Nomura (Japan) were also examined during the study. Habitus and male genitalia photos of *G. sculptus* Bollow, 1940 (Myanmar) were provided by Dr. Johannes Bergsten (Stockholm). Unfortunately, we were unable to examine *G. carus* Hinton and *G. chinensis* Mařan. The original description of *G. carus* was detailed



Figures 23–28. Male genitalia. **23–25** *Grouvellinus orbiculatus* sp. n., holotype **26–28** *Grouvellinus sagittatus* sp. n., holotype **23, 26** in ventral view **24, 27** in dorsal view; **25, 28** in lateral view. Scale bar: 0.2 mm.

and habitus photos were clear, so some differences between *G. carus* and the two new species could be observed. The type specimen of *G. chinensis* was not deposited in the National Museum, Prague, Czech Republic (NMPC) as mentioned in the original publication, so the new species could only be compared with the original description of *G. chinensis*, but the original description was simple, so the main differences observed were the body size and carinae on elytral intervals. The topotype of *G. chinensis* Maran should be collected improving the revision of *Grouvellinus*.

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A taxonomic review of the genus *Horniolus* Weise from China, with description of a new species (Coleoptera, Coccinellidae)

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Abstract

Five species of the genus *Horniolus* Weise, 1901 from China are revised, including the description of a new species, *Horniolus hainanensis* Chen & Ren, **sp. n.** *Horniolus sonduongensis* Hoàng, 1979 is reported from China for the first time. A key to the species from China is provided. Nomenclatural history, diagnoses, detailed descriptions, illustrations, and distribution for each species have been provided. A checklist of all known species of this genus is also presented.

Keywords

Coccinelloidea, checklist, Hainan Island, Scymnini, taxonomy

Introduction

The genus *Horniolus* was proposed by Weise (1901), with type species *Horniolus dispar* Weise described from Sri Lanka by monotypy. Bielawski (1961) pointed out that the species described as *Scymnus (Pullus)* sp. from Sri Lanka by him (Bielawski 1957) was *H. dispar*. Miyatake (1963) provided a detailed description of *Horniolus*, transferred

Scymnus fortunatus Lewis, 1896 to this genus, and described three additional species from Japan. Subsequently, six more species from China (Hong Kong and Taiwan), India, Malaysia (North Borneo), Thailand and Vietnam were added by him to this genus (Miyatake 1976, 1979). Hoàng (1979) described *H. sonduongensis* from Vietnam. Booth and Pope (1989) transferred *Scymnus guimeti* Mulsant, 1850 to *Horniolus* based on the examination of the type material deposited in the Hope Entomological Collections. Sathe and Bhosale (2001) described *H. mirajensis* from India, but it was synonymized with *Propylea dissecta* (Mulsant, 1850) by Poorani (2004). Recently, Poorani (2015) described *H. sororius* from India. Prior to the present study, only 14 species have been recognized in this genus, occurring from East to South and Southeast Asia, and most species are poorly represented in collections.

Horniolus has been placed in the tribe Scymnini, which was included in the subfamily Scymninae by earlier workers such as Sasaji (1971), Pang and Gordon (1986), Fürsch (1996) and Poorani (2002). However, Korschefsky (1931) assigned this genus to the tribe Ortaliini within the subfamily Coccinellinae in his catalogue. Recent phylogenetic study on classification of Coccinellidae using both molecular and morphological data has showed that *Horniolus* Weise and *Rodolia* Mulsant form a clade placed within the tribe Coccidulini in the broadly defined subfamily Coccinellinae (Seago et al. 2011).

Members of *Horniolus* mostly prey on mealybugs, such as *Planococcus lilacinus* Cockerell and *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae), infesting coffee, tea and pineapple. These beetles also feed on spiraling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), and play an important role in the biological control of this pest (Irulandi et al. 2001, Ramani et al. 2002, He et al. 2013, Poorani 2015).

In the present paper, five species of the genus *Horniolus* from China are revised, with the addition of a new species. All the species are illustrated and described in detail. A key to and distribution map for the species from China are given. A checklist of the species of *Horniolus* around the world is also provided.

Material and methods

Specimens examined were collected from China and Vietnam, and deposited in the Department of Entomology, South China Agricultural University, Guangzhou, China (SCAU). In addition, the holotypes of *H. novempunctatus* and *H. hisamatsui* were obtained from the collection of the Entomological Laboratory, Ehime University, Matsuyama, Japan (ELEU).

The morphological terms follow Ślipiński (2007) and Ślipiński and Tomaszewska (2010). Measurements were made using a micrometer attached to a SteREO Discovery V20 dissecting stereoscope and are defined as follows:

- TW** total width, across both elytra at widest part;
TH total height, at highest part of elytra in lateral view;

TL	total length, from apical margin of clypeus to apex of elytra;
PL	pronotal length, from the middle of anterior margin to the base of pronotum;
PW	pronotal width at widest part;
EW	elytral width, equal to TW;
EL	elytral length, along suture from base to apex including scutellum;
HW	head width, at widest part including eyes.

Male and female genitalia were dissected, cleared in a 10% solution of NaOH by boiling for several minutes, and placed on slides for further study. Photographs of the whole beetles and their genitalia were taken according to Chen et al. (2015).

Taxonomy

Genus *Horniolus* Weise, 1901

Horniolus Weise, 1901: 442. Type species: *Horniolus dispar* Weise, 1901, by monotypy.

Diagnosis. *Horniolus* is similar to *Scymnus* Kugelann, particularly the subgenus *Scymnus* (*Pullus*) Mulsant in general appearance. However, it can be easily distinguished from the latter by the following combination of characters: body rounded or elongate oval; antennae composed of 11 antennomeres (Fig. 1f); prosternal process with an inverted Y-shaped carina (Fig. 1b); abdominal postcoxal line complete or apically obliterated and apparently incomplete (Figs 1h, 3d); area enclosed by postcoxal line coarsely punctate; abdomen with six ventrites (Fig. 1h); tarsi with four tarsomeres (Fig. 1g); female genitalia with spermatheca tubular, long and intricately coiled (Fig. 4e).

Description. Body rounded or elongate oval, moderately convex, with dense pubescence, widest around middle of elytra.

Head transverse; frons wide (Fig. 1a). Eyes moderately large, finely faceted, inner ocular margin slightly arcuate. Clypeus truncate anteriorly, slightly expanding laterally, entirely covering antennal insertions. Antennae composed of 11 antennomeres (Fig. 1f); 1st antennomere stout, curved and distinctly rounded on inner side; 2nd firmly united with 1st, shorter and narrower than the latter; 3rd obviously trapezoidal, small, outer side nearly two times as long as inner side; 4th to 6th as wide as 3th; 7th to 11th forming a fusiform club (Fig. 1f). Labrum exposed, transverse, rounded anteriorly (Fig. 1a). Mandible bifid apically with inner tooth slightly shorter than outer one (Fig. 1e). Terminal maxillary palpomere stout, securiform, weakly broadening apically, apical margin strongly obliquely truncate (Fig. 1c). Labial palps with three palpomeres, terminal palpomere blunt, subcylindrical, shorter than penultimate one (Fig. 1d).

Pronotum moderately convex, hind margin wider than anterior one (Fig. 2b). Anterolateral angles of pronotum indistinct, blunt. Pronotal hypomeron broad without delimited foveae (Fig. 1b). Prosternum T-shaped with prosternal process bearing an inverted Y-shaped carina (Fig. 1b), area enclosed by prosternal carina smooth.

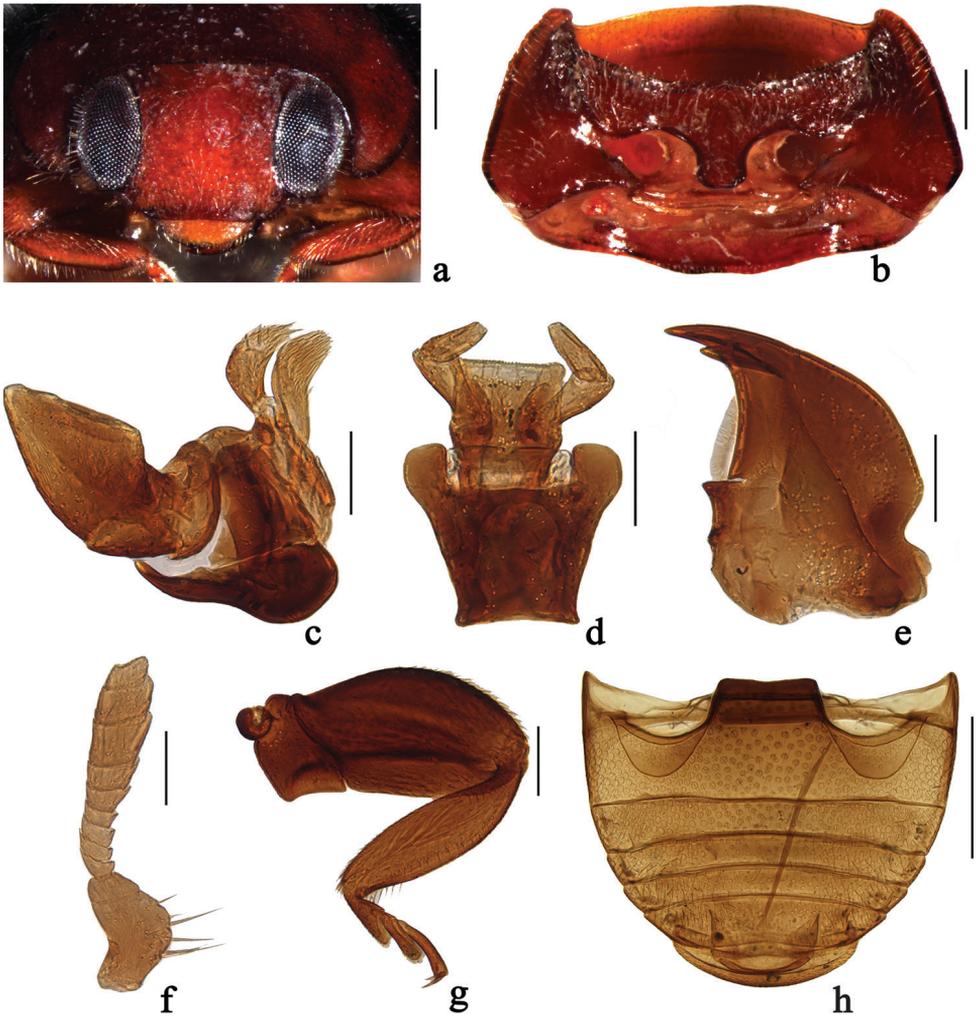


Figure 1. Main characters of the genus *Horniolus* Weise: **a–g** *Horniolus hisamatsui* Miyatake: **a** head **b** prothorax, ventral **c** maxilla **d** labium **e** mandible **f** antenna **g** hind leg **h** *Horniolus sonduongensis* Hoàng: abdomen. Scale bars: 0.2 mm (**a–b, g**), 0.2 mm (**c–f**), 0.5 mm (**h**).

Scutellum moderately large and triangular (Fig. 2a). Elytra distinctly wider than pronotum at base, surface finely punctate. Elytral epipleuron narrow and nearly horizontal, terminated at level of hind coxae. Abdomen with six ventrites (Fig. 1h). Abdominal postcoxal lines complete or incomplete (Figs 1h, 3d). Legs stout and long (Fig. 1g), not extending beyond external boundary of body; femora of hind leg broad and flattened; tibiae without apical spur; tarsi with four tarsomeres, claws bifid with sharp basal teeth.

Distribution. Bangladesh, China, India, Japan, Malaysia, Nepal, Sri Lanka, Thailand, Vietnam.

Key to the species of the genus *Horniolus* from China

- 1 Elytra dark brown to black with 4 transverse spots or orange with 6 black spots **2**
 – Elytra yellow with 9 black spots (Fig. 2a); body length 2.95 mm
 ***H. novempunctatus* Miyatake**
- 2 Head and pronotum testaceous; abdominal postcoxal line complete; penis guide shorter than parameres in lateral view **3**
 – Head and pronotum black (Figs 3a–b); abdominal postcoxal line incomplete (Fig. 3d); penis guide stout, longer than parameres in lateral view (Fig. 3h); body length 2.50–3.20 mm ***H. hainanensis* Chen & Ren, sp. n.**
- 3 Pronotum entirely reddish brown; apex of penis expanded with membranous appendage **4**
 – Pronotum dark brown with black marking (Fig. 4a–b); apex of penis convergent apically without membranous appendage, penis guide widest at base in ventral view (Sasaji, 1971); body length 2.83 mm ***H. fortunatus* (Lewis)**
- 4 Body outline narrower; first pair of elytral spots with hind margin deeply emarginated (Fig. 5a); penis stout and short (Fig. 5i); apex of penis guide strongly curved, forming a hook-shaped in lateral view (Fig. 5l); body length 2.19–2.72 mm ***H. hisamatsui* Miyatake**
 – Body outline broader; first pair of elytral spots with anterior margin deeply emarginated (Fig. 6a); sometimes the orange spots enlarged, each elytron with 2 or 3 black spots (Figs 6d–i); penis slender and long (Fig. 6k); apex of penis guide slightly curved, not forming a hook-shaped projection in lateral view (Fig. 6n); body length 2.76–3.34 mm ***H. sonduongensis* Hoàng**

Species descriptions and redescriptions***Horniolus novempunctatus* Miyatake, 1979**

Figs 2, 7

Horniolus novempunctatus Miyatake, 1979: 105; Pang et al. 2004: 89; Kovár 2007: 579.

Diagnosis. This distinctive species can easily be separated from the other species of *Horniolus* by its peculiar colour pattern on elytra.

Description. TL: 2.95 mm, TW: 2.00 mm, TH: 1.52 mm, TL/TW: 1.47, PL/PW: 0.51, EL/EW: 1.12, HW/PW: 0.63, PW/EW: 0.86.

Body elongate oval, moderately convex, dorsum covered with white pubescence (Fig. 2a–c). Head, antennae and mouthparts brown. Pronotum brown to dark brown. Scutellum black. Elytra yellow with nine black spots (Fig. 2a). Underside chestnut (Fig. 2d).

Head with fine frontal punctures, as large as eye facets, 0.5–1.0 diameter apart. Eyes finely faceted, interocular distance 0.56 times head width. Pronotal punctures



Figure 2. *Horniolus novempunctatus* Miyatake: **a** dorsal view **b** frontal view **c** lateral view **d** ventral view **e** labels of holotype. Scale bars: 1 mm.

slightly larger than those on frons, 1.0–2.0 diameters apart. Surface of elytra with punctures larger than those on pronotum, separated by 2.0–3.0 diameters. Prosternal carinae Y-shaped with stem approximately $\frac{1}{3}$ as long as arm, arms broadly separated. Abdominal postcoxal lines strongly recurved and complete laterally (Fig. 2d), reaching $\frac{4}{5}$ length of abdominal ventrite 1, area enclosed by lines coarsely punctate, narrowly smooth along line. Abdominal ventrite 5 in female with apex rounded.

Male unknown.

Type material. Holotype: female, Juisui, Taiwan, [23°31.23'N, 121°24.67'E, ca 300 m], 4. IV. 1974, Takeda S. leg (ELEU, Fig. 2e).

Distribution. China (Taiwan).

***Horniolus hainanensis* Chen & Ren, sp. n.**

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Figs 3, 7

Diagnosis. This species is similar to *Horniolus amamensis* Miyatake and *Horniolus kyushuensis* Miyatake in general appearance, but can be separated from the latter by having incomplete abdominal postcoxal lines (Fig. 3d) and broader body outline (Fig. 3a). The stout penis guide (Fig. 3g–h) and the peculiar apex of penis (Fig. 3f) are also diagnostic.

Description. TL: 2.50–3.20 mm, TW: 1.80–2.41 mm, TH: 1.22–1.53 mm, TL/TW: 1.32–1.39, PL/PW: 0.51–0.53, EL/EW: 1.01–1.02, HW/PW: 0.60–0.62, PW/EW: 0.76–0.78.

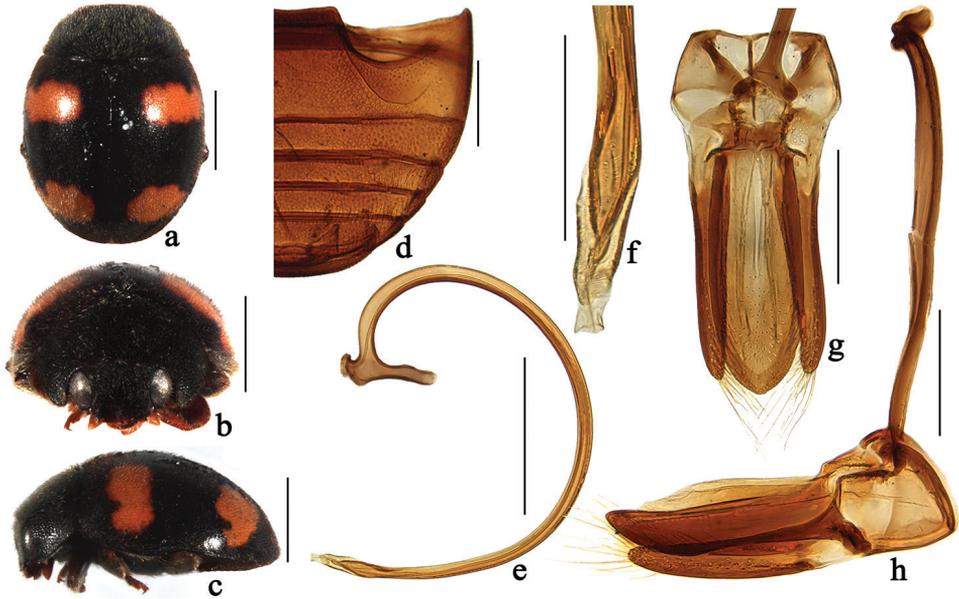


Figure 3. *Horniolus hainanensis* Chen & Ren, sp. n.: **a** dorsal view **b** frontal view **c** lateral view **d** abdomen **e** penis **f** apex of penis **g** tegmen, ventral view **h** tegmen, lateral view. Scale bars: 1 mm (**a–c**), 0.5 mm (**d–e**), 0.2 mm (**f–h**).

Body rounded, moderately convex, dorsum covered with white pubescence (Fig. 3a–c). Head black (Fig. 3b). Antennae and mouthparts dark brown. Pronotum and scutellum black. Elytra black with four yellowish spots, first pair transverse, almost straight, parallel to elytral base, situated at anterior $\frac{1}{3}$ length of elytra; second pair comma-shaped, located in apical $\frac{1}{3}$ length, not reaching suture and lateral margins (Figs 3a, 3c). Prothoracic hypomeron and prosternum black. Mesoventrite and metaventrite black. Elytral epipleuron dark brown with inner and outer margins black. Legs black except tarsi brown.

Head with coarse frontal punctures, distinctly larger than eye facets, 0.5 diameter apart. Eyes finely faceted, interocular distance 0.53 times head width. Pronotal punctures smaller than those on frons, 1.0–2.0 diameters apart. Surface of elytra with punctures larger than those on pronotum, separated by 1.0–2.0 diameters. Prosternal carinae Y-shaped with stem $\frac{1}{3}$ as long as arm, arms broadly separated. Abdominal postcoxal lines strongly recurved and distinctly incomplete laterally (Fig. 3d), reaching $\frac{4}{5}$ length of abdominal ventrite 1, area enclosed by lines coarsely punctate, narrowly smooth along line. Abdominal ventrite five weakly emarginated apically in male.

Male genitalia. Penis stout, evenly curved (Fig. 3e); penis capsule with long inner arm and short outer arm; apex of penis slightly expanded (Fig. 3f). Tegmen stout (Figs 3g–h) with penis guide parallel-sided from base to $\frac{3}{4}$ length, then tapering gradually to blunt apex in ventral view (Fig. 3g). Parameres tapering toward apex, shorter than penis guide, densely covered with long setae at apices (Fig. 3h).

Female externally similar to male but with abdominal ventrite 5 truncate apically.

Type material. Holotype: male, No. 20070320057, **CHINA: Hainan:** Tongza, 18°54.22'N, 109°40.49'E, ca 470 m, VIII. 1995, Peng ZQ leg (SCAU). **Paratypes (10): Hainan:** 1♀ with same data as holotype. 1♂, Wuzhishan National Nature Reserve, 18°47.07'N, 109°31.98'E, ca 650 m, 22. XI. 1991, Peng ZQ leg. 2♀, Wuzhishan National Nature Reserve, 18°47.07'N, 109°31.98'E, ca 650 m, 8. VI. 1994, Tian MY leg. 1♂, Wuzhishan, 18°47.0'N, 109°31.98'E, ca 650 m, VIII. 1995, Peng ZQ leg. 1♂1♀, Lianyuan, Nada, Danzhou City, 19°30.77'N, 109°29.77'E, VIII. 1995, Peng ZQ leg. 1♂, Maoyang Town, Qiongzong County, 18°56.18'N, 109°30.31'E, ca 230 m, IX. 1995, Peng ZQ leg. 1♂, Bawangling National Nature Reserve, 19°05.49'N, 109°06.38'E, ca 260 m, 3. IX. 1998, Peng ZQ leg. 1♂, Limushan National Forest Park, 19°14.05'N, 109°48.03'E, ca 160 m, 22.VII.2006, Dong XL leg (SCAU).

Distribution. China (Hainan).

Etymology. The specific epithet refers to its type locality, Hainan Island.

Horniolus fortunatus (Lewis, 1896)

Figs 4, 7

Scymnus fortunatus Lewis, 1896: 38; Ohta 1929: 10.

Scymnus (Scymnus) fortunatus: Mader 1955: 939.

Scymnus (Pullus) fortunatus: Kamiya 1961: 308.

Horniolus fortunatus: Miyatake 1963: 8; Kamiya 1966: 71; Sasaji 1971: 118; Wei et al. 1985: 67; Pang et al. 2004: 89; Kovář 2007: 579.

Diagnosis. This species closely resembles *Horniolus dispar* Weise in elytral pattern and male genitalia, but can be distinguished from it by having dark brown pronotum with black marking (Fig. 4a) and the penis guide distinctly shorter than paremeres (Sasaji 1971).

Description. TL: 2.83 mm, TW: 1.91 mm, TH: 1.24 mm, TL/TW: 1.48, PL/PW: 0.53, EL/EW: 1.08, HW/PW: 0.63, PW/EW: 0.75.

Body elongate oval, slightly convex, dorsum covered with white pubescence (Fig. 4a–c). Head, antennae and mouthparts dark brown. Pronotum dark brown with black marking at middle. Scutellum black. Elytra black with four reddish brown, transverse spots, first pair distinctly sinuated, located before middle in anterior half; second pair smaller, constricted medially, located apical ¼ length of elytra. Prothoracic hypomeron dark brown. Prosternum dark brown to black. Mesoventrite, metaventrite and elytral epipleura black. Legs dark brown.

Head with fine frontal punctures, slightly larger than eye facets, 1.0–2.0 diameters apart. Eyes finely faceted, interocular distance 0.54 times head width. Pronotal punctures as large as those on frons, 1.0–2.0 diameters apart. Surface of elytra with punctures larger than those on pronotum, separated by 2.0–3.0 diameters. Prosternal carinae Y-shaped with stem 1/7 as long as arm, arms narrowly separated. Abdominal postcoxal lines strongly recurved and complete laterally (Fig. 4d), reaching 5/6 length

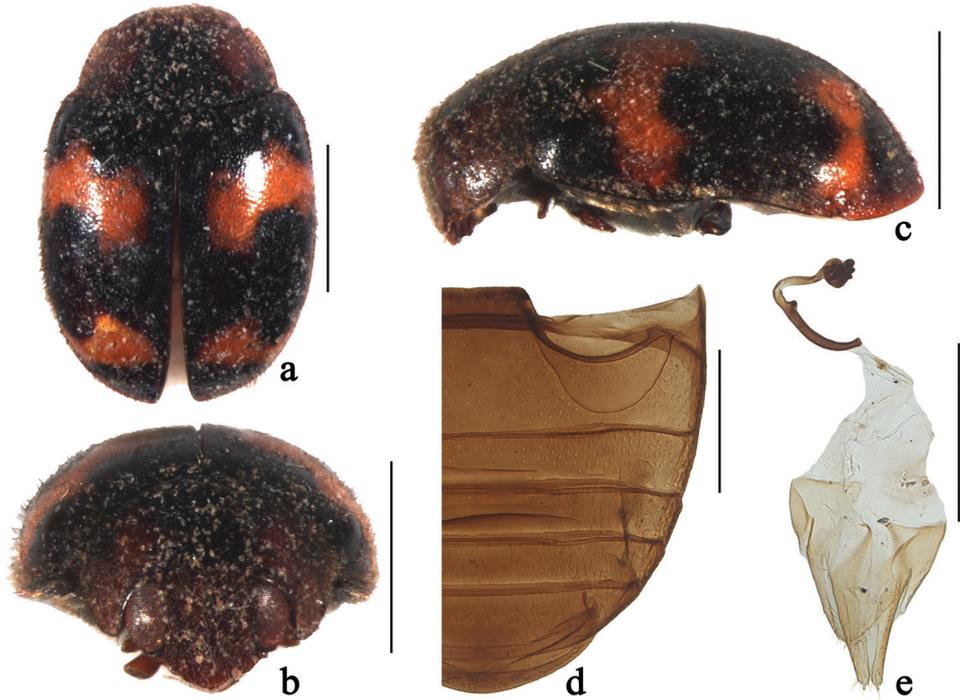


Figure 4. *Horniolus fortunatus* (Lewis): **a** dorsal view **b** frontal view **c** lateral view **d** abdomen **e** female genitalia. Scale bars: 1 mm (**a-c**), 0.5 mm (**d-e**).

of abdominal ventrite 1, area enclosed by lines coarsely and sparsely punctate, broadly smooth along line.

Male genitalia not studied in the present paper. According to the descriptions and illustrations given by Sasaji (1971: 118), the penis is stout and distinctly convergent apically; penis capsule with long inner arm and short outer arm; apex of penis simple without membranous appendage; tegmen stout with penis guide boat-shaped, widest at base, then tapering gradually to pointed apex in ventral view; parameres strongly curved at base, longer than penis guide, sparsely covered with long setae at apices.

Female externally similar to male except for sexual characters. Abdominal ventrite 5 with apex rounded. Coxites triangular, elongated, outer and inner margins almost straight, tapering to blunt apices, each with dense, long terminal setae (Fig. 4e). Spermatheca tubular, long and intricately coiled (Fig. 4e).

Material examined. CHINA: Shaanxi: 1♀, Ningqiang County, 32°49.98'N, 106°14.46'E, ca 850 m, 7. VI. 1982, Collector unknown (SCAU).

Distribution. China (Shaanxi); Japan.

Remarks. This species has variable pronotal colouration (Miyatake 1963). Lewis (1896) described this species in the genus *Scymnus* Kugelann based on only one specimen from Japan. In his original description, Lewis found a Ceylonese species which closely resembled this species. Ohta (1929) listed this species under *Scymnus* (s. str.)

without examining any specimens, and this treatment was followed by Mader (1955). Kamiya (1961) assigned this species to the subgenus *Scymnus* (*Pullus*) Mulsant due to the complete abdominal postcoxal line. He also indicated the peculiar character of prosternal carinae and described the male genitalia.

***Horniolus hisamatsui* Miyatake, 1976**

Figs 5, 7

Horniolus hisamatsui Miyatake, 1976: 29; Pang and Gordon 1986: 187; Yu and Lau 2001: 152; Canepari 2003: 262; Pang et al. 2004: 89; Kovář 2007: 579; Ren et al. 2009: 58.

Diagnosis. This species is similar to *Horniolus vietnamicus* Miyatake in general appearance and male genitalia, but can be distinguished from it by the first pair of elytral spots with hind margin deeply emarginated at middle (Fig. 5a, d) and penis guide shorter than parameres (Fig. 5l). In *H. vietnamicus*, the first pair of elytral spots has straight hind margins and penis guide is as long as parameres.

Description. TL: 2.19–2.72 mm, TW: 1.51–1.94 mm, TH: 1.02–1.22 mm, TL/TW: 1.40–1.45, PL/PW: 0.52–0.54, EL/EW: 1.04–1.09, HW/PW: 0.64–0.66, PW/EW: 0.74–0.76.

Body elongate oval, slightly convex, dorsum covered with white pubescence (Fig. 5a–f). Head, antennae and mouthparts reddish brown. Pronotum reddish brown. Scutellum dark brown. Elytra black with 4 yellowish brown, transverse spots, first pair of spots with anterior margins sinuated, hind margins deeply emarginated at middle, located behind middle in anterior half; second pair smaller, comma-shaped, located at apical $\frac{1}{4}$ length of elytra. Prothoracic hypomeron and prosternum reddish brown. Mesoventrite, metaventrite and elytral epipleura reddish brown. Legs brown.

Head with fine frontal punctures, as large as eye facets, 1.0–2.0 diameter apart. Eyes finely faceted, interocular distance 0.48 times head width. Pronotal punctures larger than those on frons, 1.0–2.0 diameters apart. Surface of elytra with punctures slightly larger than those on pronotum, separated by 2.0–3.0 diameters. Prosternal carinae Y-shaped with stem approximately $\frac{1}{3}$ as long as arm, arms broadly separated. Abdominal postcoxal lines strongly recurved and complete laterally (Fig. 5h), reaching $\frac{3}{4}$ length of abdominal ventrite 1, area enclosed by lines coarsely punctate, broadly smooth along line. Abdominal ventrite 5 in male with apex truncate.

Male genitalia. Penis stout, evenly curved (Fig. 5i); penis capsule with long inner arm and indistinct outer arm; apex of penis slightly expanded with membranous appendage (Fig. 5j). Tegmen stout (Fig. 5k–l) with penis guide parallel-sided at basal half, widest at middle in ventral view (Fig. 5k); in lateral view, penis guide robust at basal $\frac{2}{3}$ length, then abruptly narrowed in apical $\frac{1}{4}$ th and produced into a sickle-shaped, acutely pointed apex (Fig. 5l). Parameres narrowed toward apex, longer than penis guide, densely covered with long setae at apices and inner side (Fig. 5l).

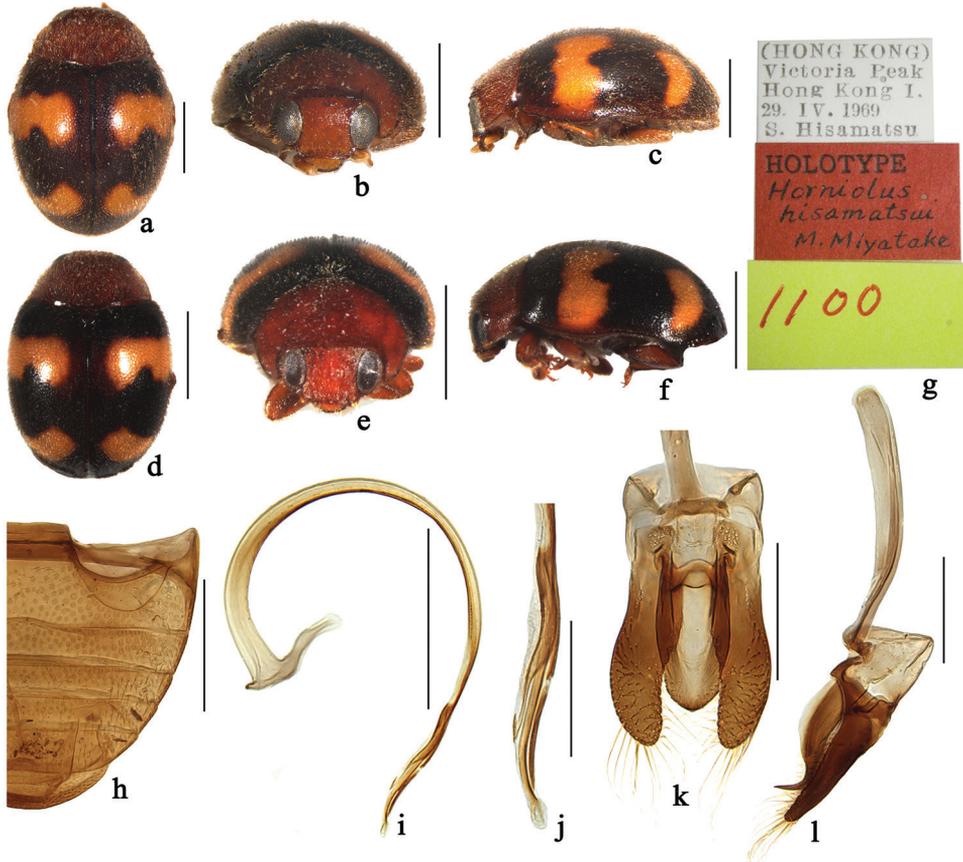


Figure 5. *Horniolus hisamatsui* Miyatake: **a–c** female, holotype **d–f** male **a, d** dorsal view **b, e** frontal view **c, f** lateral view **g** labels of holotype **h** abdomen **i** penis **j** apex of penis **k** tegmen, ventral view **l** tegmen, lateral view. Scale bars: 1 mm (**a–f**), 0.5 mm (**h–i**), 0.2 mm (**j–l**).

Female externally similar to male but with abdominal ventrite 5 rounded apically.

Type material. Holotype: female, Victoria Peak, Hong Kong, [22°16.55'N, 114°8.73'E], 29. IV. 1969, S. Hisamatsu leg (ELEU, Fig. 5g).

Other material examined. CHINA: Guangdong: 1♂, Shimentai National Nature Reserve, Yangqiu Mountains, 24°16.17'N, 113°17.54'E, ca 500 m, 6.X.2004, Wang XM leg. 2♂, South Subtropical Crops Research Institute, Zhanjiang City, 21°09.78'N, 110°16.45'E, ca 10 m, II. 2012, He YB leg. **Hainan:** 1♂, Yajia, Bawangling National Nature Reserve, 19°04.41'N, 109°09.08'E, ca 970 m, VIII.1995, Peng ZQ leg. 1♂, Tongza, 18°54.22'N, 109°40.49'E, ca 470 m, VIII. 1995, Peng ZQ leg. 2♂, Bawangling National Nature Reserve, 19°05.49'N, 109°06.38'E, ca 260m, 5. V. 2005, Wang XM leg. 1♂1♀, Baoting County, 18°37.47'N, 109°41.53'E, ca 70 m, 21.VII.1996, Peng ZQ leg. 1♀, Limushan National Forest Park, 19°14.05'N, 109°48.03'E, ca 160 m, 22.VII.2006, Dong XL leg. 1♀, Wuzhishan National Nature

Reserve, 18°47.07'N, 109°31.98'E, ca 650m, 22. XI. 1991, Peng ZQ leg. 1♀, Wuzhishan National Nature Reserve, 18°47.07'N, 109°31.97'E, ca 700m, 3. V. 1996, Peng ZQ leg. 1♀, Yinggen Town, Qiongzong County, 19°02.10'N, 109°49.84'E, ca 200 m, 15. VII. 1999, Peng ZQ leg. **Guangxi:** 1♂, Pinglongshan, Fulong, Shangsi, 21°49.88'N, 107°56.79'E, ca 160m, 29. VII. 2005, Wang XM leg. **Tibet:** 1♀, Motuo County, Linzhi City, 29°19.30'N, 95°18.33'E, ca 760 m, 17. X. 2009, Chen XS leg. 2♂, Beibeng Village, Motuo County, 29°14.31'N, 95°10.58'E, ca 800 m, 9. X. 2011, Huo LZ et al. leg (SCAU).

Distribution. China (Guangdong, Hong Kong, Hainan, Guangxi, Tibet); Nepal.

Horniolus sonduongensis Hoàng, 1979

Figs 6, 7

Horniolus sonduongensis Hoàng, 1979: 12; 1982: 122; Kuznetsov and Ren 1991: 9.

Diagnosis. This species is similar to *Horniolus vietnamicus* Miyatake and *Horniolus bimaculatus* Miyatake in general appearance and can be separated from these species only by the male genitalia.

Description. TL: 2.76–3.34 mm, TW: 2.03–2.45 mm, TH: 1.40–1.63 mm, TL/TW: 1.35–1.36, PL/PW: 0.54–0.58, EL/EW: 1.02–1.04, HW/PW: 0.60–0.63, PW/EW: 0.72–0.75.

Body rounded oval, moderately convex, dorsum covered with white pubescence (Fig. 6a–i). Head, antennae and mouthparts reddish brown. Pronotum reddish brown. Scutellum dark brown to black. Elytra castaneous to black with 4 orange spots, sinuated (Fig. 6a, c), first pair of spots with anterior margins deeply emarginated at middle, located behind middle in anterior half; second pair smaller, hind margin deeply emarginated medially, located in apical $\frac{1}{3}$ length of elytra. Underside entirely castaneous.

Head with coarse frontal punctures, slightly larger than eye facets, 0.2–0.5 diameter apart. Eyes densely faceted, interocular distance 0.54 times head width. Pronotal punctures smaller than those on frons, 2.0–3.0 diameters apart. Surface of elytra with punctures much larger than those on pronotum, separated by 1.0–2.0 diameters. Prosternal carinae Y-shaped with stem approximately $\frac{1}{3}$ as long as arm, the arms broadly separated. Abdominal postcoxal lines strongly recurved and complete laterally (Fig. 6j), reaching $\frac{3}{4}$ length of abdominal ventrite 1, area enclosed by lines coarsely punctate, narrowly smooth along line. Abdominal ventrite 5 in male with apex weakly emarginated medially.

Male genitalia. Penis slender and long (Fig. 6k); penis capsule with long inner arm and short outer arm; apex of penis swollen and curved outwardly (Fig. 6l). Tegmen stout (Fig. 6m–n) with parallel-sided at basal half, then tapering gradually to a blunt apex in ventral view (Fig. 6m). Parameres narrow and curved apically, slightly shorter than penis guide, densely covered with long setae at apices (Fig. 6n).

Female externally similar to male but with abdominal ventrite 5 truncate apically.



Figure 6. *Horniolus sonduongensis* Hoàng: **a, d, g** dorsal view **b, e, h** frontal view **c, f, i** lateral view **j** abdomen **k** penis **l** apex of penis **m** tegmen, ventral view **n** tegmen, lateral view. Scale bars: 1 mm (**a–i**), 0.5 mm (**j–k**), 0.2 mm (**l–n**).

Material examined. CHINA: Fujian: 1♂, Xiangxi Village, Huboliao National Nature Reserve, Nanjing County, 24°31.07'N, 117°17.08'E, ca 240 m, 18. VIII. 2012, Li WJ leg. Guangdong: 1♂5♀, Nankunshan National Nature Reserve, Long-

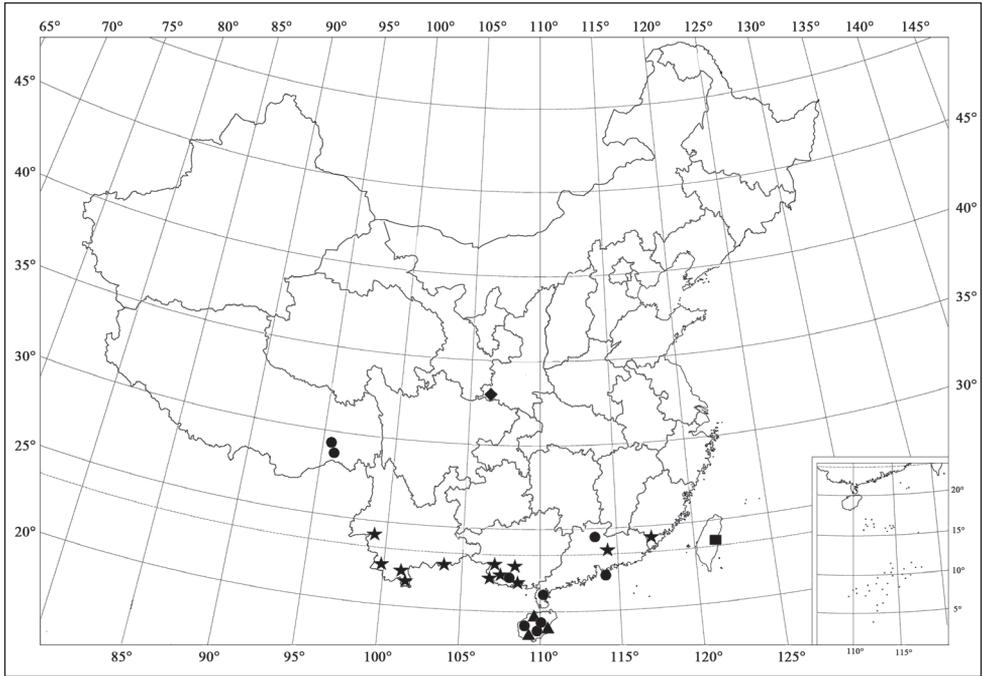


Figure 7. Distribution map. *H. novempunctatus* Miyatake (■); *H. hainanensis* Chen & Ren, sp. n. (▲); *H. fortunatus* (Lewis) (◆); *H. hisamatsui* Miyatake (●); *H. sonduongensis* Hoang (★).

men County, Huizhou City, 23°38.90'N, 113°51.58'E, 460 m, 15. VI. 2014, Ren SX leg. **Guangxi:** 1♂, Pinglongshan, Fulong, Shangsi, 21°49.88'N, 107°56.79'E, ca 160m, 29. VII. 2005, Wang XM leg. 2♂1♀, Naqin Town, Fangchenggang City, 21°49.52'N, 108°02.11'E, ca 100 m, 30.VII.2005, Zhang CW and Wang XM leg. 2♀, Nonggang National Nature Reserve, Longzhou County, 22°28.22'N, 106°57.31'E, ca 230 m, 3. VIII. 2005, Qin ZQ and Zhang CW leg. 2♀, Daqing Mountains, Pingxiang, 24°54.76'N, 113°2.83'E, 2. VIII. 2005, Zhang CW and Wang XM leg. 2♀, Longguang, 29-30. VII. 1985, Pang XF leg. **Yunnan:** 1♂, Xiaowei Mountains, Hekou, 22°53.86'N, 103°34.04'E, ca 900m, 23. IV. 2008, Hao JY leg. 1♂, Mengdui, Zhenkang, 23°53.47'N, 98°53.33'E, 1400m, 18. V. 2008, Liang JB leg. 2♀, Jinuo Mountains, Jinghong, Xishuangbanna, 22°02.21'N, 101°00.35'E, ca 900 m, 6. V. 2009, Chen XS leg. 1♀, Jinuo Mountains, Xishuangbanna, 22°02.21'N, 101°00.35'E, ca 900 m, 28. IV. 2008, Liang JB leg. 1♂2♀, Gongxin, Menglian, 22°18.27'N, 99°19.31'E, 1500 m, 8. V. 2008, Hao JY leg. 1♂, No. 213 Highway, Mengla, 21°33.77'N, 101°34.85'E, ca 700 m, 12-13. X. 2006, Wang XM leg. 1♂, Nature Reserve, Menglun, Mengla, 21°55.27'N, 101°16.64'E, ca 550m, 12-13, X. 2006, Wang XM leg. 1♂, Mengla, Xishuangbanna, 21°26.59'N, 101°38.01'E, ca 1160m, 23. VIII. 2005, Wang XM leg. **VIETNAM:** 1♂, Vietnam, Prov. Gialai-Contum, Buonloi, 14°06.73'N, 107°58.30'E, ca 700 m, 28. V. 1985, Zaitzev U leg (SCAU).

Distribution. China (Fujian, Guangdong, Guangxi, Yunnan) new distribution; Vietnam.

Remarks. This species has variable colour pattern on elytra. In the original description of this species, Hoàng (1979) mentioned it was similar to *H. okinawensis* Miyatake and *H. vietnamicus* Miyatake in colour pattern. In the present study, we found some specimens with orange spots enlarged, but anterior, sutural and lateral margins always castaneous, and each elytron with three black, rounded spots, first one situated on the humerus, second one situated on the middle of elytra, third one situated before apex (Fig. 6d–f); the apical spot disappeared occasionally, only with anterior and middle spots present (Fig. 6g–i).

Checklist of the species of *Horniolus* Weise, 1901

Horniolus amamensis Miyatake, 1963

Horniolus amamensis Miyatake, 1963: 10; Kamiya 1966: 71; Sasaji 1971: 120; Kovář 2007: 579.

Distribution. Japan.

Horniolus bimaculatus Miyatake, 1976

Horniolus bimaculatus Miyatake, 1976: 35.

Distribution. Malaysia.

Horniolus dispar Weise, 1901

Horniolus dispar Weise, 1901: 443; Korschefsky 1931: 110; Bielawski 1961: 397; Iablokoff-Khnzorian 1976: 376; Döbler 1987: 37; Poorani 2002: 349.

Scymnus (Pullus) sp.: Bielawski 1957: 83.

Distribution. Sri Lanka.

Horniolus fortunatus (Lewis, 1896)

Scymnus fortunatus Lewis, 1896: 38; Ohta 1929: 10.

Scymnus (Scymnus) fortunatus: Mader 1955: 939.

Scymnus (Pullus) fortunatus: Kamiya 1961: 308.

Horniolus fortunatus: Miyatake 1963: 8; Kamiya 1966: 71; Sasaji 1971: 118; Wei et al. 1985: 67; Pang et al. 2004: 89; Kovář 2007: 579.

Distribution. China (Shaanxi); Japan.

Horniolus guimeti (Mulsant, 1850)

Scymnus guimeti Mulsant, 1850: 979; Weise 1879: 145; Korschefsky 1931: 143; Booth and Pope 1989: 354.

Horniolus guimeti: Booth and Pope 1989: 355; Poorani 2002: 349.

Distribution. Malaysia, Bangladesh.

Notes. Poorani (2002, 2015) mentioned that *H. guimeti* was a doubtful record for India as suggested by Roger Booth, coccinellid expert at the Natural History Museum, London (BMNH). We agreed with Poorani's opinion and excluded India in its distribution range.

***Horniolus hainanensis* Chen & Ren, sp. n.**

Distribution. China (Hainan).

***Horniolus hisamatsui* Miyatake, 1976**

Horniolus hisamatsui Miyatake, 1976: 29; Pang and Gordon 1986: 187; Yu and Lau 2001: 152; Canepari 2003: 262; Pang et al. 2004: 89; Kovář 2007: 579; Ren et al. 2009: 58.

Distribution. China (Guangdong, Hong Kong, Hainan, Guangxi, Tibet); Nepal.

***Horniolus kyushuensis* Miyatake, 1963**

Horniolus kyushuensis Miyatake, 1963: 9; Kamiya 1966: 71; Sasaji 1971: 119; Kovář 2007: 579.

Distribution. Japan.

***Horniolus nigripes* Miyatake, 1976**

Horniolus nigripes Miyatake, 1976: 33; Poorani 2002: 350.

Distribution. India.

***Horniolus novempunctatus* Miyatake, 1979**

Horniolus novempunctatus Miyatake, 1979: 105; Pang et al. 2004: 89; Kovář 2007: 579.

Distribution. China (Taiwan).

***Horniolus okinawensis* Chûjô & Miyatake, 1963**

Horniolus okinawensis Chûjô & Miyatake in Miyatake, 1963: 11; Kamiya 1966: 71, 85; Sasaji 1971: 121; Chûjô and Chûjô 1998: 19; Kovář 2007: 579.

Distribution. Japan.

***Horniolus siamensis* Miyatake, 1976**

Horniolus siamensis Miyatake, 1976: 31; Chunram and Sasaji 1980: 476.

Distribution. Thailand.

***Horniolus sonduongensis* Hoàng, 1979**

Horniolus sonduongensis Hoàng, 1979: 12; 1982: 122; Kuznetsov and Ren 1991: 9.

Distribution. China (Fujian, Guangdong, Guangxi, Yunnan); Vietnam.

***Horniolus sororius* Poorani, 2015**

Horniolus sororius Poorani, 2015: 7.

Distribution. India.

***Horniolus vietnamicus* Miyatake, 1976**

Horniolus vietnamicus Miyatake, 1976: 36; Hoàng 1982: 121.

Distribution. Vietnam.

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Novadessus viracocha, a new genus and species of Bidessini Sharp from Peru (Coleoptera, Adepaga, Dytiscidae, Hydroporinae)

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Abstract

Novadessus viracocha **gen. n.** and **sp. n.** is described from Peru. The genus distinctly is characterized by having the following combination: (1) a transverse occipital line absent on the head; (2) the anterior clypeal margin not modified; (3) a pair of basal pronotal striae present; (4) the basal elytral stria absent; (5) the elytral sutural stria absent; (6) the elytron without longitudinal carinae; (7) the epipleuron without a transverse carina at the humeral angle; (8) the lateral lobes of the male aedeagus two-segmented; (9) the overall habitus elongate and oval, with lateral pronotal and elytral margins discontinuous; (10) without distinct denticles along the posterior margins of the abdominal sternites; (11) the male genitalia (both median lobe and lateral lobes) bilaterally symmetrical; and (12) the metatrochanter small relative to the metafemur, approximately 0.6 × the length of the metafemur. The genus is diagnostically similar to *Fontidessus* Miller and Spangler and *Neobidessodes* Hendrich and Balke, but is superficially more similar to *Liodes* Guignot. The habitus and male genitalia are illustrated, and a distribution map is provided.

Resumen

Novadessus viracocha **gen. n.** y **sp. n.** se describe de Perú. El género se caracteriza especialmente por tener la siguiente combinación: (1) línea occipital transversal ausente en la cabeza; (2) margen anterior clipeal no modificado; (3) presencia de un par de estrías basales pronotales; (4) estría elitral basal ausente; (5) estría elitral sutural ausente; (6) élitro sin carenas longitudinales; (7) epipleuron sin carena transversal en el ángulo humeral; (8) lóbulos laterales del aedeagus del macho bisegmentados; (9) hábito alargado y oval, con márgenes laterales de pronoto y élitros discontinuos; (10) sin denticulos a lo largo de los márgenes posteriores de los esternitos abdominales; (11) órganos genitales del macho (lóbulo medio y lóbulos laterales) bilateralmente simétricos; y (12) metatrocánter pequeño en relación con el metafémur, aproximadamente

0.6 × de la longitud del metafémur. El género es diagnósticamente similar a *Fontidessus* Miller y Spangler y *Neobidessodes* Hendrich y Balke, pero es superficialmente similar a *Liodessus* Guignot. Se ilustran el hábito y los órganos genitales del macho, y se proporciona un mapa de distribución.

Keywords

Water beetles, taxonomy, classification, Neotropical, *Novadessus*, new genus, Dytiscidae, Coleoptera

Introduction

The diving beetle tribe Bidessini Sharp has provided large numbers of new species and genera over the past few years, especially in the Neotropical region (Miller 2016; Miller and Garcia 2011; Miller and Montano 2014; Miller and Short 2015; Miller and Spangler 2008). The few characters that typically characterize bidessine genera come in numerous combinations making diagnoses complex and relationships difficult to establish. Bidessini is currently the largest clade of diving beetles, and the group promises to continue to grow as new diversity is discovered.

A new species was discovered among legacy specimens from the US National Collection that could not be assigned to an existing genus. Therefore, the goal of this project is to describe a new genus and new species of Bidessini from Peru.

Materials and methods

Measurements. Measurements were taken using a Zeiss Discovery V8 dissecting microscope with an ocular scale. All known specimens were measured. Measurements include: 1) total length (TL), 2) greatest width across elytra (GW), 3) greatest width of pronotum (PW), 4) greatest width of head (HW), 5) distance between eyes (EW), 6) greatest length of metafemur (FL), and 7) greatest width of metafemur (FW). The ratios TL/GW, HW/EW and FW/FL were also calculated.

Images. Illustrations were made using a drawing tube on a Zeiss Discovery V8 dissecting scope. Sketches were first done in pencil then scanned, placed into an Adobe Illustrator artboard and “inked” digitally using vector lines.

Results

Novadessus gen. n.

<http://zoobank.org/15DD642E-6A90-4721-9C59-065C4A71F7EC>

Type species. *Novadessus viracocha* sp. n., by current designation.

Diagnosis. *Novadessus* is characterized by the following combination: (1) a transverse occipital line absent on the head; (2) the anterior clypeal margin not modified;

(3) a pair of basal pronotal striae present; (4) the basal elytral stria absent; (5) the elytral sutural stria absent; (6) the elytron without longitudinal carinae; (7) the epipleuron without a transverse carina at the humeral angle; (8) the lateral lobes of the male aedeagus two-segmented; (9) the overall habitus elongate and oval, with lateral pronotal and elytral margins discontinuous; (10) without distinct denticles along the posterior margins of the abdominal sternites; (11) the male genitalia (both median lobe and lateral lobes) bilaterally symmetrical; and (12) the metatrochanter small relative to the metafemur, approximately $0.6 \times$ the length of the metafemur.

The genus is relatively similar in appearance and overall shape of the male genitalia to *Liodesus* Guignot, a rather generalized group of Bidessini species. However, *Novadessus* is missing both the transverse occipital line across the back of the head and the basal elytral striae, each of which is characteristic of *Liodesus*. In Miller and Bergsten (2016) the genus keys out to couplet 13 which separates *Fontidessus* Miller and Spangler and *Neobidessodes* Hendrich and Balke, neither of which is a convincing fit. *Fontidessus*, though Neotropical like *Novadessus*, have a characteristic ventral sclerite on the male median lobe and very large metatrochanters, neither of which are present in *Novadessus*. *Neobidessodes* have a rather different body shape (elongate oval with a continuous lateral body line), are Australian, and do not appear to be similar in any other particular way to *Novadessus*. *Spanglerodesus* Miller and García and *Amarodytes* Régimbart (both Neotropical) each also have the combination of absence of an occipital line, presence of pronotal striae and absence of elytral striae, but the first is very broad and broadly rounded laterally with a broad pronotal bead and the second has the pronotal striae generally short, curved and located somewhat laterally. In addition, each group is superficially quite distinct from *Novadessus* in many other less discrete characters such as body shape, overall coloration, surface sculpture, etc. Even so, the relationships of *Novadessus* to these other taxa, and to other Bidessini genera in general, is unknown and needs investigation.

Etymology. This genus is named *Novadessus* from the Latin word, *novus*, meaning “new” and *dessus*, a common root for genera in the Bidessini.

Modified key to Bidessini genera from Miller and Bergsten (2016)

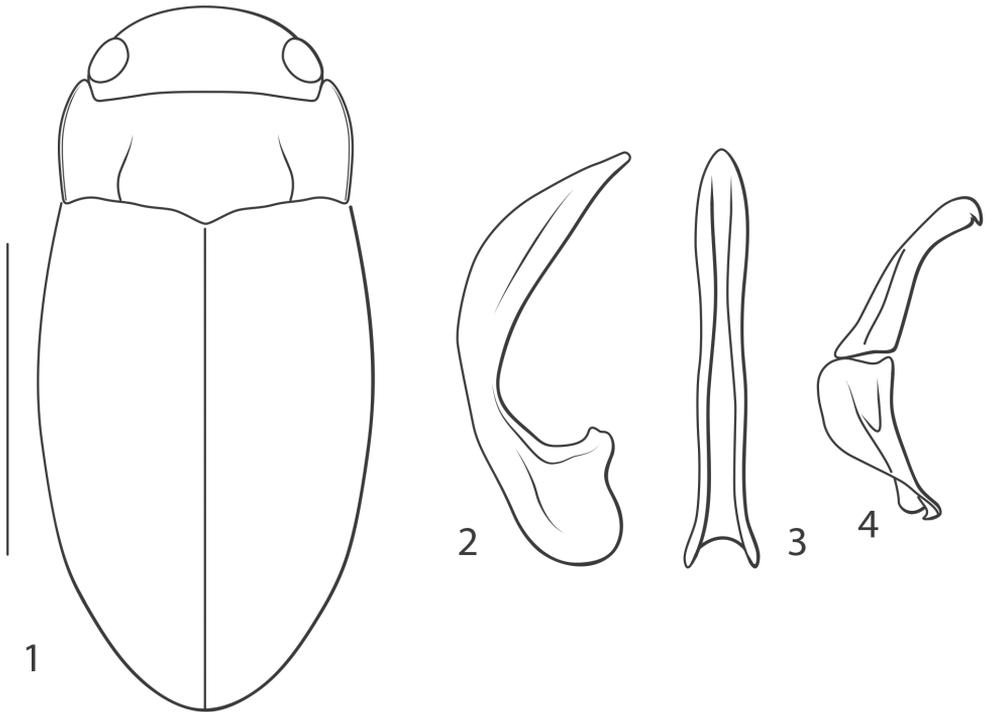
- 13a(12) Body outline approximately continuously curved between pronotum and elytron (Miller and Bergsten fig. 37.13b) **13**
 13a' Body outline discontinuous between pronotum and elytron (Fig. 1); Neotropical (Fig. 5)..... ***Novadessus* gen. n.**

Novadessus viracocha Miller, sp. n.

<http://zoobank.org/7602EF1A-6DA2-4D90-9B2F-27ABBE96D550>

Figs 1–5

Type locality. Peru, Department Huanuco, Shishmay.



Figures 1–4. *Novadessus viracocha* sp. n. **1** Dorsal habitus, scale bar = 1.0mm. **2–4** Male genitalia **2** Median lobe, right lateral aspect **3** Median lobe, ventral aspect **4** Right lateral lobe, right lateral aspect.

Diagnosis. Monotypic. The male median lobe in lateral aspect is expanded medially and evenly curved to a narrowed, apically narrowly rounded apex (Fig. 2). The coloration is overall brownish.

Description. *Measurements.* TL = 2.0–2.2mm, GW = 0.9–1.0mm, PW = 0.8–0.9mm, HW = 0.6–0.7mm, EW = 0.4–0.5mm, TL/GW = 2.1–2.2, HW/EW = 1.4–1.5. Body elongate oval, lateral outline somewhat discontinuous between pronotum and elytron (Fig. 1).

Coloration. Head, including appendages, evenly brown. Pronotum brown, somewhat darker along anterior and posterior margins. Elytron brown, lighter brown laterally. Ventral surfaces brown, legs, head and epipleuron lighter brown than thoracic and abdominal sternites.

Sculpture and structure. Head surface finely but distinctly microreticulate and micropunctate; without occipital line or modifications to evenly rounded clypeal margin. Pronotum surface smooth and shiny with few micropunctures scattered across surface; lateral margins broadly rounded with bead narrow; broadest anteriorly, narrowed posteriorly; lateral striae distinctive, extending anteriorly more than half distance across pronotum. Elytron smooth and shiny with fine, distinctive punctures distributed evenly; lateral margins subparallel anteriorly, narrowed to posteriorly pointed apex; basal and sutural striae absent. Prosternum moderately broad; prosternal process nar-

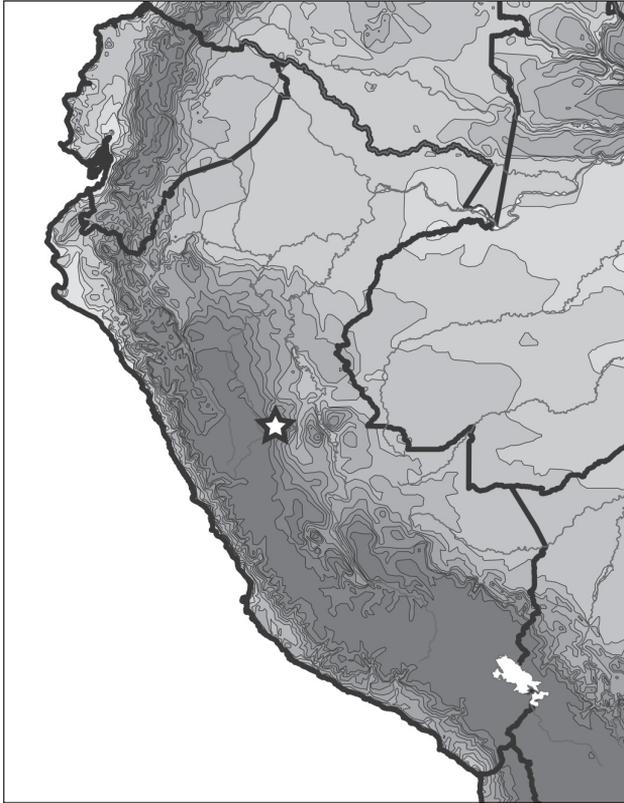


Figure 5. *Novadessus viracocha* sp. n. distribution.

row, apically sharply pointed, medially rounded. Mesoventrite and metacoxal surfaces smooth and shiny with few, scattered fine punctures; metacoxal lines anteriorly somewhat divergent. Abdominal surfaces smooth and shiny with few scattered punctures. Metatrochanter small, ventrally rounded, not strongly offset from line of metafemur; metafemur slender, unmodified.

Male genitalia. Median lobe in lateral aspect evenly curved, medially distinctly and broadly expanded, apically narrowed to elongate, narrowly rounded apex (Fig. 2); in ventral aspect slender with lateral margins somewhat sinuate to narrowly rounded apex (Fig. 3). Lateral lobe with apical segment elongate slender with small ventral hook apically; ventral segment shorter, moderately broad (Fig. 4).

Variation. Specimens vary somewhat in intensity of coloration, but are otherwise similar.

Etymology. This species is named *viracocha* after the Inca creator god.

Distribution. The species is known from two series. The type series bears label data indicating it is from “Vic. of Shishmay.” Shishmay is a small town in the Andes of Peru (Fig. 5). The other series has label data indicating “Vic. San Domingo,” in Huanuco, Peru. This second locality could not be located.

Habitat. The type series was collected from “highland lakes.” Nothing else is known of the habitat of *Novadessus viracocha*.

Type material. Holotype in United States National Entomological Collection, Smithsonian Institution (USNM), male labeled, “Peru, S. A. Sept. 15-20, 1937 F. Woytkowski. No. 3787/ Dept. of Huanuco Vic. of Shishmay Andes 3600-4100 m Highland Lakes/ HOLOTYPE *Novadessus viracocha* Miller, 2016 [red label with black line border].” Paratypes (in USNM and The Museum of Southwestern Biology, University of New Mexico), 4 labeled same as holotype. Nine labeled, “Peru, S. A. Nov. 11-23, 1937 F. Woytkowski No. 3812/ Dept. Huanuco Vic. San Domingo Andes 3000 M. el./ PARATYPE *Novadessus viracocha* Miller, 2016 [blue label with black line border].”

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Mitochondrial genotyping of an endangered bitterling *Acheilognathus typus* (Cyprinidae)

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Abstract

Genotyping of endangered species is helpful for establishing and evaluating conservation strategies. Mitochondrial sequence data was analyzed from 541 individuals of a critically endangered fish, *Acheilognathus typus* from present-day range-wide localities to re-evaluate an in-progress restoration program around Lake Izunuma-Uchinuma, Miyagi, Japan. *Acheilognathus typus* showed low sequence diversity with only eight haplotypes and π and h values of 0.59129 and 0.00118 respectively. Genetic data suggests *A. typus* is adapted to pulsed environments and prone to population flush and crash. Genotyping of populations in introduced localities revealed that their source is not from nearby localities.

Keywords

Bottleneck, Cyprinidae, conservation genetics, fish, heteroplasmy, invasive alien species, restoration

Introduction

Genotyping of endangered species offers opportunities for establishing conservation strategies, particularly for evaluating conservation unit (Crandall et al. 2000). This is especially important for freshwater fish geographically isolated by marine and land barriers. Range fragmentation by these barriers brings about geographically structured composition of populations that are potential targets of conservation programs.

Acheilognathus typus Bleeker, 1863 is a medium-sized bitterling endemic to eastern Honshu Island, Japan. It was a common freshwater fish in shallow lakes, ponds, low-land rivers, and streams several decades ago, but desperately declined after the World War II (Nakamura 1963). Recent range contraction occurred because of frequent civil engineering works, and habitats are fragmented and unstable at present (Kitajima et al. 2004) (Fig. 1). The Ministry of Environment, Japan (2015) thus acknowledged this bitterling as a critically endangered species. In Lake Izunuma-Uchinuma, Miyagi Prefecture, Japan, this bitterling was exceptionally abundant. More than 500-individual/net/day fisheries catch was recorded in autumn 1996, but completely disappeared by 2000 (Takahashi et al. 2001) with the invasion of the largemouth bass, *Micropterus salmoides* (Lacepède, 1802). A small population near the lake was found in 2001, and restoration project of the bitterling to the lake started in 2003 including transplantation activities (Kitajima et al. 2004).

In this report mitochondrial sequence data was analyzed from 541 individuals of *A. typus* from the present range-wide localities for re-evaluation of the restoration program. The dataset of this research is also helpful for conservation programs of this critically endangered fish.

Materials and methods

The samples included fin-clips of 541 individuals from 18 localities collected from 2001 through 2011 (Table 1). Specimens were collected using methods including casting netting, plastic traps, or draining off pond water. Four out of 18 localities are introduced stocks (#6-5, 6-6, 7, 8). A volunteer people collaborating with the restoration project of *Acheilognathus typus* indicated that introduction took place from #6-1 into #8. Next, individuals were taken from #8 and released to #6-6, and then from #6-6 to #6-5. Introduction into #7 took place from Katsurazawa Pond in Kashimadai before the source population declined.

DNA was extracted using QuickGene DNA Tissue kit on QuickGene-810 (Kurabo, Neyagawa, Japan). PCR primers were L16019 on the L-strand (GCTACCAAA-GCCAGAATTCTAA) (Saitoh et al. 2004) and CrpH301D on the H-strand (GTT-WARGTCCCTGATTCTATCAT) which worked for amplifying a 505 bp fragment of mitochondrial DNA encompassing 16 bp from the beginning of tRNA(Pro) gene and the left domain of control region. PCR reaction mixture of 12.5 µL contained 1 µL

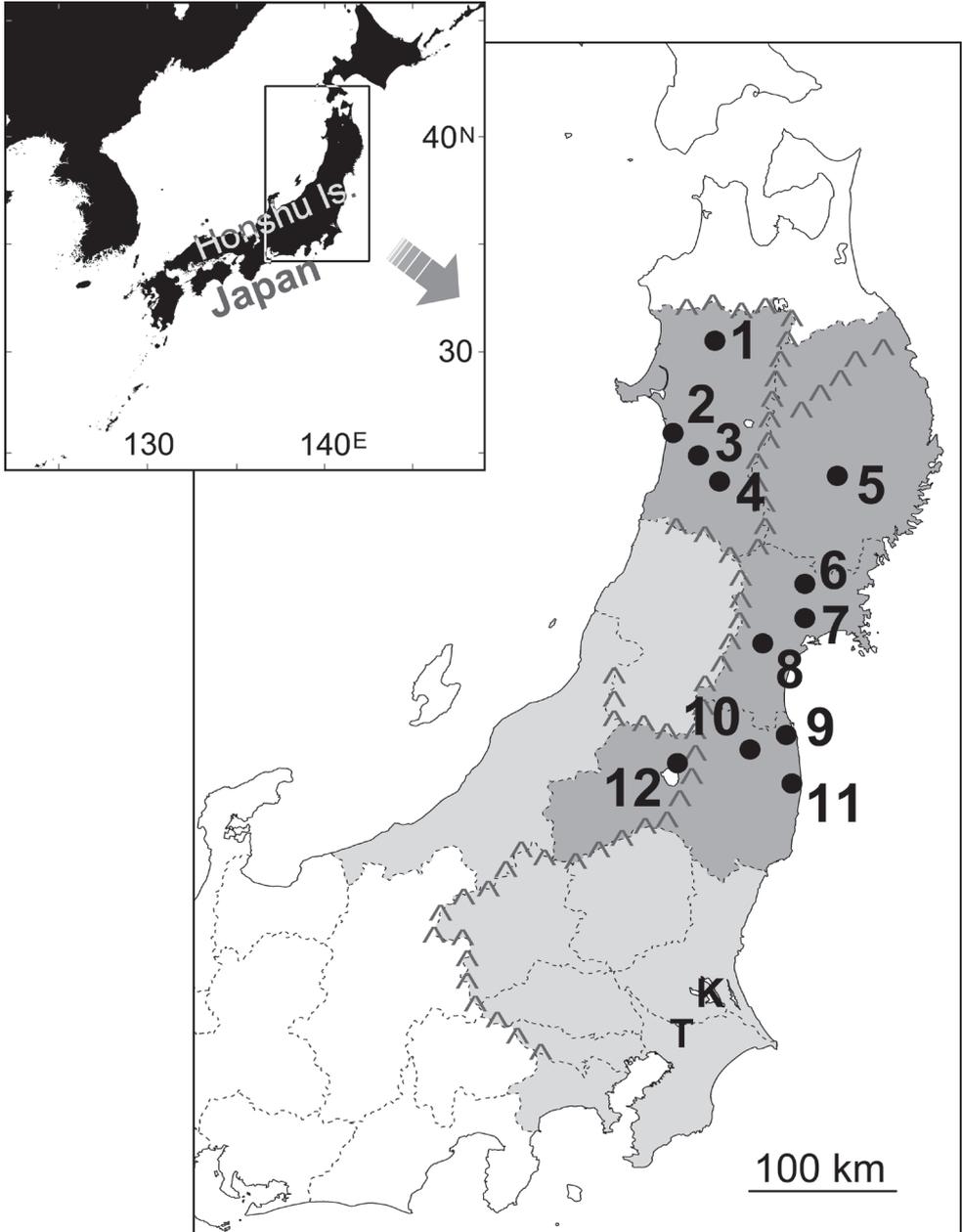


Figure 1. Collecting localities (1–12) and range contraction of *Acheilognathus typus*. Shaded areas are prefectures with past (light) and recent (dark) records. Major mountain ranges are indicated by Δ . Locality **4** contains two ponds, and **6** contains a creek and five ponds around Lake Izunuma-Uchinuma, close to each other respectively. K and T indicate locations of Lake Kasumigaura and Teganuma Lake where *A. typus* was abundant in the past.

Table 1. Localities and specimens used in this study.

#	Locality	Number of individuals
1	Small pond near Kitaakita	30
2	Shiohiki Lagoon	52
3	Small pond near Nishisenboku	37
4-1	Shinzutsumi Pond in Yasumoto near Yokote	49
4-2	Pond near Yokote	20
5	Small pond in Yasawa near Hanamaki	49
6-1	Creek near Lake Izunuma-Uchinuma	39
6-2	Small pond #125 in Izunuma-Uchinuma catchment	49
6-3	Small pond #127 in Izunuma-Uchinuma catchment	18
6-4	Two neighboring small ponds #90 connected with a ditch in Izunuma-Uchinuma catchment	6
6-5	Pond #91 in Izunuma-Uchinuma catchment	48
6-6	Small pond H004 near Lake Izunuma-Uchinuma	50
7	Small pond near Kashimadai	30
8	Pond near Sendai	4
9	Creek near Soma	42
10	River near Iidate	8
11	Creek near Kodaka	8
12	Small pond near Inawashiro	2

of template DNA, 0.96 μL of dNTP mix (2.5 nmol each), 1.2 μL of $10\times$ ExTaq buffer, 0.06 μL (0.3 U) of ExTaq (Takara, Shiga, Japan), 1 μL of primers (5 pmol each), 7.28 μL of Milli-Q grade water. PCR reaction started with 3 min at 94°C followed by 35 cycles of 30 sec at 94°C , 30 sec at 55°C , 120 sec at 72°C with final extension at 72°C for 5 min. Both L and H-strand primers worked for double-stranded sequencing with BigDye terminator v.3.1 kit run on an ABI3730 sequencer (ABI, Foster City CA, USA). Sequences used in this research cover nucleotide position 15640 to 16142 of the mitochondrial genome of *A. typus* (AB239602) (Saitoh et al. 2006). DDBJ/GenBank entries of sequences used in this research are LC148863 - LC149403.

Indices of nucleotide, haplotype, and population divergence were calculated with Arlequin v.3.5 (Excoffier et al. 2010). Parsimonious haplotype network was drawn with TCS v.1.2.1 (Clement et al. 2000). Interrelationships among populations based on pairwise net nucleotide divergence values were represented as a NJ tree with MEGA6 (Tamura et al. 2013). The net nucleotide divergence is calculated by $\pi_{xy} - (\pi_x + \pi_y)/2$ where π_{xy} is average number of nucleotide difference between populations x and y, and π_x and π_y stand for this value between individuals within populations x and y. We inferred population expansion and contraction states calculating Tajima (1989)'s D. An overall value of D was estimated in two ways; with and without normalization of number of individuals over localities. The normalization has rationale, because absolute sample sizes obtained with different methods at different opportunities do not directly indicate differences of fish abundance among localities. A normal-

ized sample size was set at 30 for each locality (541 / 18 localities ~ 30) and rounded up and down for minor and major haplotypes respectively to make them integer for calculation conveniences and approximation (Suppl. material 1: Table S1).

Results

Data for *Acheilognathus typus* showed low sequence diversity. Three individuals out of 541 in total, however, indicated heteroplasmic sequence traces from both strands with doubled fluorescence peak at one or two sites each (Suppl. material 1: Fig. S1). These sites are probably of real heteroplasmy (Shigenobu et al. 2005). For analytical convenience, we phased the heteroplasmic sites into either of the two bases of non-major haplotype in the locality where the heteroplasmic individuals came from. This manipulation makes this analysis conservative by reducing haplotype skewness or by giving moderate estimation of sequence differences among haplotypes. Overall π nucleotide diversity was 0.59129 ± 0.52518 , and \hat{h} haplotype diversity was 0.00118 ± 0.01511 upon this phasing. Upon normalization of sample size over localities, these values were 0.97484 ± 0.73356 and 0.00194 ± 0.01789 respectively.

There were ten variable sites, and eight haplotypes appeared (Hap1-8, Table 2). A haplotype network showed a major (Hap1) and minor haplotypes connected with mostly one mismatch (Fig. 2). The major haplotype appeared in the most localities (16 of 18). Seven localities out of 18 were monotypic, and others contain at most three haplotypes. Among eight haplotypes, Hap8 is somewhat distant from others with transversions and the only haplotype detected at locality #12. This overall haplotype composition structure reflects Tajima (1989)'s D value as negative (Table 3), indicating recent population expansion under neutral evolution, though eight out of 11 polymorphic localities showed positive values.

Interrelationships among localities (NJ tree) based on pairwise net nucleotide divergence indicated that introduced stocks (#6-5, 6-6, 8) are close to each other, distant from others and somewhere in between localities in Fukushima (#9, 10) and others (Fig. 3). Except for these introduced stocks, those around Lake Izunuma-Uchinuma (#6-1, 6-2, 6-3, 6-4) composed of Hap2 and Hap3 with Hap1. Hap2 and Hap3 were common and characteristic to these localities. Here again, #12 was distant from others.

Discussion

The low sequence diversity represented by both π and \hat{h} values with a simple haplotype network even in the fast evolving control region (Fig. 2), and negative Tajima's D value as a whole (Table 3), indicate the population experienced a bottleneck followed by expansion in the recent past. The normalization of sample size over localities reduces skewness of haplotype composition making diversity indices higher and deviation of Tajima's D value from zero smaller in the present case. This normalization thus makes our analysis

Table 2. Variable sites and haplotypes.

Haplotype	Nucleotide position	0	0	0	0	0	0	0	0	0
			0	1	1	2	2	2	2	4
		0	5	6	1	2	3	6	4	5
		6	7	4	8	0	2	8	3	0
Hap1*		T	G	T	A	T	T	G	T	A
Hap2		T	G	T	A	T	T	G	C	T
Hap3		T	A	T	A	T	T	G	T	T
Hap4		T	G	T	A	T	T	A	T	T
Hap5		C	G	T	A	T	T	G	T	T
Hap6		T	G	T	G	T	T	G	T	T
Hap7		T	G	T	A	T	C	G	T	T
Hap8		T	G	C	A	C	T	G	T	G

* Identical to AB239602.

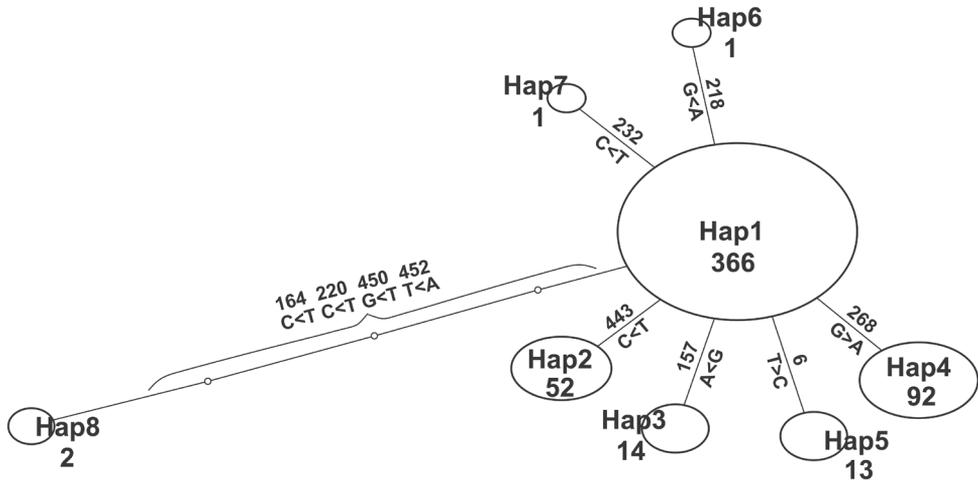


Figure 2. Haplotype network of *Acheilognathus typus* sequenced in this research. Small circles denote missing haplotypes. Numbers below haplotype ID stand for number of individuals observed. Numbers and bases at branches show nucleotide changes at these sites.

conservative but still gave similar results. Paucity in number (1-3) of haplotypes in individual localities with positive D values is due to further subsequent bottleneck after the overall geological bottleneck and expansion (Fay and Wu 1999). The locality #12 with only a distant haplotype (Hap8) has larger population genetic distances from other localities (Fig. 3) and beyond the major mountain ranges from others (Fig. 1). We thus postulate this haplotype as a remnant in recent range contraction, though we have some reservations because of limited number of individuals examined at this locality.

Acheilognathus typus is the sister species (Kawamura et al. 2014) and ecologically similar to *Acheilognathus longipinnis* Regan 1905 which is adapted to pulsed habitats (Odum et al. 1995) with annual flood-drought cycle (Ogawa 2011, Nishio et al. 2015).

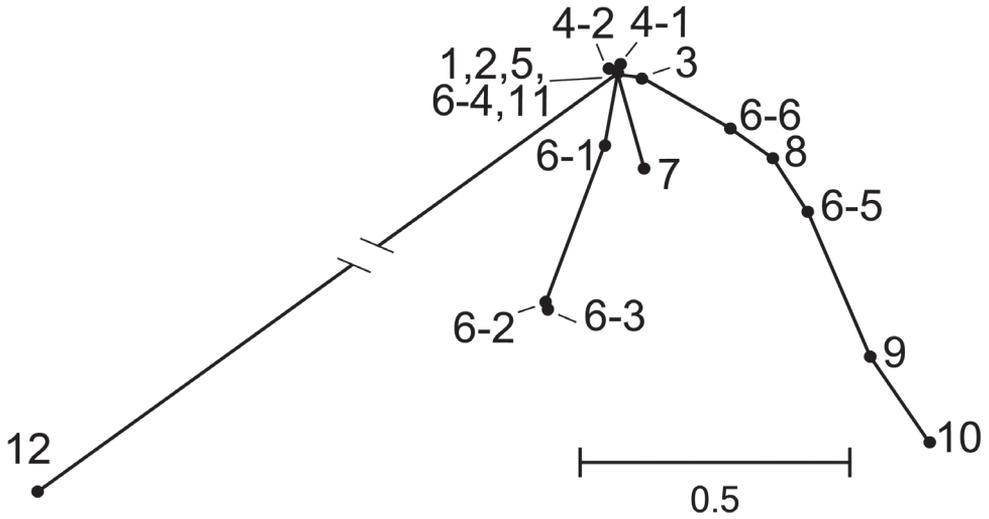


Figure 3. NJ tree based on net nucleotide divergence showing interrelationships among localities. Long branch between #12 with Hap8 and a node of #1, 2, 5, 6-4, 11 with Hap1 only is abbreviated and the real length is 2.4687.

Table 3. Haplotype composition and Tajima's D of each locality.

# \ Haplotype	Hap1	Hap2	Hap3	Hap4	Hap5	Hap6	Hap7	Hap8	D
1	30								n.a.
2	52								n.a.
3	34			3					-0.527
4-1	48					1			-1.105
4-2	19						1*		-1.164
5	49								n.a.
6-1	22	7	10						0.887
6-2	12*	33	4*						0.614
6-3	6	12							1.166
6-4	6								n.a.
6-5	23			25					1.714
6-6	29			21					1.648
7	17				13				1.578
8	2			2					1.633
9	9			33					0.681
10				8					n.a.
11	8								n.a.
12								2	n.a.
Overall	366	52	14	92	13	1	1	2	-1.246
Overall (normalized)	338	46	11	99	13	1	2	30	-0.694

* Each contains one heteroplasmic individual phased to the marked haplotype.

Acheilognathus typus actually showed population crash killing host mussels by excess spawning in a pond (Fujimoto and Shindo 2012). We therefore postulate that *A. typus* populations are prone to flush and crash, i.e., population bottleneck. Overall haplotype composition and negative Tajima's D yet positive in individual localities (Table 3) support our hypothesis.

Fish species that experience frequent population flush and crash events need wider habitats that allow spatially various phases in environmental fluctuation. In such habitats, those fish species like *A. typus* will sustain as metapopulations in which constituent subpopulations temporarily work as source or sink, and vice versa. Lake Izunuma-Uchinuma (5 km²) of 20 years ago, Lake Kasumigaura (172 km²) of more than 30 years ago, and Teganuma Lake (4 km²) of 45 years ago were such good habitats for *A. typus* (Fig. 1). Present-days known and introduced habitats are, however, small ponds (< 1 ha) in many cases, and population sustainability is questionable.

In Lake Izunuma-Uchinuma, invasion and establishment of largemouth bass have inhibited recovery of *A. typus* population (Takahashi et al. 2001). The largemouth bass exterminates bitterlings by heavy predation shortly (Fujimoto et al. 2009). The largemouth bass cleanup efforts with intensive catch at all life stages by people nearby, however, reduced the bass stock in the lake drastically, and cyprinid fishes are recovering (Ueda 2013). What were and what should be the genetic characteristics of the past and recovering *A. typus* population in the lake? Remnant populations can be characterized with specific common haplotypes (Hap2 and Hap3) (Table 3). We postulate four localities and others uncovered, if any, represent as a whole the past genetic composition of the lake population (branch of #6-1, 6-2, 6-3 in Fig. 3), and recovery of this population is the goal of the restoration program.

Under this circumstance, a good practice for restoring *A. typus* population may be to propagate them at first in ponds where the bass is absent in the lake catchment. Propagated *A. typus* then hopefully flows out from the ponds to the lake. The anticipated outflow would be a natural experiment whether *A. typus* population could establish in the lake where a small stock of the bass still remains.

Re-established stock at locality #6-5 in the lake catchment, however, may be inconvenient for recovery of the past *A. typus* population in the lake. Haplotype composition of that stock is similar to those in Fukushima (#9, 10) (Fig. 3, Table 3). The source of that stock is unknown and not from those nearby Lake Izunuma-Uchinuma. Outflow from that pond, if any, may change genetic composition of expected recovered population in the lake.

Introduction of *A. typus* into the ponds #6-6 from #8 and then from #6-6 to #6-5 took place when population at #6-1 declined temporarily. At that time it was not possible to introduce *A. typus* from #6-1 which was the only known locality near Lake Isunuma-Uchinuma. Pond #8 was then selected for the source of pond #6-6 based on the unverified information that it was introduced from #6-1, but it was misleading.

A lesson from above is importance of intensive survey of habitats, both known and unknown, before introduction. Population at #6-1 recovered in 2011 (Table 1) indicating actual population crash and flush. Populations in a few ponds in the lake catchment were

also found after intensive survey (#6-2, 6-3, 6-4). We conducted this genetic research after the finding of these populations, and identified the introduced populations near the lake (#6-5, 6-6) were not representatives of the past Lake Izunuma-Uchinuma population.

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KSa conceived this research. KSa analyzed data and drafted the manuscript. YF and TS conceived the entire project including this research and coordinated writing the manuscript. KSh, YF, and KT conducted field survey. All authors read, improved, and approved the manuscript. This research was financially supported by Research and Development Projects for Application in Promoting New Policy of Agriculture Forestry and Fisheries, Japan (#21062). Hideki Sugiyama (Akita Prefectural Institute of Fisheries) and Osamu Inaba (Minamisoma City Museum) helped in the collection of specimens from some localities. Takahiro Ohtsuka (Nat'l Res Inst Fish Sci) helped us for laboratory works. Michael H. Doosey provided invaluable comments for improvement of this manuscript. We have no financial conflict of interests about contents of this publication. Introduction of *Acheilognathus typus* into ponds around Lake Izunuma-Uchinuma was done under the Restoration Project of *Acheilognathus typus* (2003-) by the Miyagi Prefectural Izunuma-Uchinuma Environmental Foundation.

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Supplementary material I

Table S1, Figure S1

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Data type: sample data

Explanation note: **Table S1.** Normalized haplotype composition of localities.

Figure S1. Sequence traces of both directions with double-peaked sites.

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