

Taxonomy of reproductive Nereididae (Annelida) in multispecies swarms at Ambon Island, Indonesia

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Abstract

Multispecies, or mass, spawning of different invertebrate species is well known for coral reef systems; however, incidences involving polychaetes are poorly documented. In this study we report on mass swarming, prior to spawning, of Nereididae at Ambon Island, Maluku, on three occasions: in 1866, inferred from an historical sample deposited in Naturalis, Leiden, and in March, 2009 and 2014, based on newly collected samples. The 2009 and 2014 events co-occurred with spawning of other polychaetes, known locally as wawo and including the widespread Indo-Pacific eunicid, *Palola viridis* (Gray in Stair). Ten species of reproductive Nereididae are described, including *Composetia marmorata* (Horst) new combination, formerly *Ceratonereis marmorata*; epitokous modifications are described for both sexes of each species including taxonomically important features such as body colour and number of pre-natatory chaetigers. Three distinct types of natatory region morphologies are recognized, which appear to characterise groups of genera. The ten new records brings to 13 the total number of nereidid species known to undergo mass swarming at Ambon Island; a key to the 13 species is provided. Species composition varies slightly between the three time periods: four species were common between all three periods, five species were in common between 1866 and 2014, and four species were in common between 1995 and 2009/14. Two species of *Neanthes* and one of *Nereis* are identified as potentially new and will be described in subsequent papers.

Keywords

Systematics, heteronereid, epitoke, new species, Polychaeta, Maluku, Wallacia

Introduction

Synchronised swarming of polychaetes at the surface of the sea for the purpose of breeding is perhaps best known among the family Eunicidae, in particular the Indo-Pacific species *Palola viridis* Gray in Stair, 1847. However, swarming is also common among Nereididae and Syllidae and occurs also in at least 15 other families (Clark 1961). Two main strategies are employed: either the adult worm metamorphoses into a swimming form called an epitoke or heteronereid (many Nereididae, some Syllidae), or separate, independent reproductive individuals, called stolons, are formed (Syllidae, some Eunicidae such as *Palola* species); the epitokes and stolons then congregate (swarm) at the water surface to spawn, usually by rupture of the body wall. The epitokous strategy of Nereididae causes non-reversible modifications in adults that usually results in the death of the adult shortly after spawning. The timing of swarming in both strategies is regulated by both environmental as well as endogenous stimuli (Clark 1961; Watson et al. 2003).

In Ambon and surrounding islands of Maluku, mass swarming of polychaetes occurs every year from February to April. Locals refer to the worms as ‘wawo’ or, more popularly, as ‘laor’. The swarming has been known for a very long time, as occurring either in February and March (Rumphius 1705) or March and April (Radjawane 1982). Animals emerge from their coral substrate habitat and enter the water column two times each year, usually on the second to fourth night after a full moon immediately after sunset and continue for about two hours. In that two hour period, wawo are caught by the islanders in fishing nets (Ambonese: siru-siru) then cooked and eaten. The activity of catching the worms is known as ‘timba laor’ by the locals (Pamungkas 2011). The entire species composition of wawo from the present collections (2009, 2014), comprising 5 families and 25 species, has been listed by Pamungkas (2015); there are similar numbers of species of Eunicidae and Nereididae, but wawo is dominated in mass by the larger-bodied eunicids.

The only previous taxonomic studies of Ambonese wawo are those of Horst (1904 in English, 1905 in Dutch) and Martens et al. (1995). A small collection of epitokous worms from Ambon Island by Dutch biologist D.S. Hoedt in 1866, now at Naturalis (Leiden), was never published on, and the identity of these worms – all Nereididae – is reported in this study. The two investigations by Pamungkas (2009, 2011) were more concerned with, respectively, sexual behaviour and anthropological aspects of the worms rather than their taxonomic aspects. Since none of the above-mentioned taxonomic research was published in Indonesian (and because of their limited availability in Indonesian libraries), the natives of Ambon Island have been more influenced by Radjawane (1982), an Ambonese researcher who identified the eunicid *Lysidice oele* Horst, 1905 as the only wawo species in Maluku waters, and popularized the mass spawning phenomenon of wawo among locals. Reports of swarming Nereididae in other tropical regions are equally scarce: Hutchings and Howitt (1988) found 16 nereidid species in multiple swarming events at Lizard Island, northern Great Barrier Reef, Australia, between October and January, 1985/86 – none of the species were identified beyond

family, but a later review of the nereidids of this island (Glasby 2015) indicated that at least some species are in common with those at Ambon Island.

In this study we describe the taxonomy of the nereidid component of wawo collected in 2009/14 and nereidids in Hoedt's collection (1866), in particular the epitokous modifications of each sex and species. Because all samples contained a small fraction of spent worms (i.e., lacking coelomic gametes), we have assumed that spawning occurred about the time the swarming worms were collected. The species composition of the two collections, almost 150 years apart, is compared with that reported by Martens et al. (1995).

Material and methods

Sexually mature polychaetes (wawo) were collected during the swarming period on 14 March, 2009 and 18–19 March, 2014, from Ambon coastal waters (Table 1, Fig. 1). At each station, the worms were collected using a small, fine-mesh hand net and were immediately fixed with 10% formaldehyde solution for at least 24 hours. A lantern or torch was used to illuminate the surroundings and attract the worms. Collected worms were rinsed with tap water to remove both the fixing agent and salt crystals, and were then preserved in 70% ethanol.

In the laboratory, the epitokous nereidids were separated from other species of wawo by gross differences in the body form. The primary characteristics used to identify the different types of nereidid wawo were form of the parapodia and head morphology including eyes, antennae number and proboscis form. Voucher specimens were deposited at the Museum and Art Gallery of the Northern Territory, formerly Northern Territory Museum (NTM), Darwin, Australia, the Museum Zoologicum Bogoriense (MZB), Bogor, Indonesia, and the Reference Collection LIPI Ambon (RCLA), Indonesia (RCLA belongs to the Research Center for Deep Sea, Indonesian Institute of Sciences). Comparative material was sourced from the former Zoological Museum Amsterdam (ZMA) and Naturalis (formerly Rijksmuseum van Natuurlijke Historie, RMNH), Leiden; the ZMA collection is now integrated into the RMNH collection.

Nereidid samples collected by D.S. Hoedt and V.D. Velde were studied during a visit to Naturalis (RMNH), Leiden, in 2009 by CJG. The former comprises hundreds of epitokous Nereididae in one jar (Fig. 2), which was at the time uncatalogued. The sample was received by Naturalis (formerly the Leiden Museum) in 1867 according to the register. The entire sample was studied, and selected male and female representatives of each putative species separated. A voucher collection was registered separately with the NTM.

Preserved specimens were examined using stereo (Nikon SMZ 1500 and Nikon SMZ 645) and compound (Nikon ECLIPSE 80i and Nikon ECLIPSE 50i) light microscopes. Macrophotographs of preserved animals were taken with a Canon 5D Mark II with a Canon MPE-65 Macro Lens.

Potential new species were flagged and identified using informal names or 'cf.'; for these taxa there is currently insufficient information on morphological varia-

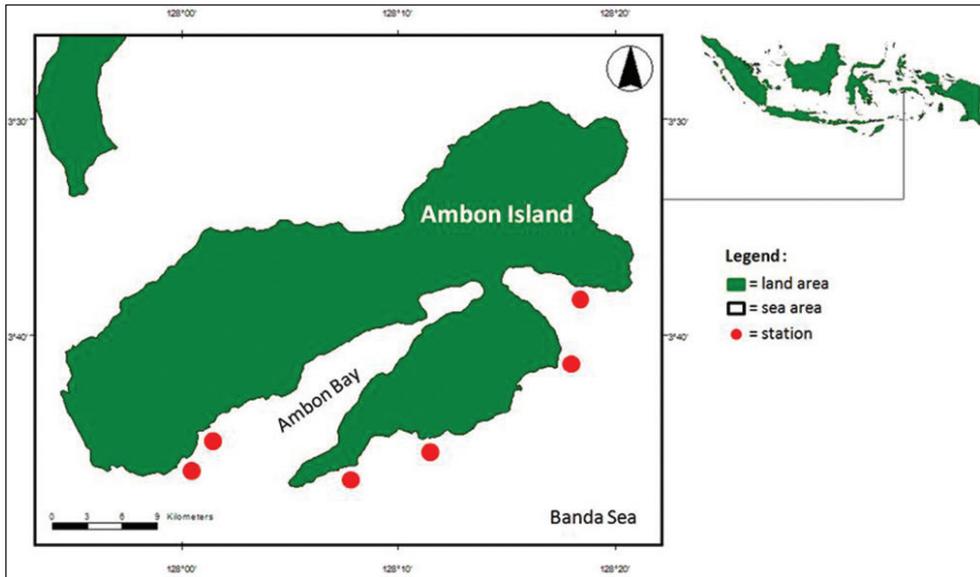


Figure 1. Map of Ambon Island showing location of stations, from left to right: Airlouw, Mahia, Hutumuri, Suli, Lilibooi, Alang; see Table 1 for co-ordinates and collection times.

Table 1. Stations, collection dates and co-ordinates of *wawo* sampling.

Station	Collection dates	Coordinates
Alang	14 March 2009, 19 March 2014	3°46'18.2"S, 128°00'24.6"E
Lilibooi	19 March 2014	3°45'08.8"S, 128°01'24.6"E
Suli	18–19 March 2014	3°37'38.2"S, 128°18'25.0"E
Hutumuri	14 March 2009, 19 March 2014	3°41'27.5"S, 128°17'56.3"E
Mahia	18–19 March 2014	3°44'42.6"S, 128°11'24.6"E
Airlouw	14 March 2009, 19 March 2014	3°46'32.5"S, 128°07'53.5"E

tion to assign a Linnean binomen. The informal species epithet takes the general format 'colloquial name_voucher number_name of person recognising the species', for example, *Neanthes 'sp_Ambon_NTMW19037'* Glasby. Species are arranged alphabetically by genus and species, with informally named species at the end of each genus. Terminology for parapodial features follows Bakken and Wilson (2005) and for metamorphosed individuals (Read 2007), and for the paragnath form follows Bakken et al. (2009). Each morphospecies is diagnosed using a combination of paragnath form and pattern, epitokous modifications, and ethanol-preserved colour patterns of the body and eggs. In the absence of a robust phylogeny for Nereididae – and therefore the possibility of using a phylogenetic-based species concept – we use the morphospecies species concept as defined by Cronquist (1978), i.e., species are the smallest groups that are consistently and persistently distinct, and distinguish-

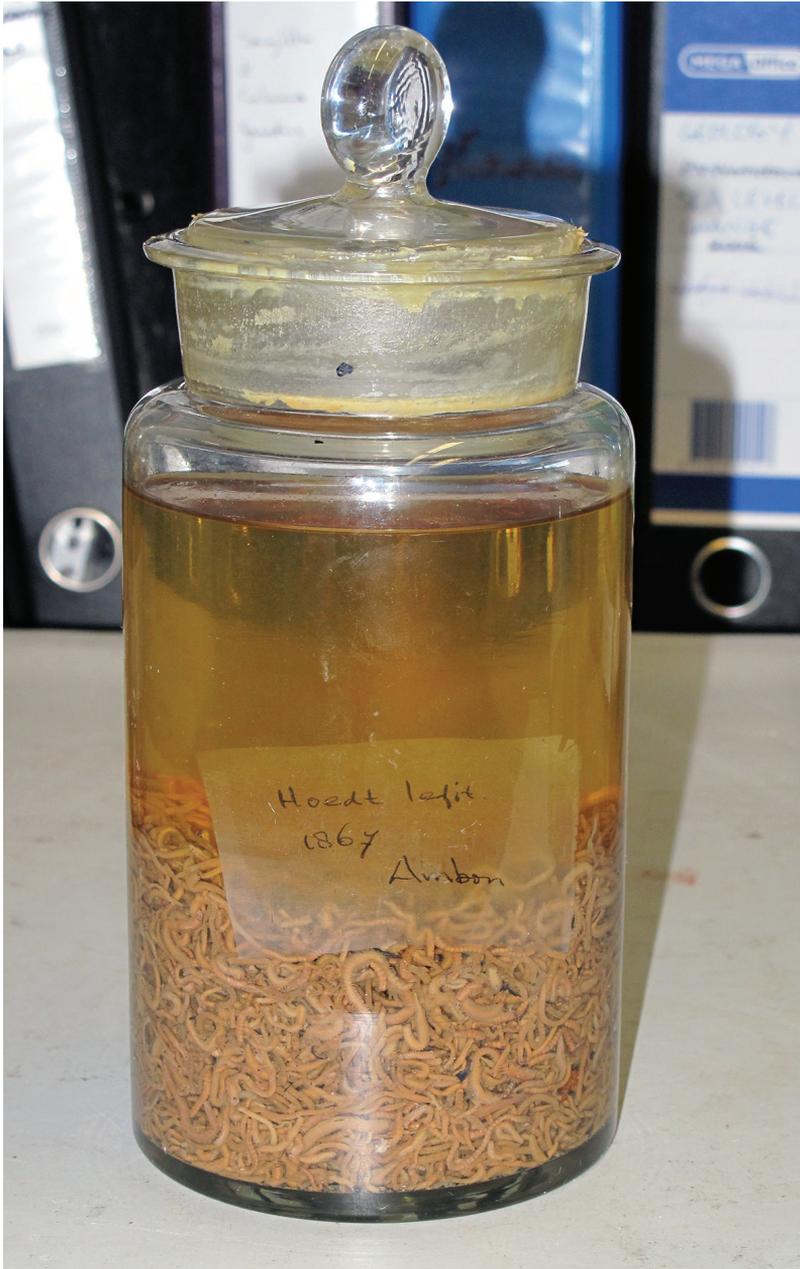


Figure 2. Sample of mixed-species nereidid epitokes collected Dutch biologist D.S. Hoedt in 1866, as found in Naturalis, Leiden.

able by ordinary means. Our proposed species are therefore hypotheses which are falsifiable when independent data, for example morphological synapomorphies and DNA sequences, become available.

Taxonomy

Family Nereididae Blainville, 1818

Ceratonereis Kinberg, 1865

Ceratonereis singularis australis Hartmann-Schröder, 1985

Fig. 3A, B

Ceratonereis (*Ceratonereis*) *singularis australis* Hartmann-Schröder, 1985: 46–47, figs 48–58.

Ceratonereis (*Ceratonereis*) cf. *singularis australis*. – Martens et al. 1995: 12, figs 3–12.

Nereis (*Ceratonereis*) *tentaculata*. – Horst 1924: 36–38, Pl. 35, figs 4–7. Non Kinberg.

Type locality. Exmouth, Western Australia, Australia.

Material examined. 1 female (NTM W25886), 1 male (NTM W25890), 2 females (MZB.Pol.00175), 2 females (RCLA.Ann.048), all from Suli, Ambon Island, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 8 ex. including 5 females (NTM W23808), Banda, Maluku Province, Indonesia, coll. V.D. Velde, May 1921 (donated to NTM, formerly ZMA VPol 0962).

Comparative material. 1 ex.(NTM W22557), 1 ex.(NTM W23939), 1 ex.(NTM W23950), 1 ex.(NTM W23983), Lizard Island, northern Great Barrier Reef, Australia, coll. CREEFS surveys 2008–2010.

Size range. Female: length (11–15 mm), maximum width (2.9–3.0 mm). Male: length (25 mm), maximum width (3.1 mm).

Diagnosis. *Ceratonereis* species with females having dark brown bands on chaetiger 2 and 3 dorsally, and sometimes weaker brown bands on chaetigers 5–14; pigment apparently absent in males (1 specimen examined) (Figs 3A, B). Eyes black in Ambon specimens but purple (perhaps faded?) in Banda specimens. Paragnaths conical, arranged as follows: Areas I: 0; II: 11–16; III: 7–11; IV: 15–18; absent areas V–VIII. Apart from pigmentation, male and female epitokes identical, i.e., males lacking scalloped dorsal cirri. Pre-natatory region comprising 16–17 chaetigers; dorsal cirri of anterior chaetigers unmodified; ventral cirri of anterior chaetigers unmodified; natatory region confined to mid-body, with posterior body unmodified; pygidium not observed. Fertilised eggs adhered to body surface in some specimens, green coloured.

Remarks. The specimens examined agree well with Hartmann-Schröder's (1985) type description especially the characteristic pigmentation pattern that we observed in female specimens, and paragnath count and form of the unmodified parapodia. It is the same as the material identified by Horst (1924) as *Nereis* (*Ceratonereis*) *tentaculata* from eastern Indonesia and as *Ceratonereis* (*Ceratonereis*) cf. *singularis australis* from Ambon Island by Martens et al. (1995). Specimens of *C. australis* from Lizard Island, northern Great Barrier Reef described by Glasby (2015) are also likely to be conspecific.

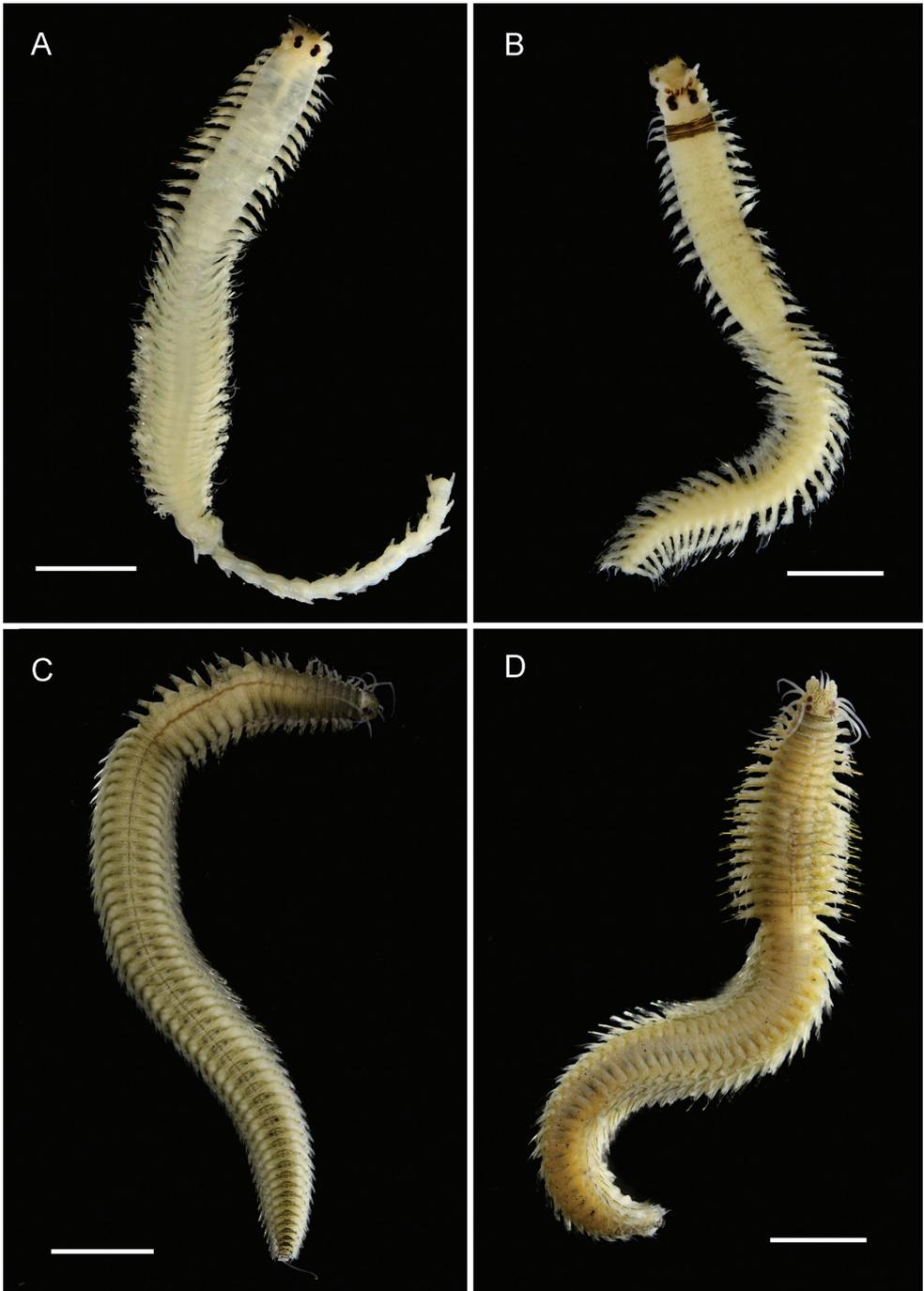


Figure 3. Nereidid epitokes, preserved specimens, dorsal view. **A** *Ceratonereis singularis australis*, male **B** *Ceratonereis singularis australis*, female (unmodified tail section missing) **C** *Compositia marmorata*, male **D** *Compositia marmorata*, female. Scales bars: 1 mm.

Ceratonereis (*Ceratonereis*) cf. *perkinsi* by Martens et al. (1995) appear to correspond to specimens observed by CJG from northern Australia, near the type locality (Broome, Western Australia) in terms of parapodial form and pigmentation pattern. Specimens reported by these authors as *Ceratonereis* (*Ceratonereis*) sp., do indeed belong to the *Ceratonereis* sensu stricto as described by Hartmann-Schröder (1985), as indicated by the illustrated deeply cleft prostomium (fig. 13); however, the suggestion that they are similar to *C. japonica* is incorrect because the latter species has a very different type and arrangement of paragnaths, more in line with a species of *Solomononereis* (see Glasby 2015).

Distribution. Eastern Indonesia and Australia (widespread).

Composetia Hartmann-Schröder, 1985

Composetia marmorata (Horst, 1924), comb. n.

Fig. 3C, D

Nereis (*Ceratonereis*) *marmorata* Horst, 1924: 177–178, pl. 34, figs 13–16.

Type locality. between Gisser and Seram-Laut, Maluku, Indonesia.

Material examined. 3 males (RCLA.Ann.042), 1 female (RCLA.Ann.043), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; 3 males (RCLA.Ann.044), 2 females (RCLA.Ann.045), Suli, Ambon, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 1 male (RCLA.Ann.046), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemala, 19 March 2014; 3 males (MZB.Pol.00161), 2 females (MZB.Pol.00162), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; 1 male (NTM W25889), 1 female (NTM W25888), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 1 ex. (NTM W23811), Salawati, Raja Ampat, Indonesia coll. unknown, 18 August 1899 (donated to NTM, formerly ZMA VPol 0980).

Comparative material. *Nereis* (*Ceratonereis*) *marmorata* syntypes: 17 ex. (ZMA Vpol 0869) and 5 ex. (RMNH 1352), Siboga Stn. 172, Gisser anchorage, between this island and Seram-Laut, Maluku, Indonesia, 3°53'9.2"S, 130°51'56.2"E, coll. 26 August 1899. *Composetia marmorata*: 1 ex. (NTM W22530), 1 ex. (NTM W22615), 1 ex. (NTM W22797), Lizard Island, northern Great Barrier Reef, Australia, coll. CREEFS surveys 2008–2010.

Size range. Male: length (20–40 mm), maximum width (3.0–5.0 mm). Female: length (17–31 mm), maximum width (2.5–4.9 mm).

Diagnosis. *Composetia* species having distinctive marmorated stripes on the prostomium and anterior body (Fig. 3D). Paragnaths conical, arranged as follows: Areas I: 1–2; II: 7–14; III: 9–13; IV: 14–16; absent areas V–VIII. Male and female epitokes similar (Fig. 3C, D), except male has pygidial rosette (lacking in female) and natatory

region dorsal cirri are sub-distally swollen (not scalloped) but unmodified in female; pre-natatory region comprising 17–18 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 6 or 7; basally swollen ventral cirri on chaetigers 1 to 5; natatory region extends to pygidium. Female with eggs unpigmented.

Remarks. The distinctive marmorated (=veined) stripes on this species of *Composetia* facilitate identification. The markings are as described by Horst (1924: 177) for *Nereis* (*Ceratonereis*) *marmorata*, and paragnath counts match well, although Horst reports considerable variation. Examination of paragnath numbers on the syntype material confirmed the identification. Hartmann-Schröder's (1985) tentative allocation of the species to *Composetia* is confirmed, and formalized here. Specimens from Lizard Island, northern Great Barrier Reef described by Glasby (2015) are likely to be conspecific.

Distribution. Indonesia. Lizard Island, Australia.

Neanthes Kinberg, 1865

Neanthes unifasciata (Willey, 1905)

Fig. 4A, B

Nereis unifasciata Willey, 1905: 271–272, pl. 4, figs 85–88.

Type locality. Cheval Paar, Sri Lanka

Material examined. 4 males (RCLA.Ann.020), 6 females (RCLA.Ann.021), Alang, Ambon Island, Indonesia, 3°46'18.2"S, 128°00'24.6"E, coll. J. Pamungkas, 19 March 2014; 10 males (RCLA.Ann.022), 11 females (RCLA.Ann.023), Lilibooi, Ambon Island, Indonesia, 3°45'08.8"S, 128°01'24.6"E, coll. J. Pamungkas, 19 March 2014; 111 males (RCLA.Ann.024), 57 females (RCLA.Ann.025), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; 2 females (RCLA.Ann.026), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 38 males (RCLA.Ann.027), 41 females (RCLA.Ann.028), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemia, 18 March 2014; 6 males (RCLA.Ann.029), 5 females (RCLA.Ann.030), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemia, 19 March 2014; 3 males (MZB.Pol.00169), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemia, 18 March 2014; 1 male (MZB.Pol.00170), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 4 males (NTM W23791), 5 females (NTM W23792), Hutumuri, Ambon Island, Indonesia, 3°42.1'S, 128°17.5'E, coll. J. Pamungkas, 14 March 2009; 2 males (NTM W23796), Alang, Ambon Island, Indonesia, 3°46'18.2"S, 128°00'24.6"E, coll. J. Pamungkas, 14 March 2009; 1 ex. (specimens extracted from a jar of many hundred worms in the collection of Naturalis, Leiden) (NTM W23806), Ambon Island, Indonesia, coll. D.S. Hoedt, 1866.

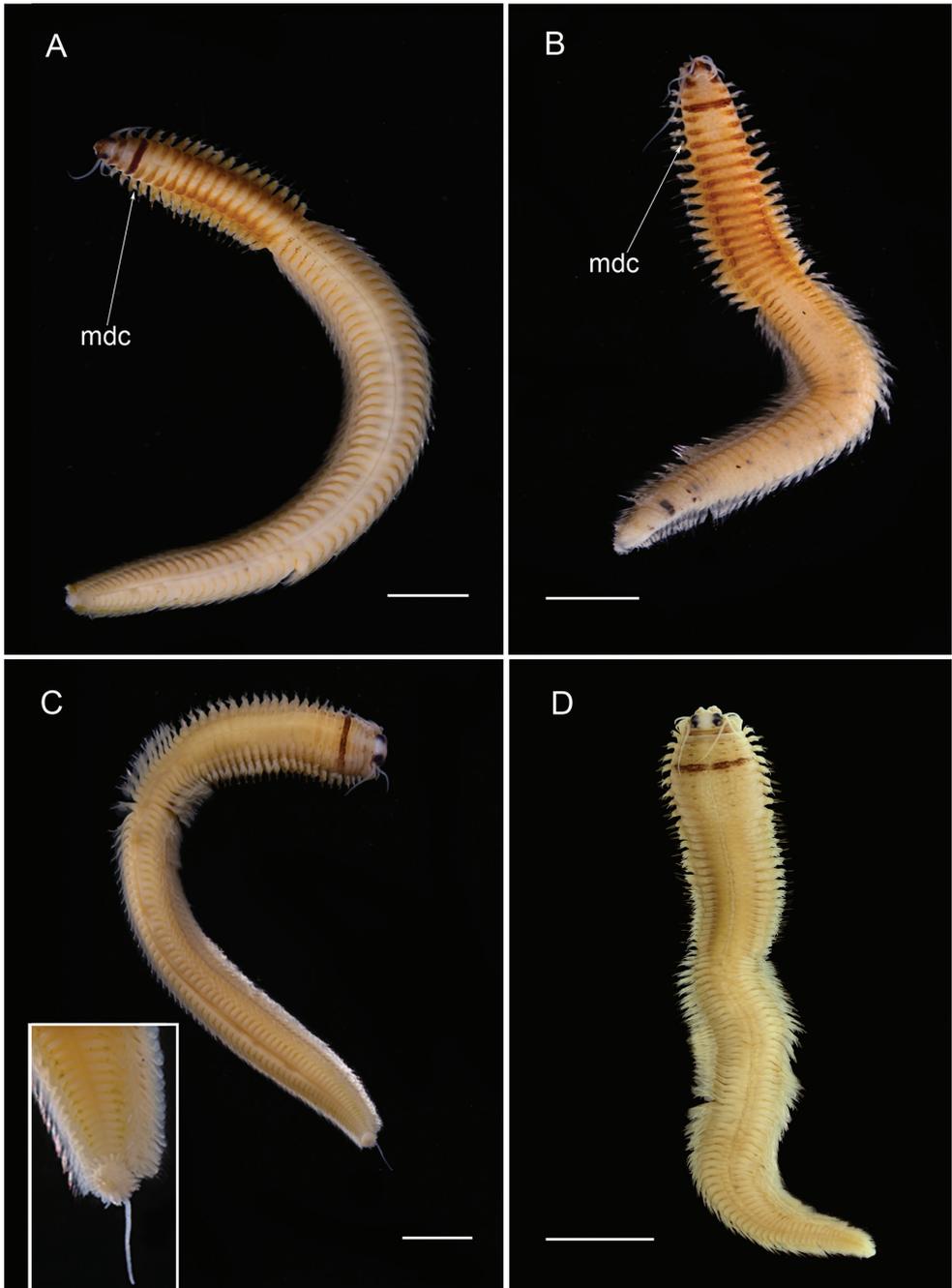


Figure 4. Nereidid epitokes, preserved specimens, dorsal view. **A** *Neanthes unifasciata*, male **B** *Neanthes unifasciata*, female **C** *Neanthes* sp. cf. *N. gisserana* male, inset showing close up of pygidial rosette **D** *Neanthes* sp. cf. *N. gisserana* female. mdc = modified dorsal cirri. Scales bars: 2 mm (**A**, **B**), 3 mm (**C**), 4 mm (**D**).

Size range. Male: length (15–25 mm), maximum width (1.5–2.5 mm). Female: length (18–28 mm), maximum width (2.0–3.0 mm).

Diagnosis. *Neanthes* species having dark brown band on dorsal surface of chaetiger 2 and lighter bands on following pre-natatory chaetigers (Fig. 4A, B). Paragnaths conical, arranged as follows: Areas I: 1; II: 14–16; III: 22–25; IV: 25–30; V: 0; VI: 6–12, very small; VII–VIII: 4–6 in one line. Male and female epitokes both with natatory region extending to pygidium, but differ in number of pre-natatory chaetigers and number modified dorsal and ventral cirri. Male has pre-natatory region comprising 16 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 7; basally swollen ventral cirri on chaetigers 1 to 5 or 6. Female has pre-natatory region comprising 19–20 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 5; basally swollen ventral cirri on chaetigers 1 to 5. Males differ from females in having subdistally swollen and scalloped dorsal cirri (smooth in females) and modified rosette pygidium.

Remarks. The specimens examined in this study agree well the description of Willey (1905), which however is not very detailed. This species of *Neanthes* may be distinguished from other wawo *Neanthes* by the brown band on the dorsal side of chaetiger 2.

Distribution. Indo-west Pacific (widespread).

Neanthes pachychaeta (Fauvel, 1918)

Ceratonereis pachychaeta Fauvel, 1918: 506–508, fig. 3a–h

Neanthes pachychaeta. – Glasby, Wilson and Bakken 2011: 363–371, figs 1–7, 8d (full synonymy provided).

Type locality. Djibouti and Madagascar.

Material examined. Ambon Island, 1 specimen (RMNH inreg.), collected D.S. Hoedt, 1866 [specimen extracted from a jar of many hundred worms in the collection of Naturalis, Leiden; Fig. 2].

Size range. Not available.

Remarks. This species was recently re-described by Glasby et al. (2011); epitokes were described by Horst (1924) under the junior synonym name of *Nereis* (*Ceratonereis*) *ramosa*.

Distribution. Indo-Pacific, widespread.

Neanthes sp. cf. *N. gisserana* (Horst, 1924)

Fig. 4C, D

Nereis (*Lycoris*) *gisserana* Horst, 1924: 151–152, pl. 30, figs 6, 7.

Material examined. 5 males (RCLA.Ann.031), 3 females (RCLA. Ann.032), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014;

1 male (RCLA.Ann.033), 1 female (RCLA.Ann.034), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 3 males (RCLA.Ann.035), 2 females (RCLA.Ann.036), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemia, 18 March 2014; 2 males (RCLA.Ann.037), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemia, 19 March 2014; 2 males (MZB.Pol.00167), 2 females (MZB.Pol.00168), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; 1 male (NTM W23797), Alang, Ambon Island, Indonesia 3°46'18.2"S, 128°00'24.6"E, coll. J. Pamungkas, 14 March 2009; 1 female (specimen extracted from a jar of many hundred worms in the collection of Naturalis, Leiden); Fig. 2 (NTM W23804), Ambon Island, Indonesia, coll. D.S. Hoedt, 1866.

Comparative material. *Neanthes gisserana* (Horst, 1924). Syntypes 2 ex. (ZMA VPol 0854), Siboga Stn. 172, Gisser anchorage, between this island and Seram-Laut, Maluku, Indonesia, 18 m, coll. 26 August 1899. *Neanthes cf gisserana*. 1 ex. (NTM W22501), Lizard Island, northern Great Barrier Reef, Australia, coll. CREEFS surveys 2008–2010.

Size range. Male: length (16–25 mm), maximum width (2.0–4.0 mm). Female: length (16–32 mm), maximum width (3.0–4.0 mm).

Diagnosis. *Neanthes* species having brown band on dorsal surface of chaetiger 4 (Fig. 4C, D). Paragnaths comprise cones and bars arranged as follows: Areas I: 4 (in a longitudinal line); II: 14; III: 19–22 (including lateral groups); IV: 26 (includes cones and bars); V: 0; VI: 3–6; VII–VIII: 5–7 in transverse line. Male and female epitokes with similar body pigmentation and natatory region extending to pygidium, but differ in number of pre-natatory chaetigers and number modified dorsal and ventral cirri. Male having pre-natatory region comprising 21–22 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 7; basally swollen ventral cirri on chaetigers 1 to 5 or 6. Female having pre-natatory region comprising 26 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 6; basally swollen ventral cirri on chaetigers 1 to 5. Also, male has pygidial rosette (Fig. 4C) and sub-distally swollen (not scalloped) dorsal cirri, lacking in female.

Remarks. The specimens examined in this study were compared with Horst's, syntypes, which they resemble closely, especially in the paragnath count and pattern; however, the present specimens are much larger than those of Horst's, the modified chaetigers start later in the present specimens, and Horst does not mention the presence of a brown band which we observed on chaetiger 4. Therefore our material possibly represents a new species; it is very similar to specimens also referred to as *Neanthes* sp. cf. *N. gisserana* by Glasby (2015).

***Neanthes* sp. cf. *N. masalacensis* (Grube, 1878)**

Fig. 5A, B

Nereis (Lycoris) masalacensis Grube, 1878: 75–76, pl. 5, fig. 4.

Material examined. 22 males (RCLA.Ann.038), 16 females (RCLA.Ann.039), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; 5 males (RCLA.Ann.40), 1 female (RCLA.Ann.41), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 1 male (MZB.Pol.00171), 5 females (MZB.Pol.00172), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; 3 males (NTM W25892), 3 females (NTM W25891), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; Ambon Island, 4 females, 2 males (NTM W23805), collected D.S. Hoedt, 1866 (specimen extracted from a jar of many hundred worms in the collection of Naturalis, Leiden; Fig. 2).

Size range. Male: length (30–47 mm), width (2.5–3.0 mm). Female: length (30–50 mm), width (2.0–3.0 mm).

Diagnosis. *Neanthes* species having uniform brown pigmentation on dorsal surface, apparently darker in male compared to female (Fig. 5A, B). Paragnaths conical arranged as follows: Areas I: 0; II: 10–13; III: 19–25; IV: 14–20; V: 0; VI: 1–2; VII–VII: 2–5 in line. Male and female epitokes both with natatory region extending to pygidium, but differ in number of pre-natatory chaetigers and number modified dorsal and ventral cirri. Male has pre-natatory region comprising 28–30 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 7; basally swollen ventral cirri on chaetigers 1 to 6. Female has pre-natatory region comprising 32 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 5; basally swollen ventral cirri on chaetigers 1 to 5. Also male has pygidial rosette and sub-distally swollen, scalloped dorsal cirri in anterior part of natatory region (dorsal cirri unmodified posteriorly), lacking in female. Female with brown pygidium.

Remarks. The present material bears a general resemblance to Grube's species, which was described from Masalac, Philippines, in terms of pigmentation pattern and paragnath arrangement and counts. Our specimens differed in lacking paragnaths in Area I (Grube illustrates 2 paragnaths) and in the relatively longer dorsal cirri (up to 3 times length of parapodia in anterior chaetigers), whereas in Grube's specimens the dorsal cirri appear to be slightly longer than the parapodia, but the angle of the illustration makes it difficult to ascertain how much longer. It probably represents a new species.

Hartman (1959) referred *Neanthes masalacensis* to *Pseudonereis* based on the comparisons of earlier workers (e.g., Fauvel, Gravier) with other species of *Pseudonereis*. However, examination of the holotype of *Nereis (Lycoris) masalacensis* by Hutchings and Glasby (1985), showed the absence of elongated dorsal notopodial lobes in posterior chaetigers, and the original figure by Grube (1868) showed the paragnaths in Area II to be conical shaped rather than p-bars (sensu Bakken). Both features strongly suggest that the species does not belong to *Pseudonereis*. Hutchings and Glasby (1985) consider it to be indeterminable unless other material from the type locality (Masalac, Philippines) can be examined (and the distinctive pigmentation pattern matched). With this new material, from the same general biogeographic region (Western Coral Triangle) that resembles closely the type description of *N. masalacensis*, we suggest the species be considered a member of *Neanthes*, as listed in Pamungkas (2015).



Figure 5. Nereidid epitokes, preserved specimens, dorsal view. **A** *Neanthes* sp. cf. *N. masalacensis*, male **B** *Neanthes* sp. cf. *N. masalacensis*, female **C** *Nereis* 'sp_AmbonNTMW19037' Glasby, male **D** *Solomononereis merauensis*, female. Scales bars: 2 mm (**A, B, D**), 1 mm (**C**).

Nereis* Linnaeus, 1858**Nereis* 'sp_Ambon_NTMW19037' Glasby**

Fig. 5C

Material examined. 1 female (RCLA.Ann.052), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemala, 19 March 2014; 1 male (MZB.Pol.00173), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; 1 female (MZB.Pol.00174), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 1 male (NTM W19155), Waimahu Beach, Ambon Island, coll. Mr Talakua, 27 March 1997; 1 male (NTM W25893), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014.

Size range. Female: length (15 mm), maximum width (2.0 mm). Male: length (16 mm), maximum width (2.0 mm).

Diagnosis. *Nereis* species lacking pigmentation (Fig. 5C). Paragnaths include cones and bars arranged as follows: Areas I: 0–1 cone; II: 15–20 cones; III: 19–26 cones; IV: about 45 in large crescentic patch includes cones and bars; V: 0; VI: 15–20 small patch of minute cones; VII–VIII: one narrow band of 50–60 cones with several additional rows at right angles. Male and female epitokes both with natatory region extending to pygidium, but differ in number of pre-natatory chaetigers and number modified dorsal and ventral cirri. Male has pre-natatory region comprising 22–23 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 7; basally swollen ventral cirri on chaetigers 1 to 5 or 6. Female has pre-natatory region comprising 26 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 5; basally swollen ventral cirri on chaetigers 1 to 4. Also male has pygidial rosette and scalloped dorsal cirri in natatory region (except near pygidium where dorsal cirri become smooth and tapered again), rosette and scalloped dorsal cirri lacking in female.

Remarks. This appears to be a new species, and will be described when further specimens, including atokous individuals become available. It was listed by Pamungkas (2015) as *Nereis* sp.

Perinereis* Kinberg, 1865**Perinereis helleri* (Grube, 1878)**

Fig. 6A, B

Nereis (*Perinereis*) *helleri* Grube, 1878: 81–82

Perinereis cultrifera. – Martens et al. 1995: 15. Not Grube.

Perinereis helleri. – Hutchings et al. 1991: 254–255, fig. 9a–c.

Type locality. Bohol, Philippines.

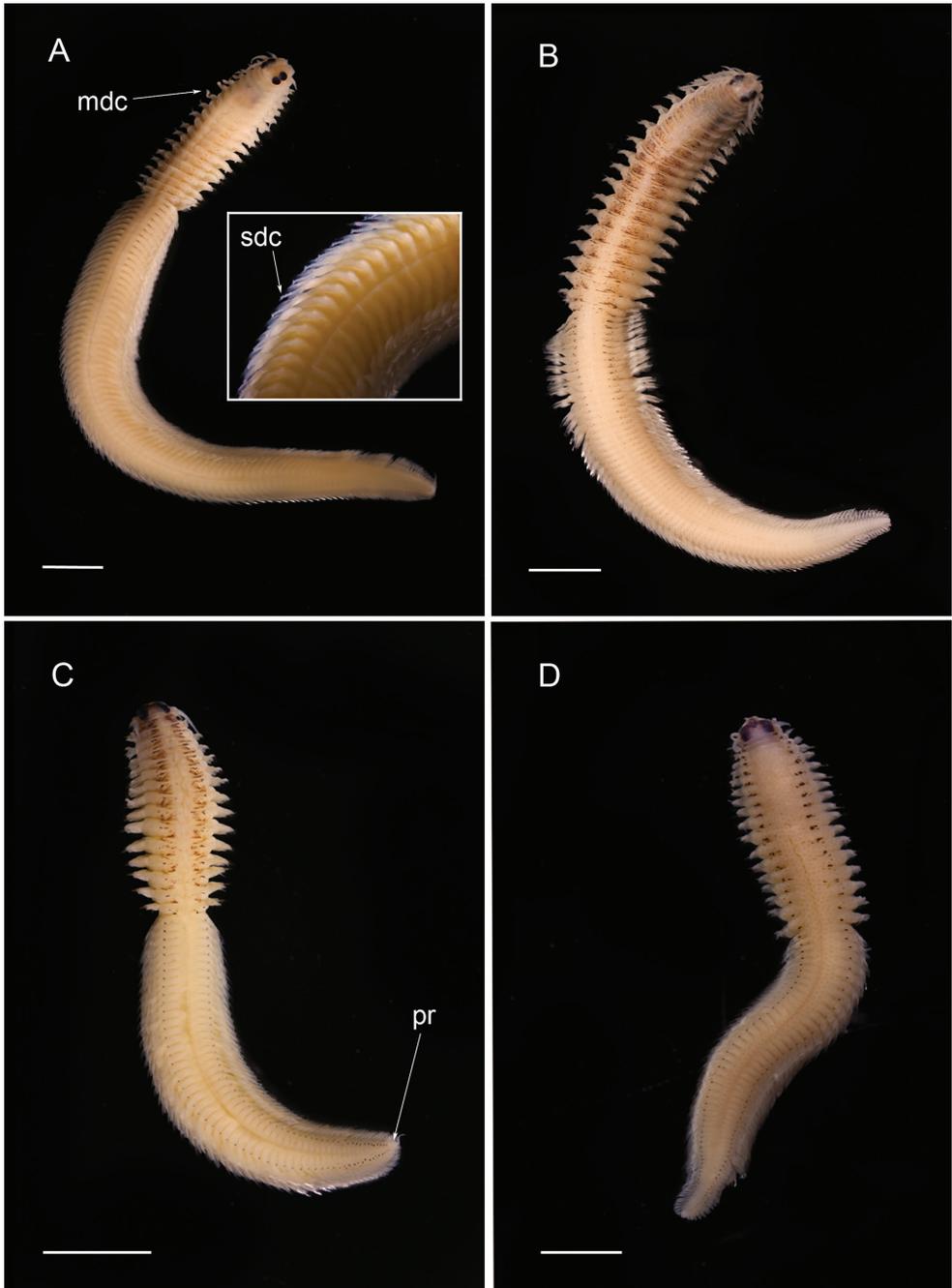


Figure 6. Nereidid epitokes, preserved specimens, dorsal view. **A** *Perinereis helleri*, male, inset shows close up of modified parapodia of natatory region **B** *Perinereis helleri*, female **C** *Perinereis nigropunctata*, male **D** *Perinereis nigropunctata*, female. mdc = modified dorsal cirri; sdc = scalloped dorsal cirri; pr = pygidial rosette. Scales bars: 2 mm (**A**, **B**), 3 mm (**C**, **D**).

Material examined. 4 males (RCLA.Ann.001), 2 females (RCLA.Ann.002), Alang, Ambon Island, Indonesia, 3°46'18.2"S, 128°00'24.6"E, coll. J. Pamungkas, 19 March 2014; 6 males (RCLA.Ann.003), 6 females (RCLA.Ann.004), Hutumuri, Ambon Island, Indonesia, 3°41'27.5"S, 128°17'56.3"E, coll. E. Moniharapon, 19 March 2014; 1 female (RCLA.Ann.005), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemia, 18 March 2014; 11 males (RCLA.Ann.006), 10 females (RCLA.Ann.007), Airlouw, Ambon Island, Indonesia, 3°46'32.5"S, 128°07'53.5"E, coll. F. E. de Soysa, 19 March 2014; 5 males (MZB.Pol.00163), 5 females (MZB.Pol.00164), Airlouw, Ambon Island, Indonesia, 3°46'32.5"S, 128°07'53.5"E, coll. F. E. de Soysa, 19 March 2014; 2 males (NTM W23794), 2 females (NTM W23795), Alang, Ambon Island, Indonesia 3°46'18.2"S, 128°00'24.6"E, coll. J. Pamungkas, 14 March 2009; 2 males (NTM W23799), 1 female (NTM W23800), Airlouw, Ambon Island, Indonesia, 3°46'32.5"S, 128°07'53.5"E, coll. J. Pamungkas, 14 March 2009; 5 ex. (specimens extracted from a jar of many hundred worms in the collection of Naturalis, Leiden); Fig. 2 (RMNH unreg.), Ambon Island, coll. D.S. Hoedt, 1866.

Size range. Male: length (25–47 mm), maximum width (3.0–6.0 mm). Female: length (28–70 mm), maximum width (4.0–6.0 mm).

Diagnosis. Large *Perinereis* species having brown streaky pigmentation on dorsal surface (Fig. 6A, B). Paragnaths conical arranged as follows: Areas I: 2–3; II: 6–10; III: 14–18 (including lateral groups); IV: 17–24; V: 3; VI: 1 bar; VII–VIII: 25–30. Male and female epitokes with similar body pigmentation and natatory region extending to pygidium, but differ in number of pre-natatory chaetigers and number modified dorsal and ventral cirri. Male has pre-natatory region comprising 17 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 7; basally swollen ventral cirri on chaetigers 1 to 6. Female has pre-natatory region comprising 19–20 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 6; basally swollen ventral cirri on chaetigers 1 to 5. Also male has pygidial rosette, and scalloped dorsal cirri; lacking in female.

Remarks. The specimens examined in this study agree well the re-description of Hutchings et al. (1991) of this species. This is one of the largest of the wawo nereidids. The specimens reported as *Perinereis cultrifera* by Martens et al. (1995) are most likely *P. helleri*, as they fit well the paragnath number and pattern described here for this species (see also Hutchings et al. 1991), although there is a discrepancy in the number of anterior unmodified chaetigers: Martens et al. (1995) report this as 14 and 18 (male and female respectively), but our observations for this species are 17 and 19–20 (ditto). We believe Martens et al. may have confused *P. helleri* with *P. nigropunctata*, which were also found in their samples, as the latter has about 14 and 17 anterior unmodified chaetigers for male and female respectively (see following account).

Distribution. Indo-Pacific (widespread).

***Perinereis nigropunctata* (Horst, 1889)**

Fig. 6C, D

Nereis nigropunctata Horst, 1889: 171, pl. 8, figs 1–3.*Perinereis cultrifera*. – Martens et al. 1995: 15–16. Not Grube.*Perinereis nigropunctata*. – Hutchings et al. 1991: 256–257, fig. 10a–e.**Type locality.** Malaysia.

Material examined. 7 males (RCLA.Ann.008), 9 females (RCLA.Ann.009), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 18 March 2014; 19 males (RCLA.Ann.010), 17 females (RCLA.Ann.011), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 18 males (RCLA.Ann.012), 32 females (RCLA.Ann.013), Hutumuri, Ambon Island, Indonesia, 3°41'27.5"S, 128°17'56.3"E, coll. E. Moniharapon, 19 March 2014; 1 male (RCLA.Ann.014), 4 females (RCLA.Ann.015), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemia, 18 March 2014; 1 male (RCLA.Ann.016), 1 female (RCLA.Ann.017), Mahia, Ambon Island, Indonesia, 3°44'42.6"S, 128°11'24.6"E, coll. A.S. Leatemia, 19 March 2014; 1 male (RCLA.Ann.018), 4 females (RCLA.Ann.019), Alang, Ambon Island, Indonesia, 3°46'18.2"S, 128°00'24.6"E, coll. J. Pamungkas, 19 March 2014; 5 males (MZB.Pol.00165), 3 females (MZB.Pol.00166), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 1 ex. (NTM W23793), 1 female (NTM W23798), Alang, Ambon Island, Indonesia, 3°46'18.2"S, 128°00'24.6"E, coll. J. Pamungkas, 14 March 2009; 3 females (NTM W23801), Airlouw, Ambon Island, Indonesia, 3°46'32.5"S, 128°07'53.5"E, coll. F. E. de Soysa, 14 March 2009; 5 ex. be (specimen extracted from a jar of many hundred worms in the collection of Naturalis, Leiden) (RMNH unreg.), Ambon Island, Indonesia, coll. D.S. Hoedt, 1866.

Size range. Male: length (10–20 mm), maximum width (2.0–3.0 mm). Female: length (11–23 mm), maximum width (2.0–4.0 mm).

Diagnosis. *Perinereis* species having brown, streaky pigmentation on dorsal surface (Fig. 4C, D). Paragnaths conical arranged as follows: Areas I: 7–9; II: 15–21; III: 22–35 (including lateral groups); IV: 24–36; V: 3; VI: 1 bar (rarely 2 on one side); VII–VIII: 32–43. Male and female epitokes with similar body pigmentation and natatory region extending to pygidium, but differ in number of pre-natatory chaetigers and number modified dorsal and ventral cirri. Male has pre-natatory region comprising 14 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 7; basally swollen ventral cirri on chaetigers 1 to 5. Female has pre-natatory region comprising 17 chaetigers; basally swollen dorsal cirri on chaetigers 1 to 5; basally swollen ventral cirri on chaetigers 1 to 5. Also male has pygidial rosette and scalloped dorsal cirri, lacking in female.

Remarks. The specimens examined in this study agree well the description of Hutchings et al. (1991) of this species. This is the smaller of the two wawo *Perinereis*.

Distribution. Indo-west Pacific (widespread).

Solomononereis* Gibbs, 1971**Solomononereis merauensis* Gibbs, 1971**

Fig. 5D

Solomononereis merauensis Gibbs, 1971: 152–153, fig. 8a–h. – Hutchings and Reid 1991: 59–60.

Type locality. Solomon Islands.

Material examined. 1 female (MZB.Pol.00176), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014; 1 female (NTM W25887), Suli, Ambon Island, Indonesia, 3°37'38.2"S, 128°18'25.0"E, coll. R. Alik, 19 March 2014.

Size range. Male: not available. Female: length (20 mm), maximum width (3.0 mm).

Diagnosis. *Solomononereis* species lacking body pigmentation (Fig. 5D). Paragnaths elongate-conical (i.e., rods), arranged 8 discrete groups as follows: Areas I: 12; II: 11; III: 3 patches with 10 in each; IV: 15–20. Female epitokes with pre-natatory region, natatory region extending to mid body, followed by highly modified tail region bearing short segments and reduced parapodia. Female has pre-natatory region comprising 23–24 chaetigers; dorsal and ventral cirri on anterior chaetigers unmodified. Female eggs are green coloured. Male epitoke unknown.

Remarks. The present specimen fits the type description closely.

Distribution. Indonesia, Northern Australia, Solomon Islands.

Websterinereis* Pettibone, 1971**Websterinereis foli* (Fauvel, 1930)**

Leptonereis foli Fauvel, 1930: 520, fig. 3.

Websterinereis foli. – Pettibone 1971: 23–25, figs 10, 11.

Nicon sp. – Martens et al. 1995: 17, figs 20–24.

Type locality. Ile de Pins, New Caledonia.

Remarks. The specimens reported as *Nicon* sp. by Martens et al (1995) are almost certainly *Websterinereis foli*. We reached this conclusion based on a comparison of their figures of parapodia and chaetae with those of Pettibone (1971: figs 10, 11) and their description of the pigmentation pattern compared to that described for this species by Pettibone (1971: fig. 10a) and Glasby (2015: figs 42, 43). Martens et al. (1995) are likely to have overlooked the pharyngeal (oral ring) papillae in their specimens as they are very small and only observable when the pharynx is fully everted: this explains the incorrect generic assignment.

Distribution. Western Pacific, Indonesia, Lizard Island (Australia).

Key to reproductive Nereididae (wawo) of Ambon Island

The following key provides a means of identifying male and females of each species of Nereididae known to swarm at Ambon Island. It includes species reported both in the present study and in Martens et al. (1995).

- 1 Body with 2 regions (largely unmodified pre-natatory region and natatory region with modified parapodia and chaetae)..... **5**
- Body with 3 regions (pre-natatory and one or two distinct natatory regions)... **2**
- 2 Single natatory region, restricted to mid-body **3**
- Two natatory regions, extending to pygidium (mid-body region with modified parapodia and oval-shaped posterior region with extremely reduced parapodia) ***Solomononereis merauensis***
- 3 Pre-natatory region with up to 36 chaetigers..... **4**
- Pre-natatory region with 37–40 chaetigers; unpigmented (females) or head and anterior segments red-brown lines and spots (males) ***Websterinereis foli***
- 4 Pre-natatory region with 14 chaetigers (female); brown pigment restricted to head region ***Ceratonereis* sp. cf. *C. perkinsi***
- Pre-natatory region with 16–17 chaetigers (male and female); females with dark brown bands on chaetigers 2 and 3 ... ***Ceratonereis singularis australis***
- Pre-natatory region with 28–33 (male) or 34–36 (female) chaetigers; brown pigment restricted to head and pygidium..... ***Ceratonereis* sp. sensu Martens et al. 1995**
- 5 Dark brown band on dorsal surface of chaetiger 2 (fainter brown bands on subsequent chaetigers); anterior region with 16 (male) and 19–20 (female) unmodified chaetigers..... ***Neanthes unifasciata***
- Dark brown band on dorsal surface of chaetiger 4; anterior region with 21–22 (male) and 26 (female) unmodified chaetigers.. ***Neanthes* sp. cf. *N. gisserana***
- Body pigmentation not as above, or absent..... **6**
- 6 Neuropodia of natatory region with distinctive ramified lamellae ***Neanthes pachychaeta***
- Neuropodia of natatory region with smooth-edged lamellae **7**
- 7 Female epitokes with 17 or 18 pre-natatory region chaetigers **8**
- Female epitokes with 19 or 20 pre-natatory region chaetigers (male 17) ***Perinereis helleri***
- Female epitokes with about 26 pre-natatory region chaetigers (male 22–23) ***Nereis* sp. *Ambon_NTMW19037***
- Female epitokes with about 32 pre-natatory region chaetigers (male 28–30). ***Neanthes* cf. *masalacensis***
- 8 Male epitokes with about 14 pre-natatory region chaetigers (female 17) ***Perinereis nigropunctata***
- Male epitokes with 17 or 18 pre-natatory region chaetigers (female also 17–18)..... ***Composetia marmorata***

Discussion

Taxonomic utility of epitokal modifications. Sexual dimorphism among reproductive Nereididae is well known – it includes differences in the number of basally-swollen anterior dorsal and ventral cirri, the number of pre-natatory region chaetigers, and the presence of undersurface scalloping on the natatory dorsal cirri and pygidial rosettes in males only (Schroeder and Hermans 1975 and references therein). All are useful taxonomic features at the species level, but as noted by Read (2007) they are infrequently known. The present study adds to the knowledge of these species-specific epitokal modifications for a number of different species. In addition, we found that males as a rule appear to have an equal number, or fewer, pre-natatory chaetigers compared to females. Further, possibly there exists colour sexual dimorphism in *Ceratonereis singularis australis*, as the presence of dorsal banding was absent in the only confirmed male specimen examined, but present in all females examined.

Less well known is that Nereididae appears to exhibit epitokal modification patterns at the generic level. Combining data from this study with literature information on *Platynereis* (Horst 1924) and on *Websterinereis* (Martens et al. 1995), we can recognize three types of natatory region morphologies: natatory regions extending over the mid- and posterior body to the pygidium (*Composetia*, *Neanthes*, *Nereis*, *Perinereis*, *Platynereis*; Figs 3C, D, 4A–D, 5A–C, 6A–D); natatory regions restricted to the mid-body and followed by an unmodified region extending to the pygidium (*Ceratonereis*, *Websterinereis*; Fig. 3A); and natatory regions of two types (mid-body part with typically modified parapodia; and posterior oval-shaped part with extremely reduced parapodia extending to pygidium (*Solomononereis*; Fig. 5D). Further, anterior dorsal and ventral cirri may be modified, i.e., basally swollen (*Composetia*, *Neanthes*, *Nereis*, *Perinereis*, *Platynereis*; Fig. 4A, B) or unmodified (*Ceratonereis*, *Solomononereis*). Finally, the dorsal cirri in the natatory region in males may be scalloped (some *Neanthes*, *Nereis*, *Perinereis*, *Platynereis*; Fig. 6A); or smooth (*Ceratonereis*, *Composetia*, *Neanthes* cf. *gisserana*). On the last point, our observations indicate that *Neanthes* is polymorphic for dorsal cirri scalloping; Schroeder and Hermans (1975) also noted variability in scalloping among *Nereis* species. This is further indication that the large nereidid genera such as *Neanthes* and *Nereis* are non-monomorphic, as suggested by Bakken and Wilson (2005).

Finally, some caution must be attributed to the generality of these patterns of epitokal modifications as possibly they are dependent on the state of sexual maturity of the individual and, of course, they are based on observations of very few species per genus.

How many species? The number of species recognised as comprising wawo in Maluku is increasing as we collect more intensively and overcome taxonomic problems. In the first publication on wawo, Rumphius (1705) considered that wawo was a single species, which he called Vermiculi Marini; however, it is clear that he recognised several different forms under this name. As this was more than a century before any formalised evolutionary thought, presumably he attributed the variation to different forms of a creature specially created. As the name Vermiculi marini was suggested

about 50 years before Linnaeus's, binomial taxonomy system was in place, it is not accepted by the International Code of Zoological Nomenclature.

Rumphius (1999) describes one form that resembles a millipede:

... about the thickness of an oaten pipe, quite like young Millipedes, of a mixed green, brown, and white, and look indeed somewhat disgusting, but these have a special name, and are not considered the true Wawo.

And further, that the millipede form:

... illumines at night, giving off a clear light, which makes people avoid them even more, since they share this attribute with the Millipedes.

The millipede form may represent the shorter-bodied nereidids with their prominent parapodia; however, luminescence has, as far as we know, not been reported previously in swarming members of this family, so doubt must remain over the identity of Rumphius's, 'millipedes'. The more abundant true wawo referred to by Rumphius (1999) as 'like silken Floss, all entangled in small clumps' are most likely *Palola viridis* and possibly other eunicid species (see Pamungkas 2015).

Horst (1904; 1905) also claimed that wawo is a single species, namely *Lysidice oele* (Eunicidae) based on collections from Banda waters during the Dutch Siboga Expedition (1899–1900). However, by the end of the twentieth century, Martens et al. (1995) had clarified the multispecies nature of wawo. He recognised 13 different species (five families) from Ambon Island waters with the eunicid *Palola viridis* as the dominant species; 7 out of 13 species of wawo were nereidids. Three of these species were the same as reported in this study, viz. *Ceratonereis singularis australis*, *Neanthes unifasciata* and *Perinereis nigropunctata* (Table 2). In the present study, six species of Nereididae were identified in the collections of Hoedt and nine species in the recent collections (Table 2; also Pamungkas (2015)). The Hoedt nereidids are all now in one jar (Fig. 2), so we do not know for sure whether they were collected from more than one site and/or on more than one occasion. However, assuming samples from all different periods each represented a single spawning event, the differences in species diversity between the three periods – 1866 (6 species), 1995 (7 species), 2009 and 2014 (9 species) – is entirely consistent with spatial or short term temporal variation and does probably not indicate long term change.

Finally, it is worth noting that wawo have been reported to swarm in islands nearby Ambon, such as Haruku and Nusalaut (JP pers. obs.) and therefore are probably widespread in Maluku Province. In the Banda Islands (south-east of Ambon Island), the natives call them 'oele'. Further, studies of Nereididae by Horst (1889, 1924) from the 'Malay Archipelago' (includes present-day eastern Indonesia, Malaysia, Brunei, Timor Leste) yielded 44 nereidid species, many of which were reproductive; four species were the same as found in our recent collections (i.e., *Ceratonereis singularis australis*, *Compositia marmorata*, *Nereis gisserana* and *Perinereis nigropunctata*), with the latter two having widespread distributions throughout the western Coral Triangle (the actual

Table 2. Comparison of swarming nereidid species collected by Hoedt (1866), reported by Martens et al. (1995), and found in this study (2009/2014). See discussion for explanation of taxonomy.

Species	1866	1995	2009/2014
<i>Ceratonereis</i> sp. cf. <i>C. perkinsi</i>	Absent	Present	Absent
<i>Ceratonereis singularis australis</i>	Present	Present	Present
<i>Ceratonereis</i> sp. <i>Martens et al.</i>	No	Present	Absent
<i>Compositia marmorata</i>	Absent	Absent	Present
<i>Neanthes</i> sp. cf. <i>N. gisserana</i>	Present	Absent	Present
<i>Neanthes</i> sp. cf. <i>N. masalacensis</i>	Absent	Absent	Present
<i>Neanthes pachychaeta</i>	Present	Absent	Absent
<i>Neanthes unifasciata</i>	Present	Present	Present
<i>Nereis</i> sp. <i>Ambon_NTMW19037</i>	Absent	Absent	Present
<i>Perinereis helleri</i>	Present	Present	Present
<i>Perinereis nigropunctata</i>	Present	Present	Present
<i>Solomononereis merauensis</i>	Absent	Absent	Present
<i>Websterinereis foli</i> (= <i>Nicon</i> sp. sensu Martens et al (1995))	Absent	Present	Absent

number of species widespread in the region is likely to be much higher). Therefore, it is highly likely that polychaete swarming events throughout the western Coral Triangle region will be found to contain not only *Palola viridis* and other eunicids, but a range of nereidid species, including those found in this study and other species.

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Two new species of Asellota (Crustacea, Isopoda) from coral reefs on Iriomote Island, Okinawa, Japan

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Abstract

Pleurocope iriomotensis sp. n. and *Prethura tuberculata* sp. n. are described from Iriomote Island, Ryukyu Archipelago, southern Japan. These are the first records of *Pleurocope* from the Pacific and of *Prethura* from the Asian Pacific coast. *Pleurocope iriomotensis* differs from its congeners in having lateral spine-like processes on pereonite 4 and coxal plates of pereonite 7. *Prethura tuberculata* can be distinguished from its single congener in having a lateral short projection of protopod of pleopod 2.

Keywords

Isopoda, Pleurocopidae, *Pleurocope*, Santiidae, *Prethura*, Japan

Introduction

Iriomote Island (Fig. 1) in the southwestern part of the Ryukyu Archipelago is located in a subtropical climate zone for terrestrial environments, but its marine fauna is more tropical, being strongly influenced by Kuroshio, a prominent warm ocean current. The island is surrounded by coral reefs and a variety of mangroves, sandy or rocky shores.

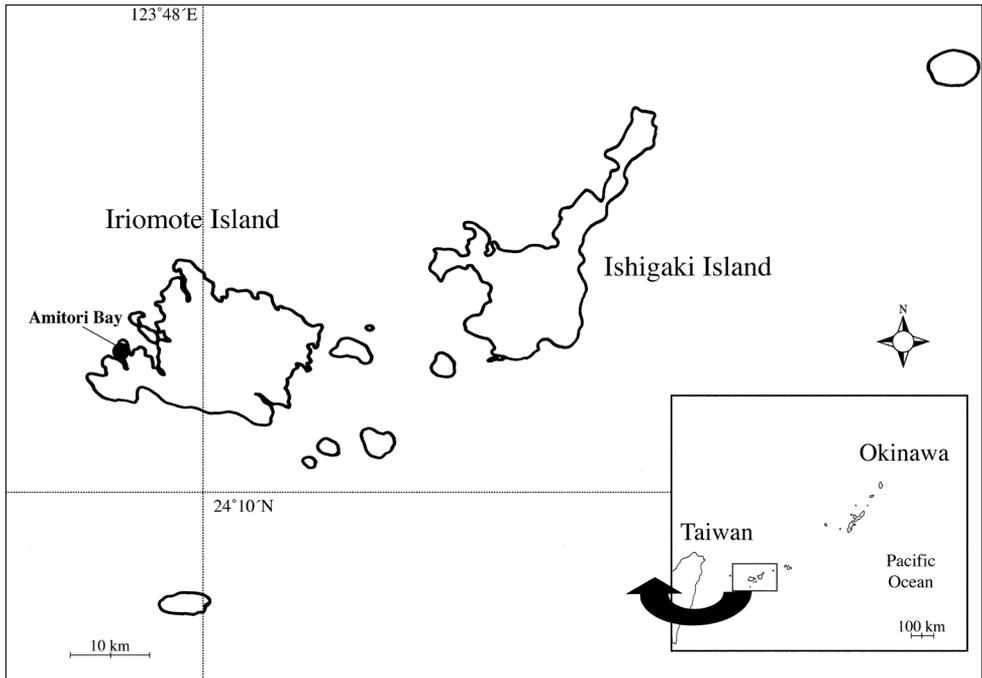


Figure 1. Map showing collection site (solid circle).

The marine isopod fauna of the Ryukyus has been studied by several authors including the families Anthuridae Leach, 1814, Bopyridae Rafinesque, 1815, Cymothoidae Leach, 1818, and Gnathiidae Leach, 1814 (e.g., Nunomura 1992; Ota 2012; Shiino 1939; Williams and Williams 1986). With regards to marine asellote isopods around the Ryukyus, only the deep water species *Mastigoniscus microcephalus* (Gamô, 1989), *Munnopsis megacephalus* Shimomura & Ohtsuka, 2005 and *Heterosignum elegans* Shimomura & Mawatari, 2002 have been recorded (Gamô 1989; Shimomura and Ohtsuka 2005). The shallow-water asellote isopods have remained uninvestigated; hence we have been participating in the faunal survey of the shallow-water asellote isopods of Iriomote Island.

The shallow-water crustacean faunal survey of Amitori Bay, Iriomote Island in 2012 by SCUBA, yielded two species of asellote isopods of the families Pleurocopidae and Santiidae. Based on this material, two new species, *Pleurocope iriomotensis* sp. n. and *Prethura tuberculata* sp. n. are described and illustrated.

Material and methods

Collections of isopods were obtained by the Collaborative Research Program funded by the Ryukyu University in Iriomote Island in 2012. Pieces of coral rubble collected

by hand near the coral reefs during SCUBA were washed in a bucket, and isopods were extracted by decanting the suspension through a sieve with a mesh size of 0.3 mm. All specimens obtained were fixed and preserved in 80% ethanol. Each individual was dissected and prepared for observation by a light microscope (Nikon E600). The total length as indicated in "Material examined" was measured from the tip of the head to the end of the pleotelson.

The type specimens are deposited in the Kitakyushu Museum of Natural History and Human History (KMNH).

Systematics

Family Pleurocopidae Fresi & Schiecke, 1972

Pleurocope Walker, 1901

Pleurocope Walker, 1901: 297; Kensley and Schotte 2002: 1435.

Type species. *Pleurocope dasyura* Walker, 1901 (by original designation and monotypy).

Species included. *P. dasyura* Walker, 1901, Mediterranean; *P. floridensis* Hooker, 1985, Gulf of Mexico; *P. wilsoni* Kensley & Schotte, 2002, Thailand and Seychelles; *P. iriomotensis* **sp. n.**, Iriomote Island, Ryukyu Islands (present study).

Diagnosis (modified from Kensley and Schotte 2002). Antennular flagellum composed of 4 articles. Antennal flagellum composed of 6–7 articles. Mandible without palp. Maxillula medial lobe rudimentary, without setae; lateral lobe with 7–9 setae apically. Maxilla lateral 2 lobes each with 2 setae apically. Some pereonites with spine-like process laterally, often bearing 1–2 stout setae. Pereopod 1 subchelate. Pereopods 2–7 with single claw. Coxal plates of pereopods 5–7 visible in dorsal view. Some coxal plates with spine-like process laterally, with 1–3 stout setae. Uropods composed of protopod, endopod and exopod, inserted dorsolaterally on proximal pleon. Pleotelson tapering posteriorly to acute apex. Male pleopod 1 tapering posteriorly, with some short setae on apex. Male pleopod 2: protopod narrow, lanceolate, with single long seta on apex; second article reaching or surpassing apex of protopod.

Remarks. Walker (1901) established the genus for his new species *P. dasyura* from a depth of 18 m at the Mediterranean. A second species, *P. floridensis*, was described by Hooker (1985) from a depth of 30 m at the Florida Middlegrounds in the northeastern Gulf of Mexico. Kensley and Schotte (2002) redefined the genus with the description of the new species of *P. wilsoni* from a depth of 77 m off Phuket Island, Thailand, and a depth of 6–16 m at Picard Island, Aldabra Atoll.

The main changes in this new diagnosis are to accommodate the number of seta on maxillae.

***Pleurocope iriomotensis* sp. n.**

<http://zoobank.org/D687B687-10AE-491F-94CA-63C99CF6A3C3>

Figs 2–4

Material examined. Holotype. ♂ (0.9 mm), 24°20'N, 123°41'E, Amitori Bay, Okinawa, Japan, 19 July 2012, dead coral, 25 m, coll. TN (KMNH IvR 500,729).

Paratypes. 3♂♂ (0.5–0.9 mm), Amitori Bay, Okinawa, Japan, 19 July 2012, dead coral, 4 m, coll. MS (KMNH IvR 500,730–500,732).

Description of the holotype. *Body* (Fig. 2) 1.6 times as long as maximum width (including spine-like processes), widest at pereonite 3, with many small granules on dorsum, without dorsal setae. Head (including eyestalks) 2.4 times as broad as long, head without eyestalks 1.2 times as broad as long, broader than pereonite 1; frontal and posterior margins of head convex. Eyes each with 2 ommatidia; eyestalks slender, long, reaching near distal end of article 1 of antenna 1. Pereonites 1 and 5–7 laterally rounded, without lateral spine-like processes; pereonites 2 and 3 with pair of long lateral spine-like processes bearing 2 or 3 robust setae; lateral spine-like processes of pereonites 2 and 3 reaching to tip of second article of antennula in length; pereonite 4 with pair of short lateral spine-like processes bearing single robust seta and 2 short teeth distally; lateral spine-like processes of pereonite 4 half as long as ones of pereonite 3. Pereonite 1 shortest; pereonite 2 three times as long as pereonite 1; pereonites 3 and 4 longest, subequal in length; pereonite 5 0.7 times as long as pereonite 4, pereonites 5–7 subequal in length. Pereonites 1 to 3 increasing in width; pereonite 4 narrower than pereonites 3; pereonites 4 to 7 decreasing in width. Coxal plates dorsally visible on pereonites 5–7, laterally with spine-like process and 2 robust setae. Pleotelson (Fig. 2) approx. 2.2 times as long as wide, widest at anterior one seventh, tapering to sharply rounded apex, with 12 robust setae distally.

Antennula (Fig. 3A) consisting of 6 articles. Article 1 longest and broadest, twice broader-like than eyestalks, with 1 long and 1 short simple setae laterodistally; article 2 nearly 0.8 times as long as article 1, distally with 1 short and 2 long, stout simple setae and 1 broom seta; article 3 about half as long as article 2, without setae; article 4 approx. 0.6 times as long as article 3, without setae; article 5 approx. 1.8 times as long as article 4, with 1 aesthetasc distally; article 6 shortest, with 1 long simple seta and 1 aesthetasc apically.

Antenna (Fig. 3B): peduncle consisting of 4 short and 2 long articles, and flagellum of 7 short articles. Articles 1 and 2 subquadrate, without setae; article 3 with a simple seta laterodistally and 4 simple setae on lateral protrusion; article 4 as long as articles 1 and 2 combined, without setae; article 5 longer than article 4, with single simple seta medially and laterally; article 6 as long as article 5, with 2 simple setae distomedially and 2 broom-like setae laterodistally; flagellum as long as peduncular articles 4–6 together, flagellar articles 1–6 each with 0, 1, 0, 2, 2 and 0 simple setae distally; flagellar article 7 with 2 simple setae and 1 aesthetasc.

Maxillula (Fig. 3E) with medial lobe short, lacking setae; lateral lobe with 7 setae distally. *Maxilla* (Fig. 3F) with medial lobe bearing some fine and 2 stout setae on margin; lateral 2 lobes each with 2 stout setae apically.

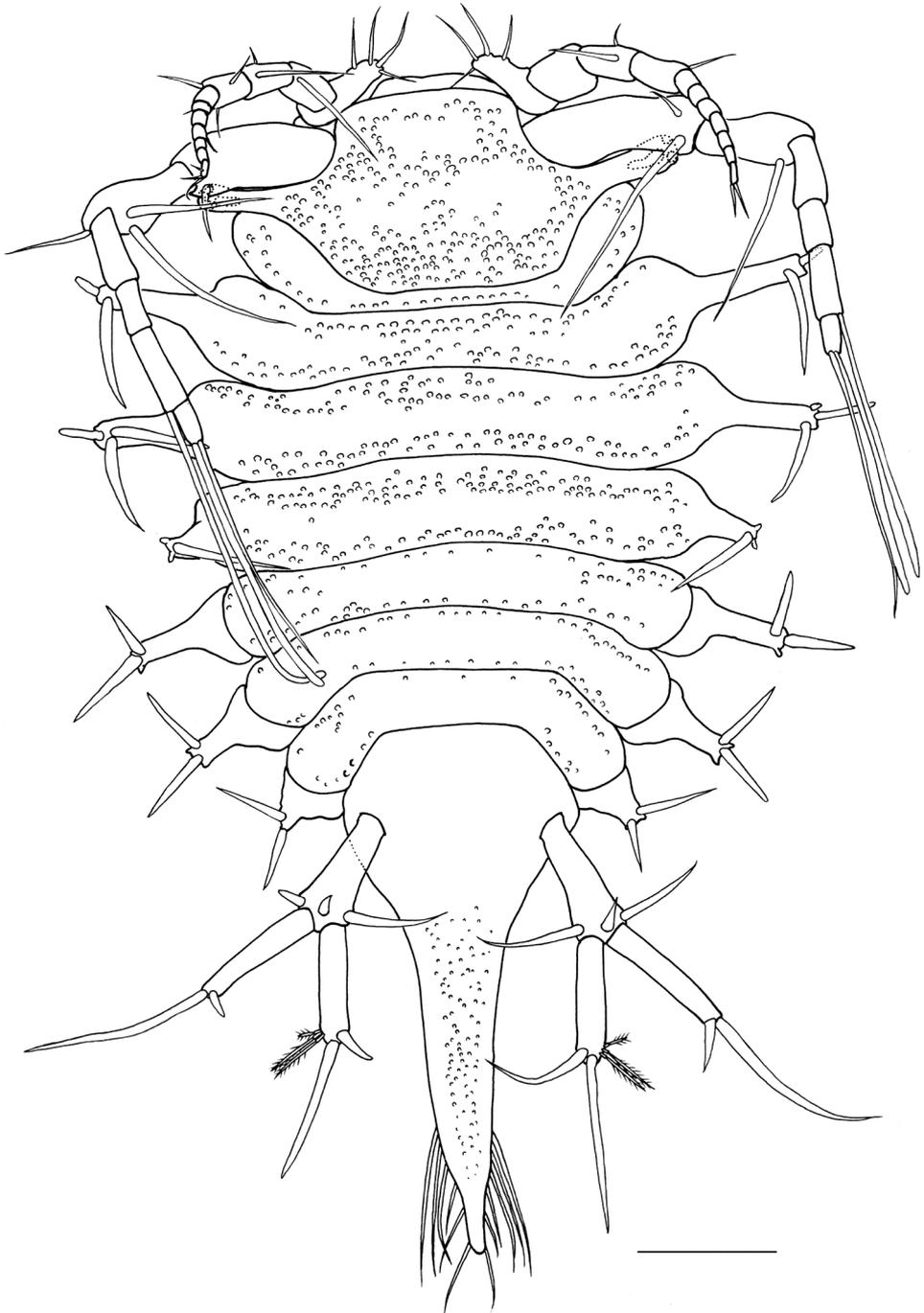


Figure 2. *Pleurocope iriomotensis* sp. n., holotype male, habitus, dorsal. Scale bar: 100 μ m.

Maxilliped (Fig. 3G) palp slender, twice as long as basis: article 1 shortest, without setae; articles 2 to 4 subequal in length, each with single distal seta; article 5 narrowest, with a short seta medially and 2 stout setae apically; basis quadrate with broad endite bearing 2 simple setae distally and 1 coupling hook medially; epipod crenulated laterally, 2.6 times as long as width, 1.5 times as long as basis.

Pereopod 1 (Fig. 3H): basis 0.8 as long as ischium, with 1 dorsal seta; ischium the longest article, with short tooth and 2 long setae dorsally; merus pentagonal, with a ventral and a dorsal seta; carpus trapezoidal, as long as merus, with distoventral tapering projection terminating with 1 stout seta and 1 slender setae ventrally; propodus ovate, 2.3 times as long as width, with 1 proximoventral and 1 distodorsal small setae; dactylus as long as propodus, with 1 triangulate tooth and 1 short seta ventrally and 2 setae subapically, unguis half as long as dactylus and 1 slender seta apically.

Pereopod 2 (Fig. 3I) shorter than pereopods 3–7; basis with 1 distoventral seta; ischium 2.1 times as long as basis, dorsally with 2 robust setae; merus shorter than half length of ischium, with 2 simple setae ventrally and 1 robust seta dorsally; carpus longer than merus, with 2 robust setae ventrally and 1 simple seta dorsodistally; propodus as long as ischium, ventrally with 3 robust unequally bifid setae and 1 fringed scale on distal third, and with 2 simple and 1 robust setae dorsally; dactylus slender, 3.1 times as long as width, with 1 simple seta, 1 stout unguis and 1 accessory claw apically, and with 2 simple setae subapically. *Pereopods 3–7* (Figs 3J–K, 4A–C) subequal in shape and slightly increasing in length posteriorly; bases with 1 ventrodorsal seta; ischia longer than bases, with 1–2 simple ventrally and 1–2 robust setae dorsally; meri less shorter than half length of ischia, with 2 setae ventrally and 1–2 robust setae dorsally; carpi longer than meri, with 1–2 simple and 2 robust setae ventrally and 0–1 simple and 0–1 robust seta dorsally; propodi longer than ischia, with 3 robust setae ventrally and 2–3 robust and 1 broom-like seta dorsally; dactyli with 2 simple setae, 1 stout unguis and 1 short accessory claw apically and 2 simple setae subapically.

Pleopod 1 (Fig. 4D) about 3.4 times as long as maximum width, tapering to apex, apically with 3 pairs of short setae. *Pleopod 2* (Fig. 4E, F): protopod 3 times as long as wide, bearing long seta apically; apical seta 1.2 times as long as protopod; endopod surpassing tip of protopod, with slender, long second article; second article 1.2 times as long as protopod; exopod narrower than endopod first article. *Pleopod 3* (Fig. 4G): endopod 4.7 times as long as width, bearing 3 stout plumose setae distally; exopod 0.9 times as long as endopod, bearing 2 simple setae apically.

Uropod (Fig. 2) half as long as pleotelson. Protopod wide posteriorly, with 3 robust setae distally; exopod as long as protopod, with 2 robust setae distally; endopod slightly shorter than exopod, with 3 broom-like and 2 robust setae distally.

Description of the paratypes. *Body* (Fig. 4H) flattened, without spines and projections on dorsum. *Left mandible* (Fig. 3C) incisor 4 conical, directed anteriorly, without setae and teeth; lacinia mobilis with 3 teeth. *Right mandible* (Fig. 3D) incisor with 2 teeth apically, without setae.

Female. Not known.

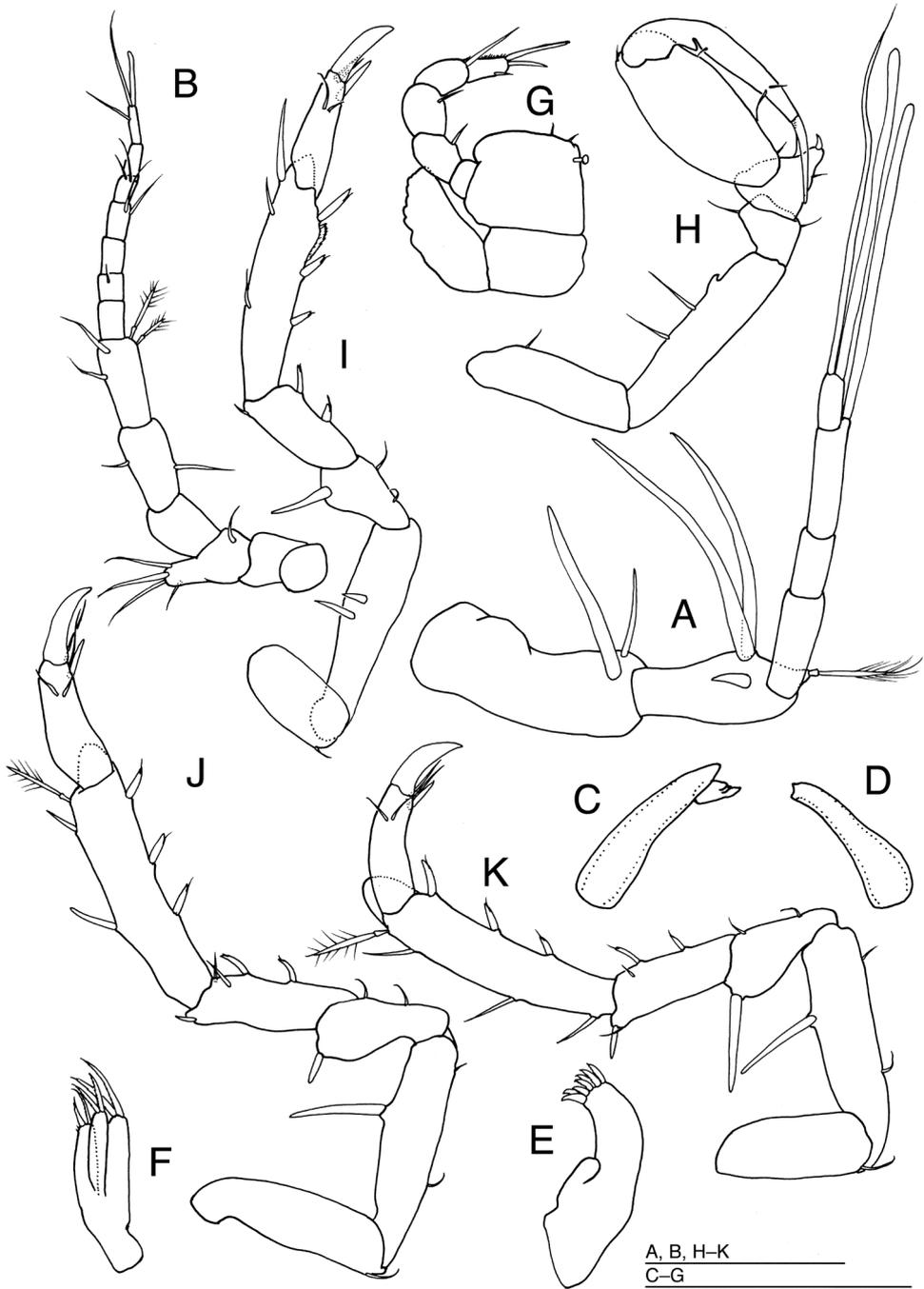


Figure 3. *Pleurocope iriomotensis* sp. n. **A–B, E–K** holotype male **C–D** paratype male (KMNH IvR 500,732): **A** left antennula, dorsal **B** left antenna, ventral **C** left mandible, dorsal **D** right mandible, dorsal **E** left maxillula, ventral **F** left maxilla, ventral **G** left maxilliped, dorsal **H** left pereopod 1, medial; **I** left pereopod 2, medial **J** left pereopod 3, medial **K** left pereopod 4, medial. Scale bars: 100 µm.

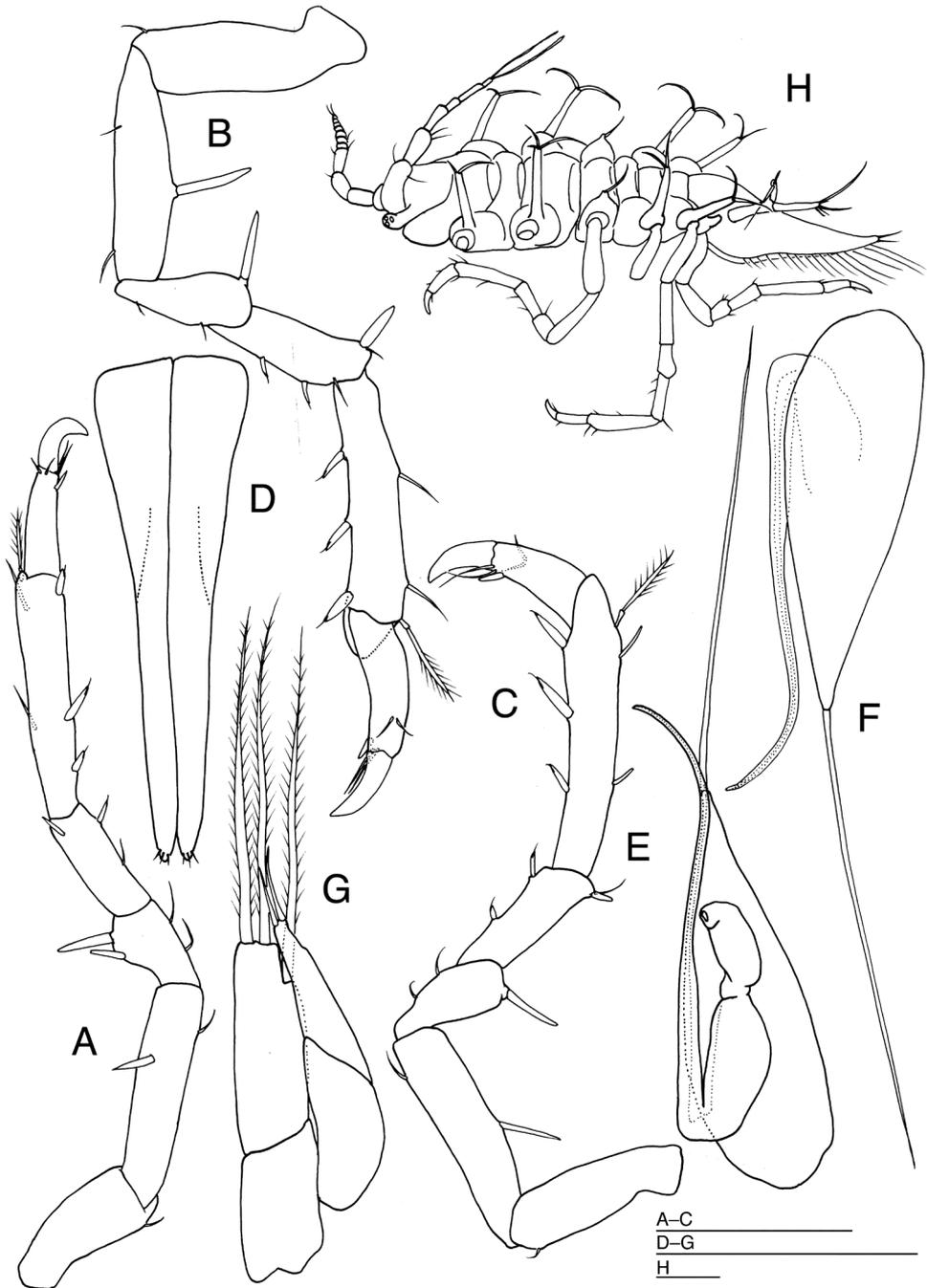


Figure 4. *Pleurocope iriomotensis* sp. n. **A–G** holotype male **H** paratype male (KMNH IvR 500,731): **A** left pereopod 5, medial **B** left pereopod 6, medial **C** left pereopod 7, lateral **D** pleopod 1, ventral **E** left pleopod 2, dorsal **F** left pleopod 2, ventral **G** left pleopod 3, dorsal **H** habitus, lateral. Scale bars: 100 μ m.

Remarks. *Pleurocope iriomotensis* sp. n. can be identified by the following combination of characters: pereonite 1 lacking lateral spine-like processes; pereonites 2–4 and coxal plates of pereonites 5–7 each with lateral spine-like process; pleotelson twice as long longer as wide; flagellum of antenna consisting of 7 articles. Pereonite 1 without a spine-like process links the new species to *Pleurocope floridensis* Hooker, 1985, from the Gulf of Mexico and to *P. dasyura* Walker, 1901, from the Gulf of Naples, Italy. *Pleurocope iriomotensis* is distinguished from *P. floridensis* by the following characters (those of *P. floridensis* in parentheses): pereonite 4 and coxal plate of pereonite 7 with spine-like process (without spine-like process); eyestalks not reaching to second article of antennula (surpassing second article of antennula); fifth article of antennula with 1 aesthetasc (with 2 aesthetascs); ischium of pereopod 1 with dorsal projection (without dorsal projection); pleopod 1 apically with 3 pairs of short setae (apically with 5 pairs of short setae). *Pleurocope dasyura* differs from the new species in having pereonite 4 and coxal plate of pereonite 7 without spine-like processes, pereon with 6 long dorsal setae, antenna with long projection, and pleopod 1 apically with 4 pairs of short setae.

Etymology. The species is named after the type locality.

Key to the species of *Pleurocope*

- | | | |
|---|---|-------------------------------|
| 1 | Lateral spine-like process on pereonite 4 and coxal plate of pereonite 7 absent..... | 2 |
| – | Lateral spine-like process on pereonite 4 and coxal plate of pereonite 7 present..... | <i>P. iriomotensis</i> sp. n. |
| 2 | Lateral spine-like process on pereonite 1 absent; pleotelson twice longer than width..... | 3 |
| – | Lateral spine-like process on pereonite 1 present; pleotelson twice as long as width..... | <i>P. wilsoni</i> |
| 3 | Lateral seta on pereonite 4 absent; dorsal short tooth of ischium of pereopod 1 absent..... | <i>P. floridensis</i> |
| – | Lateral seta on pereonite 4 present; dorsal short tooth of ischium of pereopod 1 present..... | <i>P. dasyura</i> |

Family Santiidae Wilson, 1987

Prethura Kensley, 1982

Prethura Kensley, 1982: 255; Wolff 1989: 181.

Type species. *Prethura hutchingsae* Kensley, 1982; by original designation and monotypy.

Species included, *P. hutchingsae* Kensley, 1982, Great Barrier Reef, Australia; *P. tuberculata* sp. n., Iriomote Island, Ryukyu Islands (present study).

Diagnosis (modified from Wolff 1989). Pleotelson subtriangular. Eyes well-developed, on short peduncles. Mandibular palp lacking. Coxal plates of pereonites 5 visible dorsally. Male pleopod 1 distally twisted, apically acute. Male pleopod 2 protopod enormously expanded. Uropods pedunculate, protopod enlarged, inserted ventrolaterally; medial ramus short. Female operculum pyriform.

Remarks. The generic diagnosis is slightly modified from that of Wolff (1989). The significant change in this new diagnosis is to accommodate the presence of well-developed eyes on short peduncles on the new species described below.

***Prethura tuberculata* sp. n.**

<http://zoobank.org/D79017CF-FDB4-4DB2-8643-968E2EA7E87E>

Figs 5–6

Material examined. Holotype. ♂ (0.9 mm), 24°20'N, 123°41'E, Amitori Bay, Okinawa, Japan, 19 July 2012, dead coral, 4 m, coll. MS (KMNH IvR 500,733).

Description of the holotype. *Body* (Fig. 5A) 2.6 times as long as maximum width, widest at head, with many small black chromatophores. Head 2.3 times as broad as long, broader than pereon, with pair of rounded protuberances dorsally; frontal margin concave; posterior margin slightly convex. Eyes each with 43 ommatidia. Pereonites 1 to 3 increasing in length posteriorly, each with 3 rounded protuberances dorsally; pereonite 4 longer than pereonite 3, without protuberances; pereonite 5 shortest; pereonite 6 slightly longer than pereonite 5; pereonite 7 as long as pereonite 6. Pereonites 1 to 3 increasing in width; pereonite 4 narrower than pereonite 3; pereonite 5 wider than pereonite 4; pereonite 6 narrower than pereonite 5; pereonite 7 narrowest. Pleonite half as long as pereonite 7, without pigmentations and setae dorsally. Pleotelson (Fig. 5A, E) 1.7 times as long as wide, with 3 pairs of short setae laterally and 4 pairs of short setae marginally.

Antennula (Fig. 5B) consisting of 5 articles. Article 1 with many granules, without setae; article 2 broadest, as long as article 1, with many granules, and with 1 simple seta and 1 broom-like seta distomedially and 1 broom-like seta distolaterally; article 3 and 4 subequal in length, without setae; article 5 longest, approx. 3.3 times as long as article 4, with 3 simple setae and 1 aesthetasc apically.

Antenna (Fig. 5C): articles 1 and 2 without setae; article 3 narrower than article 2, with 2 short setae distally; articles 4–6 and flagellar articles broken.

Left mandible (Fig. 6A) incisor with 4 teeth, 4-toothed lacinia mobilis and 3 serrated setae; molar process cylindrical, with one large and four short teeth, and 1 seta distally. *Right mandible* (Fig. 6B) incisor with 4 teeth and 4 serrate setae; molar process cylindrical, with 1 seta distally.

Maxillula (Fig. 6C): medial lobe with 5 setae apically and some fine setae medially; lateral lobe with 12 setae apically and some fine setae laterally. *Maxilla* (Fig. 6D) with medial lobe bearing some fine and 7 stout setae medially; two lateral lobes each with 4 stout setae apically.

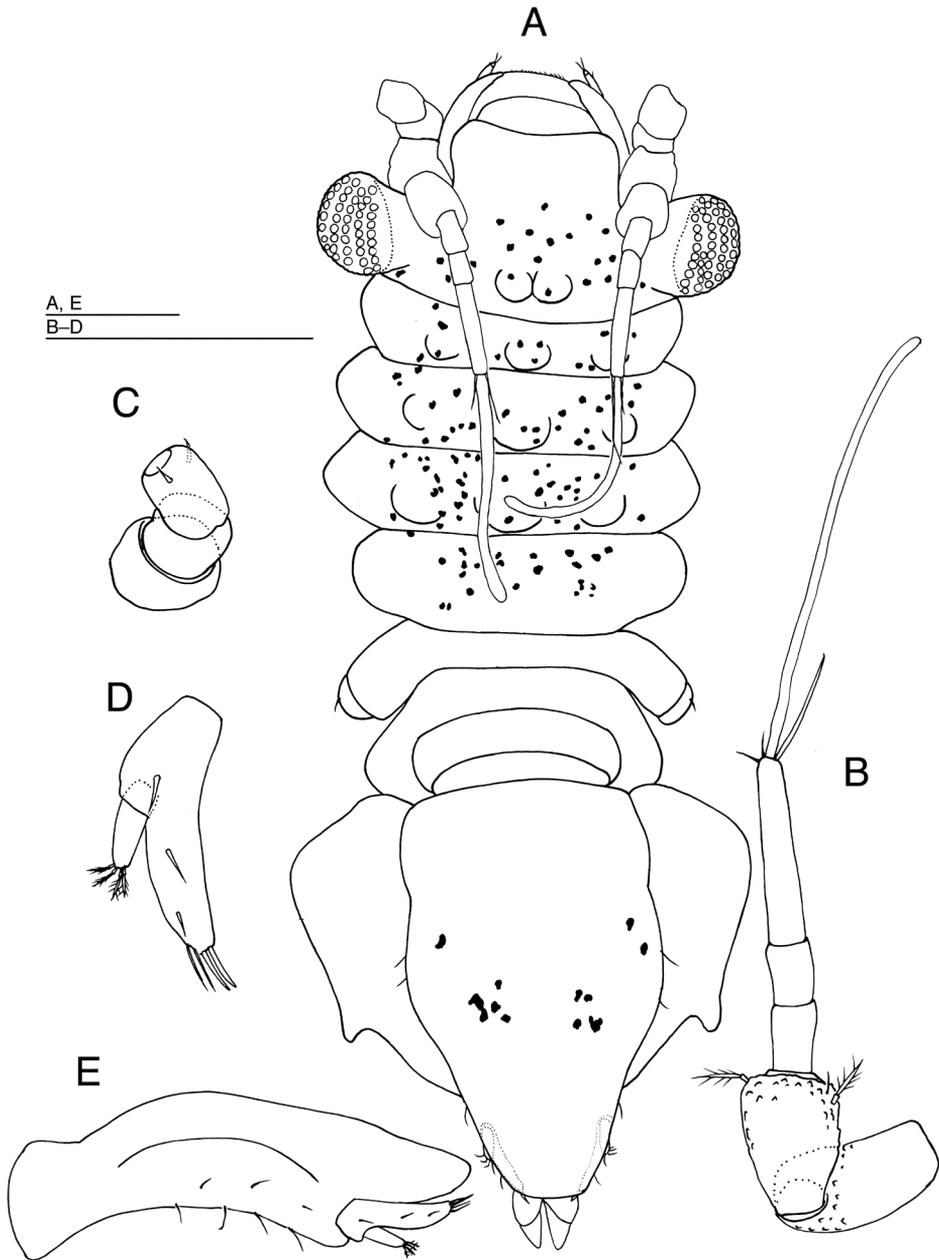


Figure 5. *Prethura tuberculata* sp. n. **A–E** holotype male: **A** habitus, dorsal **B** right antenna, dorsal **C** articles 1–3 of antenna 2, dorsal **D** left uropod, lateral **E** pleotelson and uropod, lateral. Scales = 100 μ m.

Maxilliped (Fig. 6E) palp slender, approx. 0.8 times as long as basis: article 1 without setae; articles 2 about twice as long as article 1, with 1 medial seta; article 3 longer than half of article 2, with 1 seta medially and laterally; article 4 longest, with small

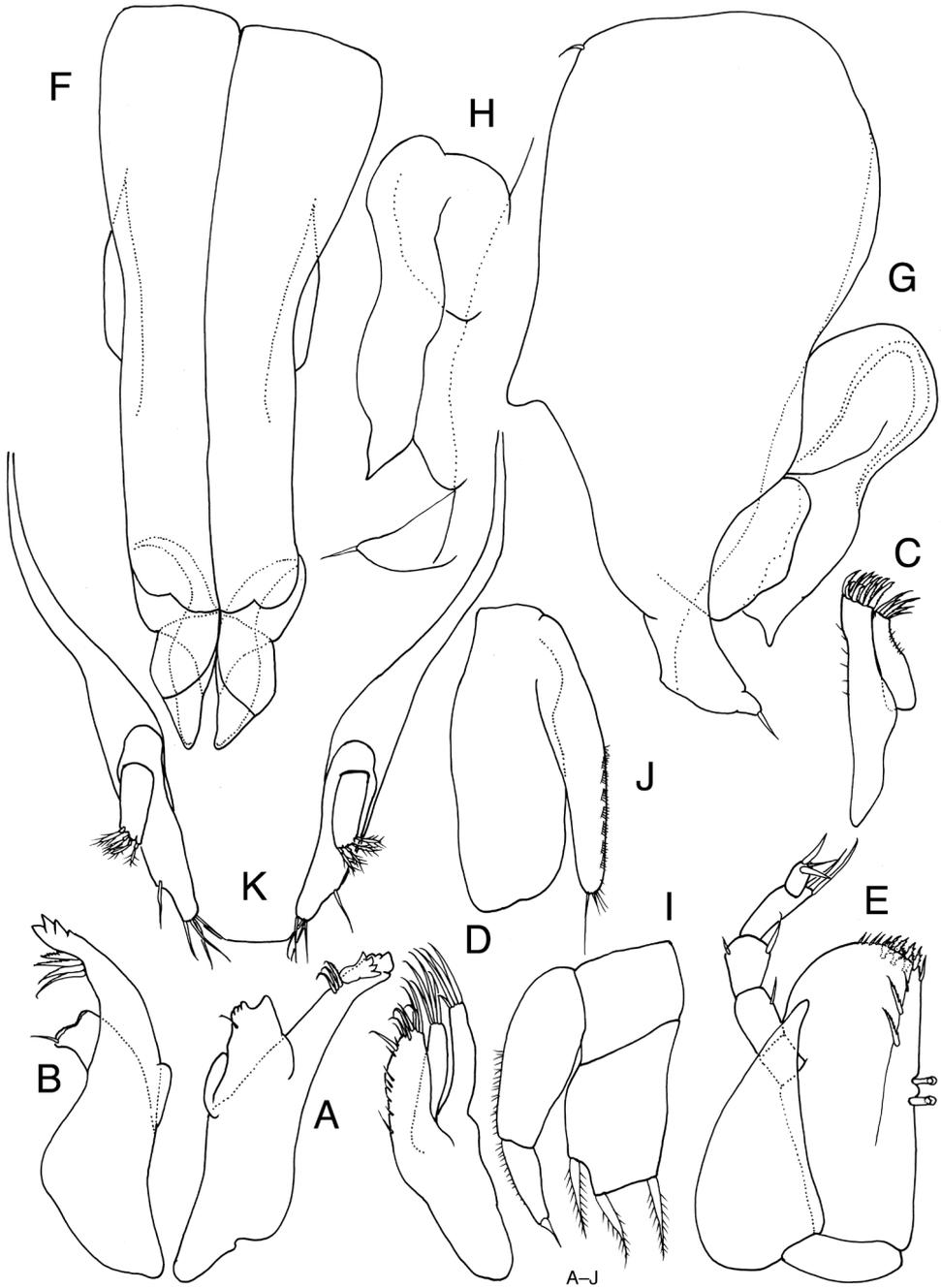


Figure 6. *Prethura tuberculata* sp. n. **A–K** holotype male: **A** left mandible, medial **B** right mandible, dorsal **C** left maxillula, dorsal **D** left maxilla, ventral **E** left maxilliped, dorsal **F** pleopod 1, ventral **G** right pleopod 2, ventral **H** endopod of right pleopod, dorsal **I** left pleopod 3, dorsal **J** left pleopod 4, ventral **K** pleotelson and uropods, ventral. Scale bar: 100 μ m.

distomedial lobe bearing 2 long setae; article 5 shortest, with 3 apical setae. Basis quadrate, bearing one simple and two pectinate setae on dorsal ridge, some simple and irregular shaped setae distally, 3 fan-shaped setae submarginally, and 2 coupling hooks medially. Epipod lanceolate, with acute apex. Pereopods broken.

Pleopod 1 (Fig. 6F) about 2.5 times as long as maximum width, without setae. *Pleopod 2* (Fig. 6G, H) with broad protopod bearing simple seta apically; protopod with short projection and simple seta laterally; endopod broad, with stout, short stylet; exopod stout. *Pleopod 3* (Fig. 6I) protopod as long as width, shorter than endopod; endopod 1.6 times as long as width, with 3 short, plumose setae distally; exopod 0.8 times as broad as endopod, bearing 1 simple seta apically and many fine setae laterally. *Pleopod 4* (Fig. 6J) endopod ovate, 2.7 times as long as broad; exopod its tip not surpassing tip of endopod, distally with 1 long slender and some short setae, and laterally with 7 spinulose scales. *Pleopod 5* broken.

Uropod (Figs 5D, 6K) 0.2 times as long as pleotelson. Protopod widest at anterior part, narrow posteriorly, with 4 simple setae distally and 3 simple setae laterally; endopod 0.3 times as long as protopod, cylindrical, with 4 broom-like setae apically.

Remarks. This new species differs from the only congener, *P. hutchingsae* Kensley, 1982, from the Great Barrier Reef, by the following characters (those of *P. hutchingsae* in parentheses): pleopod 1 lacking any setae (with setae); protopod of pleopod 2 with lateral projection (without projection); epipod of maxilliped with acute apex (blunt apex).

Etymology. From the Latin “*tuberculatus*”, referring to the lateral projection of the protopod of pleopod 1.

Acknowledgements

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DNA barcoding of euryglossine bees and the description of new species of *Euhesma* Michener (Hymenoptera, Colletidae, Euryglossinae)

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Abstract

This paper launches an open access DNA barcoding project “AUSBS” under the Barcoding of Life Datasystems (BOLD). The aims of the project are to help scientists who lack the necessary morphological knowledge to identify known species using molecular markers, to aid native bee specialists with the recognition of species groups that morphologically are difficult to define, and, eventually, to assist with the recognition of new species among known species. Using integrative taxonomy, i.e. morphological comparison to type specimens in Australian museum collections combined with phylogenetic analysis of a fragment of the mitochondrial DNA cytochrome *c* oxidase subunit I (mtCOI) gene sequences led to the recognition of four new species of *Euhesma* Michener (Hymenoptera: Colletidae: Euryglossini) collected during intensive surveys in remote Australian conservation areas, which are described. The new species are *E. micans*, *E. lyngouriae*, and *E. aulacá* in a species group associated with *Eremophila* flowers, and *E. albamala* in the *walkeriana* species group.

Keywords

Barcoding, species discovery, euryglossine bees, Bush Blitz survey, conservation

Introduction

Australia is facing a dramatic and unprecedented loss of biodiversity (Ritchie et al. 2013). However the main substance for this contention is derived from data on native vegetation (Bradshaw 2012) and vertebrates (Department of the Environment 2009). Despite the numerical dominance of invertebrates, their fundamental importance in natural ecosystems and their services to agricultural production (Tscharrntke et al. 2012), changes in their biodiversity are not well documented in Australia. This lack of information about the conservation status of Australian invertebrates is at least partly caused by a lack of knowledge of taxonomy, distribution and population dynamics of Australian invertebrate fauna (New and Sands 2004). What is not known cannot be monitored.

Australian native bees serve as a case in point. Despite their environmental and economic importance as pollinators of native plants (e.g. Houston et al. 1993), and as ecosystem services providers for crop pollination worldwide (Garibaldi et al. 2013) and in Australia (Keogh et al. 2010), only an estimated two-thirds of the Australian bee species are as yet known to science (Batley and Hogendoorn 2009). Opportunities to ameliorate this situation are constrained by a shortage of funding and career prospects for taxonomists. Hence, species may become extinct before they have been recognized.

To make native bees more accessible to the scientific community, an open access project “AUSBS” has been initiated in the Barcoding of Life Datasystems (BOLD, Ratnasingham and Hebert 2007). The aim of the project is twofold. Firstly, it will allow scientists with molecular capability but insufficient knowledge of bee taxonomy and systematics to recognize species and document local biodiversity of native bees. Secondly, for Australian native bee taxonomists, it contributes to integrative taxonomic approaches, such as elucidation of related species and clarification of problematic species groups, association of the sexes within one species, the association of larvae with adults and the identification of new species (Gibbs 2009; 2011; Packer et al. 2009; Schmidt et al. 2015).

Naturally, this initiative will only be successful if there is a wide coverage of species in the barcoding database. It is intended to regularly update the database with entries of additional species. So far, 271 sequences have been added to the project, covering 120 species in four of the five Australian bee families (Megachilidae, Apidae, Colletidae and Halictidae) that were collected during Bush Blitz surveys – intensive short-term surveys of remote, protected areas funded by the Australian Federal Government (Department of the Environment 2010).

In this publication the results of DNA barcoding are focused on species within the Euryglossinae (Colletidae). This subfamily has been relatively well studied through descriptions of new species by Houston (1992) and numerous revisions of genera by Exley (1998; 2001; 2002; 2004) and keys to the species are available for several genera and species groups. The DNA sequence data submitted to BOLD was used to assist in

delineating species into species-groups in the genus *Euhesma* (Michener), (1965) and to identify the sexes that comprise a species. Subsequent morphological comparison with type specimens allowed the recognition of four new species, which are described. The species selected for description belong to two well-delineated species groups within *Euhesma*: the *walkeriana* species group (Exley 2001), and the group associated with *Eremophila* (Myoporaceae; Exley 1998). Brief morphological characteristics of these groups are given below.

Methods

The bee specimens studied in this paper were collected during six intensive short term (1–2 week) Bush Blitz surveys (Department of the Environment 2010), at various remote locations in Australia (AUSBS 2014). Coordinates of the locations are given in decimal degrees (Suppl. material 1, Table 1). The species were caught mainly on flowering plants using a hand net, but on occasion a vehicle net or malaise traps were used. The bees were killed by freezing, pinned within a day of capture and sorted into morpho-species.

For DNA analyses a single middle leg was removed from up to five specimens per morpho-species. These legs were stored in 100% ethanol to allow preservation of the DNA, and submitted to BOLD for DNA barcoding using the cytochrome *c* oxidase subunit 1 gene. Specimen details, including collecting dates and locality information can be accessed in BOLD under the project Australian Bee Survey (accession numbers AUSBS001-12 to 190-12 and AUSBS191-13 to 380-13, e.g. http://www.boldsystems.org/index.php/Public_RecordView?processid=AUSBS131-12, and http://www.boldsystems.org/index.php/Public_RecordView?processid=AUSBS205-13). Using only BOLD BIN compliant sequence data (Ratnasingham and Hebert 2007) as input, species were delineated using Neighbor-joining trees generated by BOLD and uncorrected sequence divergence data were calculated using PAUP* version 4.0b8 (Swofford 2001). A phylogenetic tree of the barcoded data was generated using MrBayes version 3.2 (Ronquist et al. 2012).

We attempted to key all collected euryglossine specimens to species. The specimens were compared to all type specimens and all other relevant reliably named material at the Queensland Museum, the Western Australian Museum, the Australian National Insect Collection, and the South Australian Museum. Based on these morphological comparisons, several species, including four species in the genus *Euhesma*, were identified as new. These four species are described here.

The descriptions of the species and the morphological terminology follow the format used by Exley (2001) for the *walkeriana* species group and Exley (1998) for the species associated with *Eremophila* (Myoporaceae), to allow easy comparison with the other species in the same species groups. Stereomicroscope with step-less zoom and an eyepiece micrometer were used to take relative measurements of the head (following Houston 1990), whereby head width was set to 50 units.

Abbreviations

- BOLD** Barcoding of Life Database
SAMA South Australian Museum, Adelaide
WAM Western Australian Museum

Results

Molecular delineation of the taxa

BOLD barcoding of Euryglossinae resulted in DNA barcode data for 87 specimens comprising 40 species. Of these, morphological examination resulted in the identification of 17 species, 6 species were recognized as new and 17 species were identified to genus or species group level (Suppl. material 2, Fig. 1). The molecular delineation matched the morphologically identified genera and species groups for the species in the genera *Euryglossa*, *Euryglossula*, *Euryglossina*, *Pachyprosopis*, *Hyphesma*, *Xanthesma*, *Pachyprosopis*, and some species groups within *Euhesma*. However, *Euhesma* and *Callohesma* appear to be paraphyletic (Suppl. material 2, Fig. 1).

New species

A number of specimens were identified to belong to *Euhesma* species groups that previously were revised by Exley (1998, 2001). Morphological comparison of these specimens with species descriptions and with type material in the above mentioned Australian museum collections showed that, among a number of known species, five of the *Euhesma* species did not match with any of the descriptions nor with any of the examined type specimens. Molecular and morphological data showed that four of these species belong to existing species groups. These four species are described in the systematics section of this paper.

One of the four new species described here belongs to the *Euhesma walkeriana*-species group (Exley 2001, Suppl. material 2, Fig. 1). The pairwise uncorrected sequence divergence between the new species (*E. albamala* sp. n.) and the only other barcoded species (*E. bronzus* Exley 2001) in this group varied between 5.0–6.9%.

Three other new species belong to a group of *Euhesma* species that are associated with flowers of *Eremophila* (Exley 1998). Pairwise uncorrected sequence divergence between phylogenetic sister species in this group are: *E. sulcata* Exley 1998 vs. *E. aulaca* sp. n. 5.0–6.1%; *E. lyngouriae* sp. n. vs. *E. micans* sp. n. 5.8–6.1%; and, *E. aurata* Exley 1998 vs. *E. micans* sp. n. 6.0–6.3%. Because the data sets for the above species groups are incomplete, the presented divergence values are not necessarily comparisons with phylogenetically closest sister species.

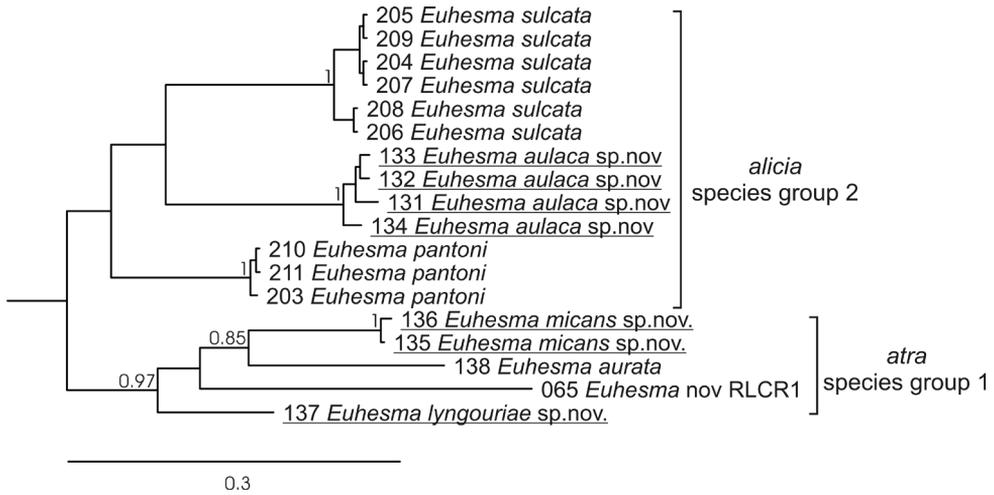


Figure 1. Phylogenetic relationships among *Euhesma* species collected on *Eremophila* flowers, based on BOLD sequence data, analysed using MrBayes (GTR-inv+gamma, partitioned by codon, 8M generations). Posterior probabilities for nodes are shown when > 0.7. The species described in this publication are underlined. The three digits preceding taxon names refer to Barcoding of Life Database: AUSBS###-12/13 specimens (AUSBS 2014).

While the molecular results group a fifth species of *Euhesma*, represented by a single barcoded male (RL1788A-AUSBS065) and some additional male and female specimens, with *Euhesma* species caught on *Eremophila*, it has a number of characters that are not consistent with this group, i.e. head and mouth parts not elongated and not caught on *Eremophila*. Thus, this species did not fit into any of the other known *Euhesma* groups and therefore the description was deferred until additional data allow improved justification for the position of this species relative to other *Euhesma* species.

Systematics

Four new species of *Euhesma* Michener

Within the subfamily Euryglossinae, the genus *Euhesma* Michener contains a large number (65) of highly diverse species (Michener 2007). The genus was erected by Michener (2000) as a “dumping ground” (Exley 2001) for species that do not fit easily elsewhere. As a result, the genus is difficult to delineate (Exley 2001, 2004). To provide more structure and delineation within the genus, Exley (1998, 2001, 2004) divided the genus into a series of species groups, which so far account for approximately half of the species included in the genus. These groups include the *walkeriana* species group (15 species; Exley 2001), the *acantha* species group (three species; Exley 2004), and three groups associated with the plant *Eremophila* (Exley 1998): the *atra*, *alicia* and *coppinensis* species groups (20 species combined).

A new species of *Euhesma* in the *walkeriana* species group

The fifteen known species in the *walkeriana* species group (Exley 2001) are small (4–6 mm), black and or with metallic sheen, often marked with yellow. Heads are wider than long with antennae low down on the face so that subantennal sutures are absent or almost so. While similar to *Xanthesma* (subgenus *Chaetohesma*), the *walkeriana* group differs from this subgenus in the following characters: the facial foveae are straight and do not curve towards lateral ocelli, the pronotum is relatively short and the basitarsi of the forelegs do not bear long, stiff setae (Exley 2001).

As only a single species is added to this group, we do not produce a modified key, but suggest modifying the key produced by Exley (2001) by inserting additional couplets after couplet 3 as follows:

4 (3)	Labrum yellow.....	5
–	Labrum brown/black.....	5A
5A (4)	Mandibles transparent white with dark tips.....	<i>E. albamala</i> sp. n.
–	Mandibles yellow, brown or black.....	6

Euhesma albamala Hogendoorn & Leijs, sp. n.

<http://zoobank.org/C018DBB3-3224-40F5-A1AD-F45D7E79A083>

Figs 2A–H, 3A–D

Material examined. *Holotype*: ♀, RL1807C, Cane River Conservation Park, Western Australia, 22.0936°S, 115.3507°E, 26 June 2011, R. Leijs, on flowers of a red flowering *Grevillea* (WAM). BOLD: AUSBS082-12. *Paratypes*: ♂, RL1811, same date and locality as holotype (WAM). BOLD: AUSBS009-12; 4♀, all same date and locality as holotype (SAMA 32-033287, -288, -289, -290).

Diagnosis. Most like *E. spinola*; however, the combination of the white mandibles with dark tips and the simple claws distinguishes this from all other species. The absence of both pothook and clubbed setae is a character shared with *E. lobata*, *E. spinola* and *E. walkeriana*.

Description. *Female*. Length approximately 5.0 mm; wing length about 3.2 mm. Head width 1.5 mm. Relative head measurements: width 50, length 41; clypeal length 11; lower interocular distance 30; upper interocular distance 28; interantennal distance 8; antennocular distance 7; interocellar distance 10; ocellocular distance 8.

Supraclypeal area, frons and mesosoma with metallic bronze-green sheen; mandibles translucent white with dark tips; labrum brown; legs amber with coxae and tibiae dark brown, and hind basitarsi pale yellow; face with long white hairs; forelegs with coxal lobes weakly developed with long hyaline unbranched setae; clubbed and pothook setae absent; hind basitibial plate with carina; hind-tibia with a row of tubercles beyond basitibial area; claws simple; metasoma brown with posterior margins of terga translucent, creating a banded effect; pygidial plate broadly spatulate.

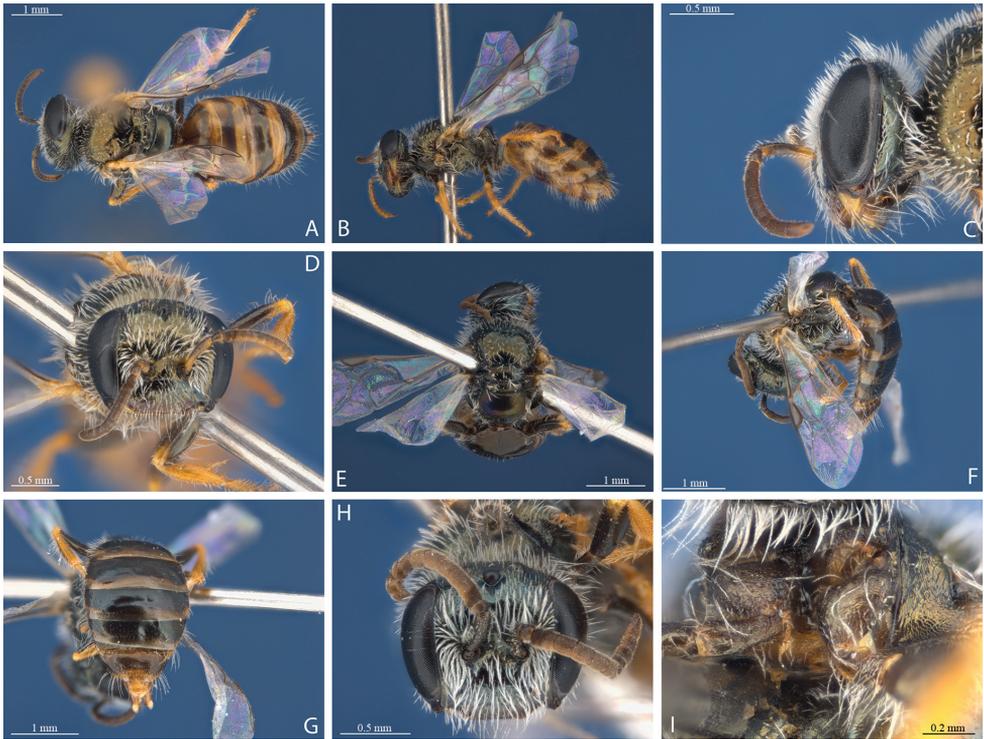


Figure 2. *Euhesma albamala* sp. n.: **A–D** Holotype female: **A** dorsal **B** lateral **C** head lateral **D** head frontal **E–H** Allotype male: **E** dorsal **F** lateral **G** tergites **H** head frontal. Fore coxae female.

Male. Length approximately 3.8 mm; wing length approximately 3 mm. Head width 1.2 mm. Relative head measurements: width 50, length 45; clypeal length 10; lower interocular distance 30; upper interocular distance 30; interantennal distance 8; antennocular distance 7; interocellar distance 10; ocellocular distance 8. Coloration and pubescence as in female, but pronotum with metallic purple sheen; metasoma dark brown with posterior margins of terga translucent. The apical five flagellar segments of the male look crumpled, however this could be an artifact. Terminalia as in Fig. 3A–D.

Etymology. The specific name refers to the white mandibles.

Three new species of *Euhesma* associated with *Eremophila*

The bees in the three known species groups associated with *Eremophila*, the *atra* (eight species), the *alicia* (eight species) and the *coppinensis* (four species) species groups, have modified heads and/or labial palps that suggest foraging on narrow, tubular flowers (Exley 1998). The distinction between the species groups is based on the total length of the labial palps, and on the relative length of segment 2 and 4 of these palps (Exley

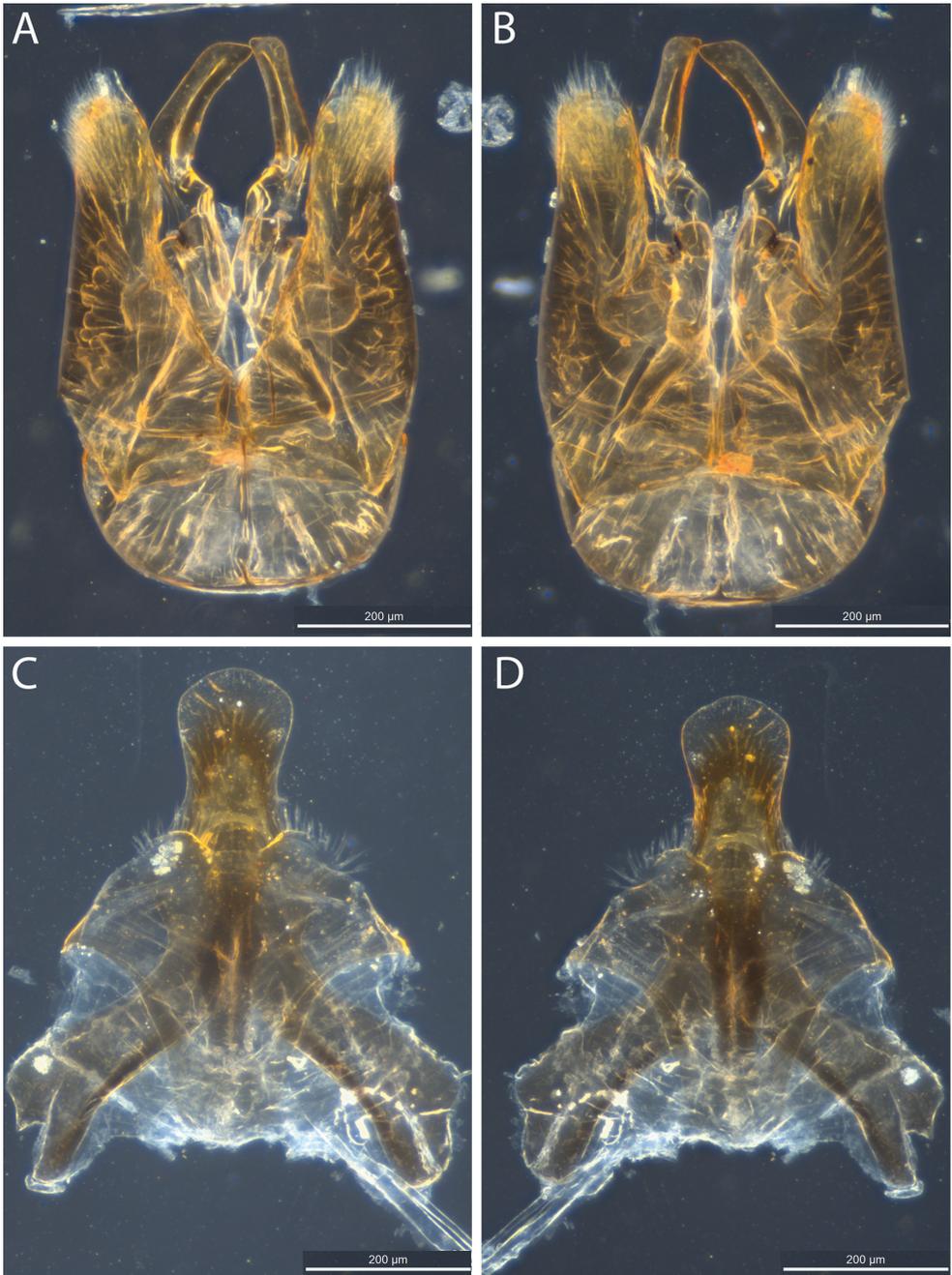


Figure 3. *Eubesma albamala* sp. n.: **A–D** Allotype male genital structures: **A** genital ventral **B** genital dorsal **C** S7+S8 ventral **D** S7+S8 dorsal. Scale bars: 200 µm.

–	Tibiae, tarsi and terminal gastral terga predominantly dark brown.....	17
16(15)	First recurrent vein distal to first submarginal crossvein	<i>E. aurata</i>
–	First recurrent vein interstitial with first submarginal crossvein	<i>E. lyngouriae</i> sp. n.
17(15)	Labial palp longer than antennal flagellum.....	18
–	Labial palp not longer than antennal flagellum	19
18(17)	Supraclypeal area almost glabrous and highly polished.....	<i>E. symmetra</i>
–	Supraclypeal area neither glabrous nor polished	<i>E. nalbarra</i>
19(17)	Dorsal surface of clypeus concave medianly (indented)	<i>E. atra</i>
–	Dorsal surface of clypeus with a slight median longitudinal furrow ...	<i>E. scoparia</i>
–	Dorsal surface of clypeus in no way concave	20
20(19)	Labial palps clearly longer than maxillary palps.....	<i>E. leonora</i>
–	Labial palps and maxillary palps about equal in length.....	21
21(20)	Fronto-clypeal suture distinct	<i>E. balladonia</i>
–	Fronto-clypeal suture absent on clypeus.....	<i>E. micans</i> sp. n.

***Euhesma micans* Hogendoorn & Leijs, sp. n.**

<http://zoobank.org/CD2BD7B3-F09C-4DD4-B953-EE4326804955>

Figs 4A–I, 5A–E

Material examined. *Holotype*: ♀, SAMA 32-03385, Bon Bon Station, South Australia, 30.5250°S, 135.5917°E, 27 October 2010, R. Leijs, on flowers of *Acacia victoriae* (SAMA). BOLD: AUSBS135-12. *Paratype*: ♂, SAMA 32-03386, same date and locality as holotype (SAMA). BOLD: AUSBS136-12.

Diagnosis. Most like *E. leonora*; however, this is the only species in which the frons above the antennae is shining, and the facial fovae are narrow and not curved towards the ocelli.

Description. *Female.* Length approximately 4.5 mm; wing length approximately 3.1 mm. Head width 0.9 mm. Relative head measurements: width 50; length 64, clypeal length 17; lower interocular distance 32; upper interocular distance 33; interantennal distance 13; antennocular distance 5; interocellar distance 16; ocellocular distance 10. Anterior margin of clypeus truncate, upper margin slightly concave; other areas of clypeus and frons convex with depressions centrally, around the anterior ocelli and antennal implants; lower part of facial fovae broadened, upper part narrow, not bent towards ocelli; antennal scapes anteriorly flattened; malar space short; labial palp segments increasing in length in the order 2, 1, 3, 4. Clypeus and frons above antennae shiny with punctures wide apart; facial fovae, interocellar area and scutum with dense reticulation and dull.

Head black; antennae brown with flagella yellowish ventrally; labial palp segments 1,2 dark brown, segments 3, 4 light brown; legs yellowish with femora dark brown. First recurrent vein of forewing interstitial with first submarginal crossvein. Scattered long white hairs on frons, clypeus, antennal scapes, vertex, mandibles, posterior genae, sides of thorax, venter and gastral tergum 5.

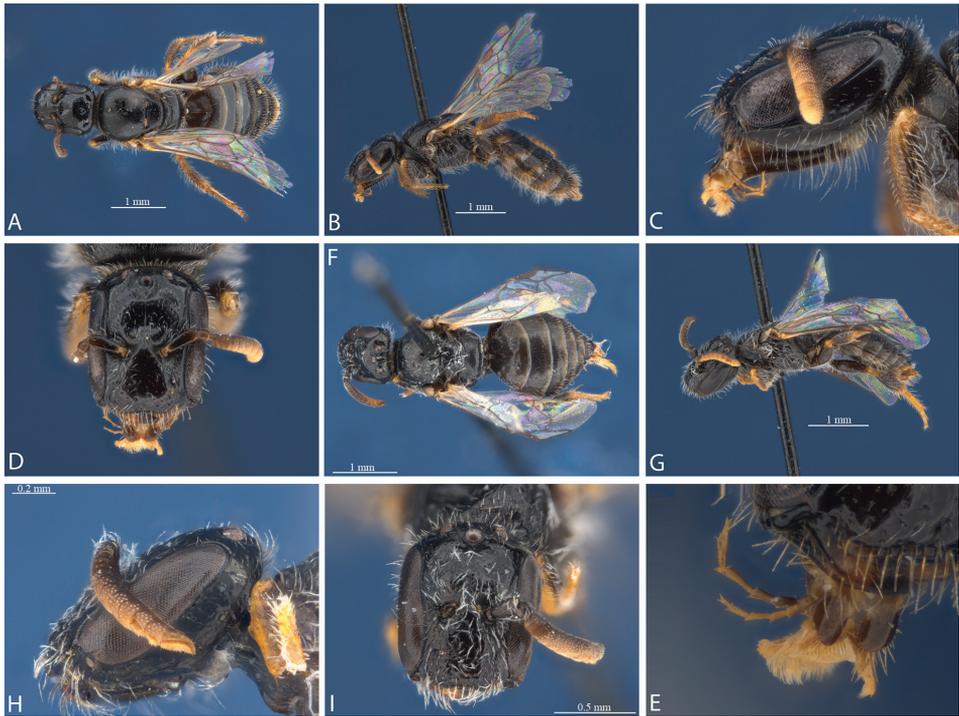


Figure 4. *Euhesma micans* sp. n.: **A–E** Holotype female: **A** dorsal **B** lateral **C** head lateral **D** head frontal **E** mouthparts **F–I** Allotype male: **F** dorsal **G** lateral **H** head lateral **I** head frontal.

Male. Length approximately 3.6 mm; wing length approximately 2.6 mm. Head width 0.8 mm. Relative head measurements: width 50; length 57, clypeal length 24; lower interocular distance 29; upper interocular distance 34; interantennal distance 10; antennocular distance 9; interocellar distance 14; ocellocular distance 10. Forewings and labial palps as in female; final three flagellar segments with indentations ventrally; inner hind tibial spur finely pectinate; frons above antennae shining with punctures wide apart. All labial segments dark; legs dark brown with fore tibia and all tarsae yellowish. Terminalia as in Fig. 5A–E.

Etymology. The specific name refers to the shiny frons.

***Euhesma lyngouriae* Hogendoorn & Leijs, sp. n.**

<http://zoobank.org/4809A089-9751-4AC1-B36D-7AC01F815ECA>

Figs 6A–D

Material examined. *Holotype:* ♀, SAMA 32-033284, Bon Bon Station, South Australia, 30.2389°S, 135.5098°E, 26 October 2010, R. Leijs, on flowers of *Swainsona stipularis* (SAMA). BOLD: AUSBS137-12. ♂, unknown.

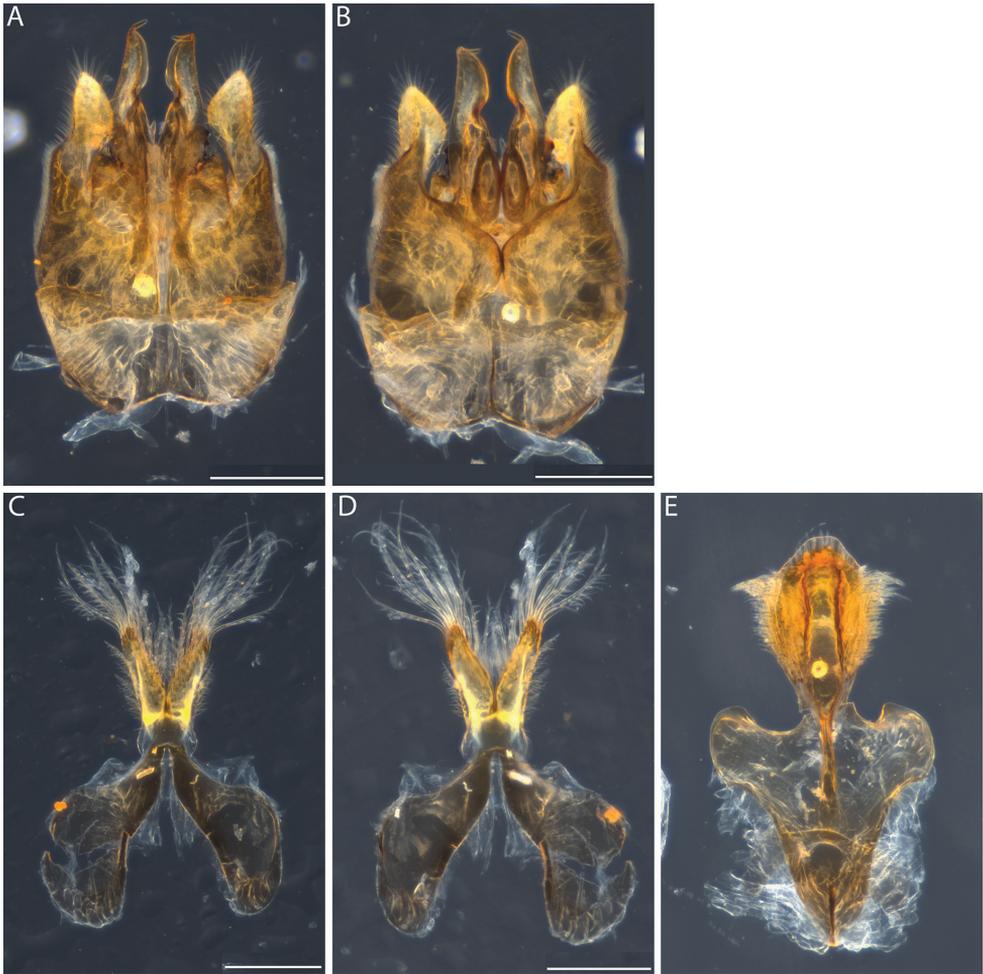


Figure 5. *Eubesia micans* sp. n.: **A–E** Allotype male genital structures: **A** genital dorsal **B** genital ventral **C** S7 dorsal **D** S7 ventral **E** S8 dorsal. Scale bars: 200 μ m.

Diagnosis. Most like *E. aurata*, however, forewing first recurrent vein interstitial with first submarginal crossvein, margin between clypeus and supraclypeal area lacking furrow, all abdominal segments amber, lacking brown bands.

Description. *Female.* Length approximately 6.0 mm; wing length approximately 3.5 mm. Head width 1.6 mm. Relative head measurements: width 50; length 50; clypeal length 14; lower interocular distance 28; upper interocular distance 32; interantennal distance 12; antennocular distance 6; interocellar distance 12; ocellocular distance 8. Anterior margin of clypeus truncate, upper margin slightly concave, lacking median furrow; frons with median area elevated; facial foveae broad, upper part slightly bent towards ocelli; antennal scapes anteriorly flattened; malar space short; labial palp

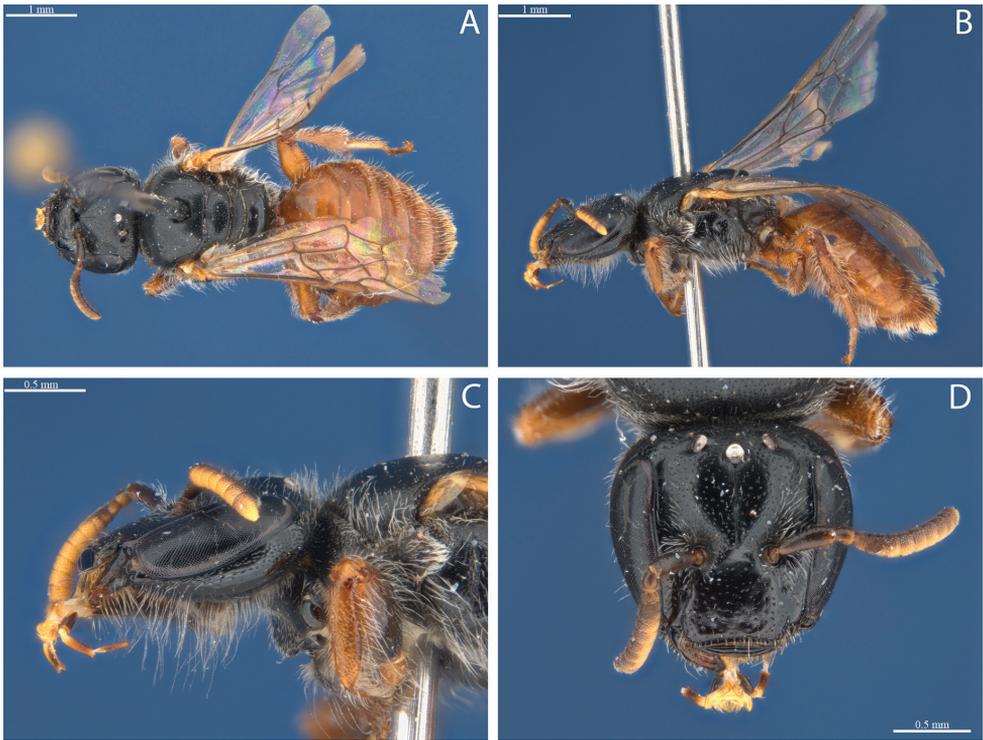


Figure 6. *Euhesma lyngouriae* sp. n.: **A–D** Holotype female: **A** dorsal **B** lateral **C** head lateral **D** head frontal.

segments increasing in length in the order 1 = 3, 2. Clypeus and frons above antennae; facial foveae interocellar area and scutum with dense reticulation and dull. Head black; antennae brown with flagella yellowish ventrally; labial palp segments 1 dark brown, 2 and 3 yellowish; legs and gaster amber, tergal foveae and fore femora posteriorly dark brown. First recurrent vein of forewing interstitial with first submarginal crossvein. Scattered long white hairs on frons, clypeus, antennal scapes, vertex, mandibles, posterior genae, sides of thorax, venter and gastral tergum 5.

Remarks. The type lacks labial palp segment 4.

Etymology. The specific name refers to the amber coloured legs and gaster of the female.

***Euhesma aulaca* Hogendoorn & Leijs, sp. n.**

<http://zoobank.org/A64B73ED-9553-4AB6-B0D1-B8095B482E0E>

Figs 7A–I, 8A–E

Material examined. *Holotype*: ♀, SAMA 32-033281, Bon Bon Station, South Australia, 30.8440°S, 135.5389°E, 27 October 2010, R Leijs (SAMA). **BOLD**: AUS-BS133-12. *Paratypes*: ♂, SAMA 32-033282, same date and locality as holotype. 3♀, Bon Bon Station, South Australia, 30.7416°S, 135.3665°E, on *Eremophila scoparia*;



Figure 7. *Euhesma aulaca* sp. n.: **A–D, I** Holotype female: **A** dorsal **B** lateral **C** head lateral **D** head frontal **I** mouthparts **E–H**: Allotype male **E** dorsal **F** lateral **G** head ventral **H** head frontal.

1♀, Bon Bon Station, South Australia, 30.5250°E, 135.5917°E; 7♀+1♂ in ethanol RL1636 all same date as holotype (SAMA). BOLD: AUSBS131,2,4-12.

Diagnosis. The species is most like *E. yellowdinensis*, but the clypeus has a distinctive deep median furrow and the total length of the labial palps is only slightly shorter than the head.

Description. *Female.* Length approximately 5.5 mm; wing length approximately 3.5 mm. Head width 1.6 mm. Relative head measurements: width 50; length 50; clypeal length 14; lower interocular distance 28; upper interocular distance 32; interantennal distance 12; antennocular distance 6; interocellar distance 12; ocellocular distance 8. Clypeus with deep median furrow, anterior margin truncate, upper margin indistinct; facial foveae broad, upper part slightly bent towards ocelli; malar space short; labial palp segments increasing in length in the order 1, 2, 3 = 4. Clypeus and frons above antennae, interocellar area and scutum with dense reticulation; clypeus and supra-clypeal area shining, frons, paraocular areas and scutum dull. Head black; antennae brown with flagella yellowish ventrally; labial palp segments 1 and 2 dark brown, 3 and 4 ribbon-like, yellowish; all femora and tibiae of middle and hind legs medially dark brown, fore tibiae, all tarsi and remaining parts yellow; gaster dark brown. Marginal zones of metasoma wide and translucent. Forewing with first recurrent vein almost in-



Figure 8. *Eubesia aulaca* sp. n.: **A–E** Allotype male genital structures: **A** genital dorsal **B** genital ventral **C** genital lateral **D** S7 dorsal **E** S8 dorsal. Scale bars: 200 μ m.

terstitial with first submarginal crossvein. Scattered long white hairs on frons, clypeus, antennal scapes, vertex, mandibles, posterior genae, sides of thorax, venter and gastral tergum 5, pygidial fimbria pale orange.

Male. Length approximately 5.2 mm; wing length approximately 3.6 mm. Head width 1.2 mm. Relative head measurements: width 50; length 60, clypeal length 17; lower interocular distance 22; upper interocular distance 30; interantennal distance 8; antennocular distance 5; interocellar distance 12; ocellocular distance 9. Forewings, labial palps and other characters as in female; inner hind tibial spur roughly pectinate; Terminalia as in Fig. 8A–E.

Remarks. Exley (1998) distinguishes the species from group 1 (the *alicia* species group) from those of groups 2 and 3 on the basis of the length of the labial palps, which are substantially shorter than the head in the species of group 1, and longer than the head in groups 2 and 3. Although the labial palps of female *E. aulaca* are shorter

than the head, the difference in length is only slight, while the shape and length of the labial palp segments are like other species in the *alicia* species group. Based on molecular and morphological data, *E. aulaca* has been classified in the *alicia* species group, and the first couplet of the key has been modified to include this species.

Etymology. The specific name means ‘with a furrow’, referring to the deep median furrow in the clypeus.

Discussion

DNA barcoding has been used to associate the sexes of species. Furthermore, integrative taxonomy (Gibbs 2009, 2011) was used to identify species that were new to science. Four new species of *Euhesma* were found and described.

Despite the small size of the present molecular database, both in terms of numbers of species and numbers of nucleotides involved, it provides some insights with respect to validity of the *Euhesma* groups described by Exley (1998, 2001), of *Euhesma* as a genus, and of some other euryglossine clades.

While Exley (1998) noted that the *Euhesma* species groups associated with *Eremophila* would not necessarily be monophyletic, the molecular data so far support monophyly. The limited molecular data (Fig. 1) also support the *alicia* (group 1) and *atra* (group 2) species groups as recognized by Exley (1998). However, it should be noted that molecular data are only available for six of the 23 described species, and do not as yet include any representatives of the *coppinensis* species group.

By contrast, and in spite of the fact that the database is far from complete, the COI phylogeny presented here (Suppl. material 2, Fig. 1) suggests that *Euhesma* as a genus is paraphyletic. This is not surprising, as the genus was erected by Michener (1965) to include species that did not fit elsewhere, and Michener himself (2007) suggested that *Euhesma* could be paraphyletic. It is envisaged that with the addition of identified species and additional DNA sequences based on mitochondrial as well as nuclear genes, eventually a robust molecular and morphological revision of euryglossine genera currently grouped with *Euhesma* should be possible.

Support was also found for some other euryglossine clades. Although results of phylogenetic analyses based on just a small fragment of mitochondrial DNA (used here as the ‘barcoding region’) are often not informative above genus level, some well supported clades (posterior probabilities > 0.8) combine species within the genera *Euryglossula*, *Euryglossina* and *Pachyprosopis*, of which their sister clade appears to be the *Euhesma-walkeriana* species group.

The creation of an open access molecular DNA barcoding project of Australian native bees enhances possibilities for scientists with molecular capabilities to document bee biodiversity (Schmidt et al. 2015) and will encourage and facilitate taxonomic work. The accession codes can also be included in other databases, such as Atlas of Living Australia and PaDil Australian Pollinators. The concatenation of molecular and morphological information for species discovery is becoming more accepted in recent

years (e.g. Gibbs 2009; 2011; Schmidt et al. 2015), and will lead to better outcomes in our understanding of Australian native bees and their conservation.

Acknowledgements

This paper would not have been completed without the support of the Bush Blitz program provided through ABRS. We thank Jo Harding, Kate Gillespie, Mim Jambrecina and the other members of the Bush Blitz team for their fabulous support in the field. We thank Susan Wright (QM), Nicole Fisher (ANIC), Brian Hanich (WAM) and Terry Houston (WAM) for providing access to the collections, Alexis Tindall for excellent photographic assistance, and Wiebe Hogendoorn for linguistic advice. This work was supported by a Bush Blitz Tactical Taxonomy Grant from the Australian Government through ABRS.

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Supplementary material 1

Type localities of new species

Authors: Katja Hogendoorn, Mark Stevens, Remko Leijts

Data type: occurrence

Explanation note: localities where type specimens were caught.

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Supplementary material 2

Phylogenetic relationships of euryglossine species based on CO1 sequence data

Authors: Katja Hogendoorn, Mark Stevens, Remko Leijts

Data type: gene tree

Explanation note: tree based on CO1 sequence data.

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Five new species of subgenus *Plesiominetia* (Diptera, Lauxaniidae, *Minettia*) in southern China, with a key to known species

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Abstract

Five species of the subgenus *Plesiominetia* Shatalkin from the southern China are described as new to science: *Minettia* (*Plesiominetia*) *flavoscutellata* sp. n., *Minettia* (*P*) *longaculiformis* sp. n., *Minettia* (*P*) *nigrantennata* sp. n., *Minettia* (*P*) *tridentata* sp. n. and *Minettia* (*P*) *zhejiangica* sp. n. One species, *Minettia longistylis* Sasakawa, is transferred to the subgenus *Plesiominetia* from *Minettia* s. str. A key to separate the known species of the subgenus is presented, along with a taxonomic list of species. The type material of the new species are deposited in the China Agricultural University, Beijing, China (CAUC).

Keywords

Minettia, Lauxaniidae, Oriental region, species key

Introduction

The subgenus *Plesiominettia* Shatalkin, 2000, of the genus *Minettia* Robineau-Desvoidy, 1830, was erected for the type species *Minettia helvola* (Becker, 1895). In the same paper, Shatalkin (2000) transferred the following species from the subgenus *Minettia* to the subgenus *Plesiominettia*: *Minettia crassulata* Shatalkin, 1998; *M. divaricata* Sasakawa, 1985; *M. flia* (Becker, 1895); *M. fuscescens* Shatalkin, 1998; *M. gemina* Shatalkin, 1992; *M. gemmata* Shatalkin, 1992; *M. helva* Czerny, 1932; *M. helvola* (Becker, 1895); *M. ishidai* (Sasakawa, 1985); *M. loewi* (Schiner, 1864); *M. omei* Shatalkin, 1998; *M. punctata* Sasakawa, 1985; *M. styriaca* (Strobl, 1892); *M. tenebrica* Shatalkin, 1992. The species *M. longistylis* Sasakawa, 2002, is here transferred into *Plesiominettia* from the subgenus *Minettia*. The subgenera of *Minettia*, as well as the species of *Plesiominettia*, can be separated using the key in this paper.

Based on a combination of the original definition by Shatalkin 2000, and the authors' observations, *Plesiominettia* is diagnosed as follows: arista pubescent, rarely bare or short plumose, rays of arista with longest setulae longer than 1/3 height of 1st flagellomere; wing uniformly hyaline, rarely pale brown at base (in *Minettia* (*P.*) *zhejiangica* sp. n.); mesonotum with 0–1+2–3 dorsocentral setae (first postsutural dorsocentral setae close to transverse scutal suture or located medially between transverse scutal suture and scutoscutellar suture); acrostichal setulae hair-like, most species with 1–2 pairs of strong setae among them, located at middle of mesonotum or in front of prescutellar acrostichal setae; male genitalia: a pair of postgonites present, postgonites rarely absent; phallic sheath absent, phallus forming a case; female terminalia: spermathecae 1+1 or 1+2. In this subgenus, the body color varies from black (most species, e.g., *Minettia* (*P.*) *divaricata*, Fig. 49) to grey pruinose (e.g., *Minettia* (*P.*) *gemmata*, Fig. 50) to yellow (e.g., *Minettia* (*P.*) *punctata*, Fig. 51).

There are 20 known species distributed in the Palaearctic and Oriental regions in the subgenus *Plesiominettia*, eight of which have been found in China.

Materials and methods

The general terminology follows Cumming and Wood (2009) and Gaimari and Silva (2010). Line diagrams were drawn using a drawing tube attached to a Nikon SMZ 1500 stereomicroscope and to a Nikon 80i compound microscope. Photographs were taken by a Nikon DS-Fi2-U3 digital camera mounted on a Nikon SMZ 1500 stereomicroscope. Genitalia preparations were made by removing and macerating the apical portion of the abdomen in cold saturated NaOH for six hours, then soaking in distilled water with a few drops of glacial acetic acid. After examination, the genitalia were transferred to glycerin and stored in a microvial pinned below the specimen. Specimens examined were deposited in China Agricultural University, Beijing, China (CAUC). Type specimens (see Appendix) are from the following museums:

Bernice Pauahi Bishop Museum; Honolulu, Hawai'i, USA (BPBM), Hungarian Natural History Museum; Budapest, Hungary (HNHM), Hrvatski Narodni Zooloski Muzej, Zagreb, Croatia (HZMZ), Władysław Rydzewski Museum of Natural History; University of Wrocław, Poland (MNHW), Naturhistorisches Museum; Vienna, Austria (NHMW), Naturhistorisches Museum der Benediktiner-Abtei Admont; Admont, Austria (NMBA), Osaka Museum of Natural History; Osaka, Japan (OMNH), Department of Natural History, National Museums of Scotland; Edinburgh, United Kingdom (RMSE), Slovenské Národné Muzeum; Bratislava, Slovakia (SNMC), Zoological Museum; University of Amsterdam, Amsterdam, Netherlands (ZMAN), Museum für Naturkunde; Berlin, Germany (ZMHB), Zoological Museum; Moscow State University, Moscow, Russia (ZMUM).

Taxonomy

Subgenus *Plesiominettia* Shatalkin, 2000

Plesiominettia Shatalkin, 2000: 52. Type species: *Minettia helvola* (Becker, 1895) (original designation).

Species descriptions

Minettia (Plesiominettia) flavoscutellata sp. n.

<http://zoobank.org/F14CA159-188B-433D-B92A-3AAD01D18EC2>

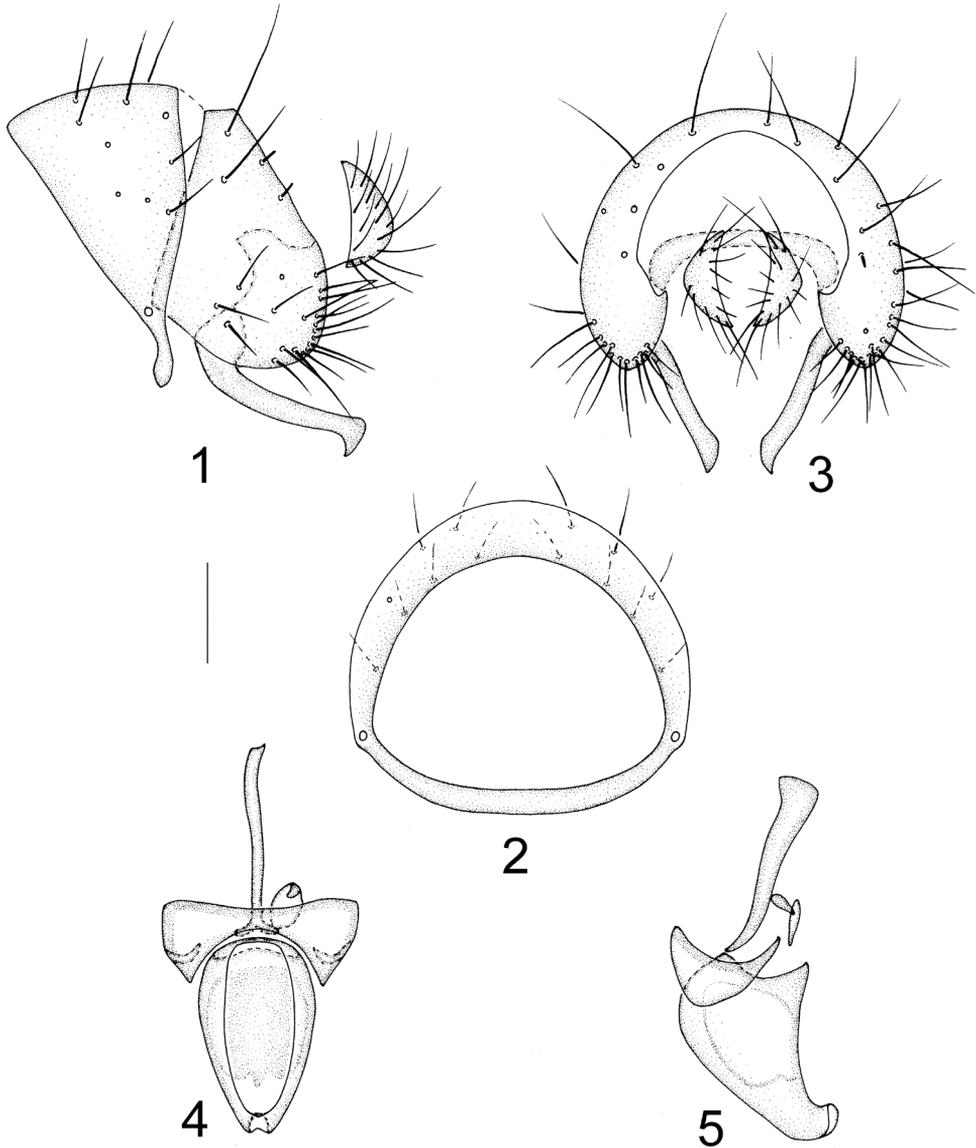
Figs 1–5, 31–33, 43

Type material. Holotype: ♂ (CAUC), CHINA, Hubei: Shennongjia National Natural Reserve, Pingqian, 1650 m, 25. vii. 2007, Qifei Liu. Paratypes: 5 ♂♂, 7 ♀♀ (CAUC), CHINA, Hubei: same data as holotype.

Etymology. Latin, *flavor*-is from the latin *flavus*, meaning yellow, + *scutellata* is from the latin *scutellatum*, meaning shield; referring to the scutellum being mostly yellow; a feminine adjective.

Diagnosis. Face slightly concave, yellowish brown to blackish brown except black ventral margin. Antenna with scape brown and pedicel brownish yellow, 1st flagellomere entirely blackish brown on outer edge but yellow on basal 1/4 on inner edge. Arista short plumose, and rays of arista with longest setulae as long as 1/2 height of 1st flagellomere. Mesonotum with weak anteriormost dorsocentral setae clearly behind transverse scutal suture. Male genitalia: surstylus with a long falcate process in lateral view; phallus wide basally and narrow apically, with a small elliptical concavity at apex. Female spermathecae 1+1, round.

Description. *Male.* Body length 3.3–3.5 mm, wing length 3.4–3.8 mm. *Female.* Body length 3.6–3.9 mm, wing length 3.9–4.3 mm.



Figures 1–5. *Minettia (Plesiominettia) flavoscutellata* sp. n. Male. **1** syntergosternite 7+8 and epandrium, lateral view **2** syntergosternite 7+8, anterior view **3** epandrial complex, posterior view **4** aedeagal complex, ventral view **5** aedeagal complex, lateral view. Scale bar: 0.1 mm.

Head black. Face slightly concave, yellowish brown to blackish brown except black ventral margin, parafacial yellow except black inner margin, with a narrow pale brown medial stripe. Frons blackish brown except yellow anterior margin; ocellar triangle black; ocellar setulae developed, longer than anterior fronto-orbital setae, anterior fronto-orbital setae reclinate, shorter than posterior fronto-orbital setae. Gena yellow,

approximately 1/6 height of eye. Antenna with scape brown and pedicel brownish yellow, 1st flagellomere entirely blackish brown on outer edge but yellow on basal 1/4 on inner edge; 1st flagellomere nearly 1.6 times longer than high; arista black, except yellow at base, short plumose; rays of arista with longest setulae as long as 1/2 height of 1st flagellomere. A blackish brown spot present between eye and base of antenna. Proboscis and palpus black.

Thorax black with sparse brownish gray pollinosity, slightly subglossy. Mesonotum with 0+3 dorsocentral setae (weak anteriormost dorsocentral setae clearly behind transverse scutal suture), acrostichal setulae in four rows; a pair of long acrostichal setulae present in front of prescutellar setae, prescutellar setae slightly longer than 1st postsutural dorsocentral setae; one strong intra-alar seta, one anepisternal seta, two katepisternal setae. Scutellum mostly yellow, except blackish brown on basal 1/4–1/3. Legs: femora black; tibiae dark yellow on basal 1/2–2/3 and blackish brown on apical 1/3–1/2; tarsi dark yellow except tarsomeres 3–5 pale brown. Fore femur with four posteroventral setae and eight posterodorsal setae, fore tibia with one short preapical anterodorsal seta and one short apicoventral seta. Mid femur with four anterior setae and one apical posterior seta, mid tibia with one strong preapical anterodorsal seta and one strong apicoventral seta. Hind tibia with one preapical anterodorsal seta and one short apicoventral seta. Wing (Fig. 43) slightly yellow, hyaline; costa with 2nd (between R₁ and R₂₊₃), 3rd (between R₂₊₃ and R₄₊₅) and 4th (between R₄₊₅ and M₁) sections in proportion of 5.3:2:1; *r-m* at middle of discal cell; ultimate and penultimate sections of M₁ in proportion of 1:1.1; ultimate section of CuA₁ approximately 1/4 of penultimate. Halter yellow.

Abdomen black with sparse brownish gray pollinosity. Male genitalia (Figs 1–5): syntergosternite 7+8 circular with dorsal setulae; epandrium broad, round apically; surstylus with a long falcate process in lateral view; hypandrium inverted-U shape, hypandrial apodeme absent; postgonite and pregonite absent; phallus wide basally and narrow apically, with a small elliptical concavity at apex; phallapodeme long, nearly as long as phallus. Female sternite 8 semicircular; spermathecae 1+1, round (Figs 31–33).

Remarks. The new species is different from other species of the subgenus in the scutellum being yellow except blackish brown on basal 1/4–1/3 and the surstylus having a long falcate process in lateral view. The 1+1 female spermathecae is distinctive from the other species of the subgenus.

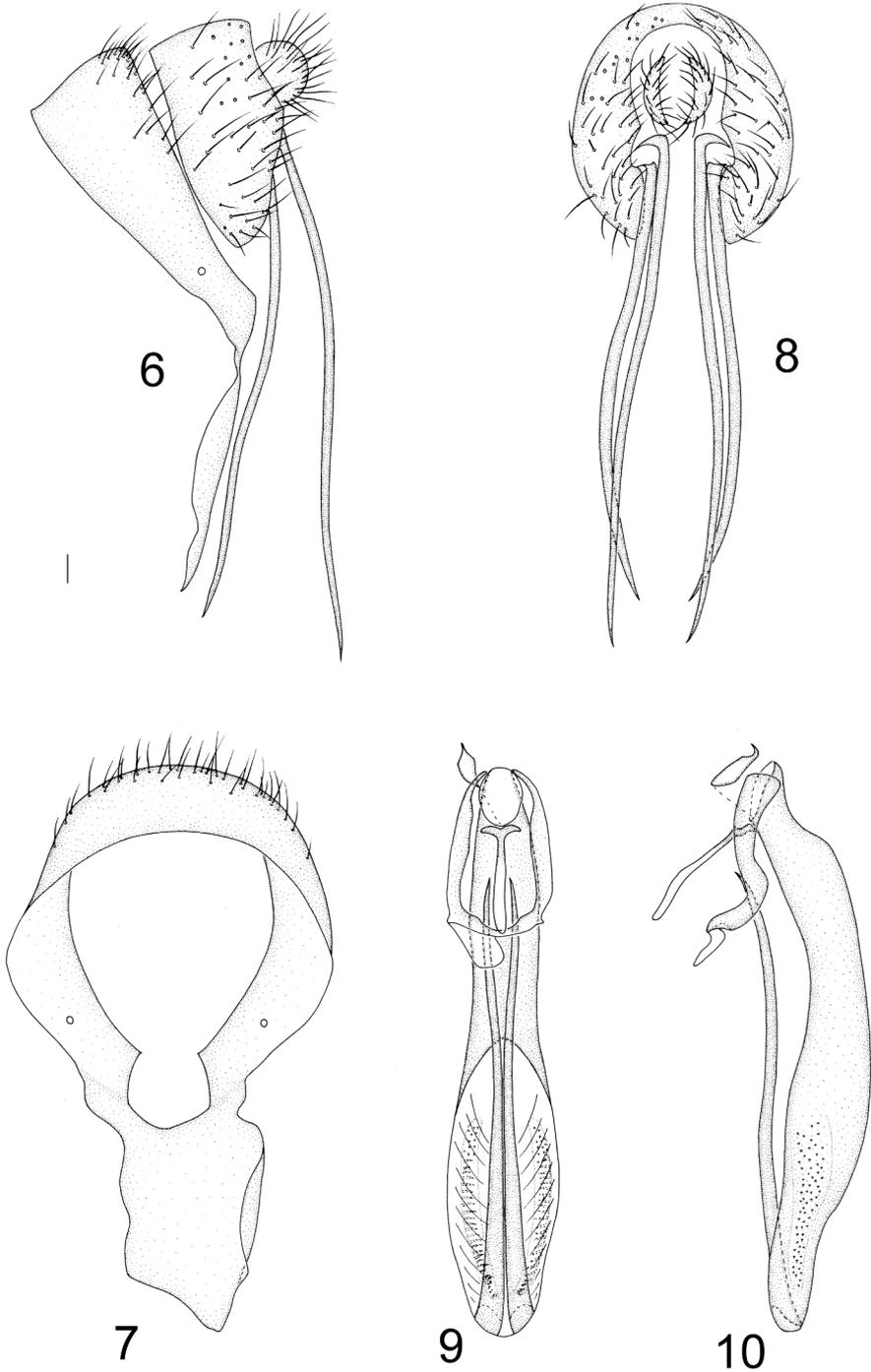
Distribution. China (Hubei).

***Minettia (Plesiominettia) longaciculiformis* sp. n.**

<http://zoobank.org/A16E8F85-E30B-43A3-A2F5-981E745BC2F4>

Figs 6–10, 34–35, 44

Type material. Holotype: ♂ (CAUC), CHINA, Zhejiang: Lin'an, Tianmushan, 19. vii. 2007, Yajun Zhu. Paratypes: 1 ♂, 6 ♀♀ (CAUC), CHINA, Zhejiang: Lin'an, Tianmushan, 18. vii. 2007, Yajun Zhu; 1 ♂ (CAUC), CHINA, Zhejiang: Lin'an, Tianmushan, Huoshandashigu, 21. vii. 2007, Yajun Zhu.



Figures 6–10. *Minettia (Plesiminettia) longaculiformis* sp. n. Male. **6** syntergosternite 7+8 and epanandrium, lateral view **7** syntergosternite 7+8, anterior view **8** epandrial complex, posterior view **9** aedeagal complex, ventral view **10** aedeagal complex, lateral view. Scale bar: 0.1 mm.

Etymology. Latin, *Longi-* is from the Latin, *longus*, meaning long; *-acicula* is the diminutive of the Latin noun *acus*, meaning needle, or pin; *-formis* is from the Latin *forma*, meaning shape; referring to the surstylus with 2 pairs of long needle-like processes; a feminine adjective.

Diagnosis. Body yellow. Mesonotum with anteriormost dorsocentral setae situated midway between transverse scutal suture and scutoscutellar suture. Legs yellow, except brown at tip of tibiae and tarsomeres 3–5 pale brown; hind tibia with one weak preapical anterodorsal seta. Male genitalia: surstylus with a pair of long needle-like processes in lateral view; phallus round apically with a pair of long processes curved upward in ventral view. Female sternite eight confluent with tergite 8, projecting on posterior margin with dense setae.

Description. Male. Body length 6.5–8.0 mm, wing length 6.5–7.0 mm. Female. Body length 7.0–8.5 mm, wing length 6.6–7.0 mm.

Head yellow. Frons with ocellar triangle yellow; ocellar setulae developed, longer than posterior fronto-orbital setae, anterior fronto-orbital setae reclinate, shorter than posterior setae. Face with sparse grayish white pollinosity, without spot; gena about 1/5 height of eye. Antenna entirely yellow, 1st flagellomere 1.7 times longer than high; arista black except yellow at base, pubescent; rays of arista with longest setulae slightly shorter than 1/3 height of 1st flagellomere. Proboscis yellow except brown tip and palpus brownish yellow.

Thorax yellow with sparse grayish white pollinosity. Mesonotum with 0+3 dorsocentral setae (anteriormost dorsocentral setae situated midway between transverse scutal suture and scutoscutellar suture), acrostichal setulae in eight rows; a pair of long acrostichal setulae present in front of prescutellar setae, prescutellar setae shorter than 1st postsutural dorsocentral setae; one strong intra-alar seta and one weak intra-alar seta situated at almost equal intervals on line between supra-alar setae and posterior dorsocentral setae; one anepisternal seta, two katepisternal setae. Scutellum yellow. Legs yellow except brown at tip of tibiae and tarsomeres 3–5 pale brown. Fore femur with six posteroventral setae and nine posterodorsal setae, fore tibia with 1 short preapical anterodorsal seta and one short apicoventral seta. Mid femur with eight anterior setae and one apical posterior seta; mid tibia with one strong preapical anterodorsal seta and two strong apicoventral setae. Hind tibia with one weak preapical anterodorsal seta and one short apicoventral seta. Wing (Fig. 44) slightly yellow, pale brown along costal margin and a brown stripe on *dm-cu*; costa with 2nd (between R_1 and R_{2+3}), 3rd (between R_{2+3} and R_{4+5}) and 4th (between R_{4+5} and M_1) sections in proportion of 5.5:1.5:1; *r-m* at middle of discal cell; ultimate and penultimate sections of M_1 in proportion of 1:1.5; ultimate section of CuA_1 approximately 1/10 of penultimate. Halter yellow.

Abdomen yellow with sparse grayish white pollinosity. Male genitalia (Figs 6–10): syntergosternite 7+8 circular with long irregular ventral process and many dorsal setae; epandrium broad, far shorter than syntergosternite 7+8, narrow apically; surstylus with a pair of long needle-like processes in lateral view; hypandrium inverted-U shape, hypandrial apodeme indistinct; pregonite and postgonite absent; phallus slender, longer than 1/2 length of abdomen, round apically with a pair of long subuliform processes

curved upward in ventral view; phallapodeme short, projecting forward. Female sternite eight confluent with tergite 8, projecting on posterior margin with dense setae; spermathecae 1+2, nearly elliptical, each with irregular short ridges. (Figs 34–35).

Remarks. The new species differs entirely from other species of the subgenus in the surstylus having a pair of very long needle-like processes and the phallus being brown, longer than 1/2 length of abdomen in ventral view.

Distribution. China (Zhejiang).

***Minettia (Plesiominettia) nigrantennata* sp. n.**

<http://zoobank.org/62C0D97E-160A-41B2-8800-FD17F2E614A4>

Figs 11–15, 45

Type material. Holotype: ♂ (CAUC), CHINA, Hunan: Changde, Shimen, Hupingshan National Nature Reserve, Zhipeng River, 450 m, 6. vi. 2008, Li Shi.

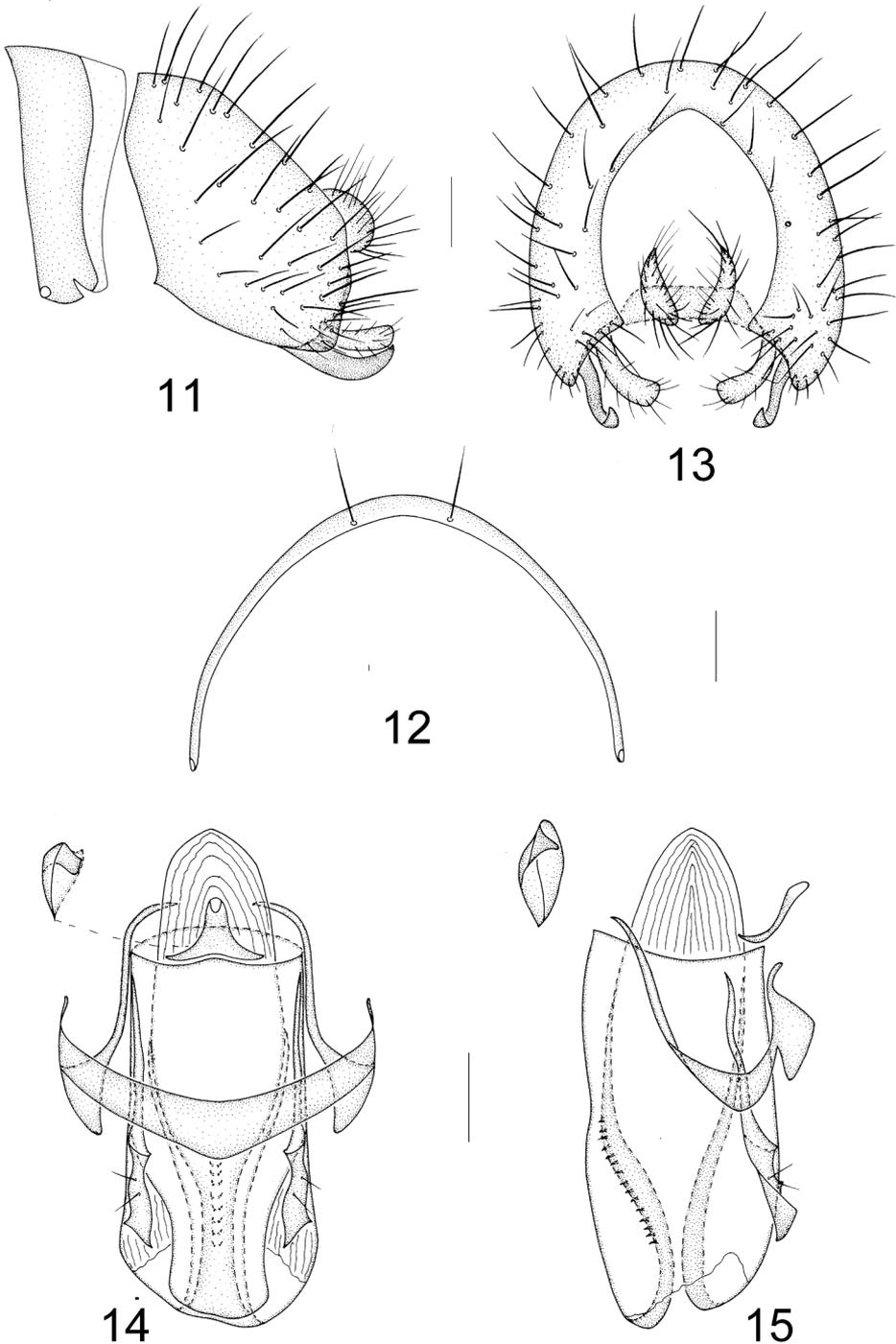
Etymology. Latin, *nigr-* is from the Latin, *nigra*, meaning black, + *antennata*, meaning antenna; referring to the blackish brown antenna; feminine adjective.

Diagnosis. Arista pubescent, rays of arista with longest setulae shorter than 1/3 height of 1st flagellomere. Mesonotum with anteriormost dorsocentral setae slightly beyond suture. Legs black, except basal tip of tibia yellow, fore tarsus brown, mid and hind tarsomeres 1–2 dark yellow and tarsomeres 3–5 brown; hind femur with a row of anteroventral seta on apical half. Male genitalia: surstylus consisting of a brown knife-like apical process and a yellow bar-like apical process with setulae in lateral view; postgonite narrow basally and broad contorted apically with 2 short setulae; phallus very broad, columnar, with a pair of inner sclerites and a row of tiny spinule, visible in dorsal view.

Description. Male. Body length 4.6 mm, wing length 5.0 mm.

Head blackish brown. Frons with narrow yellow margin; ocellar triangle grayish black; ocellar setulae developed, slightly longer than anterior fronto-orbital setae, anterior fronto-orbital setae reclinate, shorter than posterior fronto-orbital setae. Face and parafacial dark black; gena approximately 1/6 height of eye. Antenna blackish brown, 1st flagellomere 1.6 times longer than high; arista black, except pale brown at base, pubescent, rays of arista with longest setulae shorter than 1/3 height of 1st flagellomere. A blackish brown spot present between eye and base of antenna. Proboscis blackish brown and palpus black.

Thorax black with dense brownish pollinosity. Mesonotum with 0+3 dorsocentral setae (anteriormost dorsocentral setae slightly beyond suture), acrostichal setulae in 6 irregular rows, prescutellar setae as long as first postsutural dorsocentral setae; 1 strong intra-alar seta, one anepisternal seta, two katepisternal setae. Scutellum black with dense brown pollinosity. Legs black, except basal tip of tibia yellow, fore tarsus brown, mid and hind tarsomeres 1–2 dark yellow and tarsomeres 3–5 brown. Fore femur with six posteroventral setae and eight posterodorsal setae, fore tibia with one short preapical anterodorsal seta and one short apicoventral seta. Mid femur with five anterior setae



Figures 11–15. *Minettia* (*Plesiominettia*) *nigrantennata* sp. n. Male. **11** syntergosternite 7+8 and epandrium, lateral view **12** syntergosternite 7+8, anterior view **13** epandrial complex, posterior view **14** aedeagal complex, ventral view **15** aedeagal complex, lateral view. Scale bars: 0.1 mm.

and one apical posterior seta; mid tibia with one strong preapical anterodorsal seta and one strong apicoventral seta. Hind femur with a row of anteroventral seta on apical half, one short preapical anterodorsal seta and two apical posterior setae, hind tibia with one short preapical anterodorsal seta and one short apicoventral seta. Wing (Fig. 45) slightly dark yellow; costa with 2nd (between R₁ and R₂₊₃), 3rd (between R₂₊₃ and R₄₊₅) and 4th (between R₄₊₅ and M₁) sections in proportion of 6.5:1.7:1; *r-m* beyond middle of discal cell; ultimate and penultimate sections of M₁ in proportion of 1:1.3; ultimate section of CuA₁ approximately 1/9 of penultimate. Halter yellow.

Abdomen blackish brown with sparse brownish pollinosity, subglossy. Male genitalia (Figs 11–15): syntergosternite 7+8 semicircular with a pair of dorsal setulae; epandrium broad with long dorsal setae in lateral view; surstylus consisting of a brown knife-like apical process and a yellow bar-like apical process with setulae in lateral view; hypandrium slightly V-shaped, hypandrial apodeme very small; postgonite narrow basally, broad and contorted apically, with two short setulae; phallus very broad columnar, ventral sclerite narrow apically and slightly shorter than dorsal sclerite, and medial membranous section beyond base of phallus sclerites, with a pair of inner sclerites and a row of tiny spinule, visible in dorsal view; phallapodeme short, Y-shaped, slightly projecting forward.

Female. Unknown.

Remarks. See *Minettia* (*P.*) *tridentata* sp. n.

Distribution. China (Hunan).

***Minettia* (*Plesiominettia*) *tridentata* sp. n.**

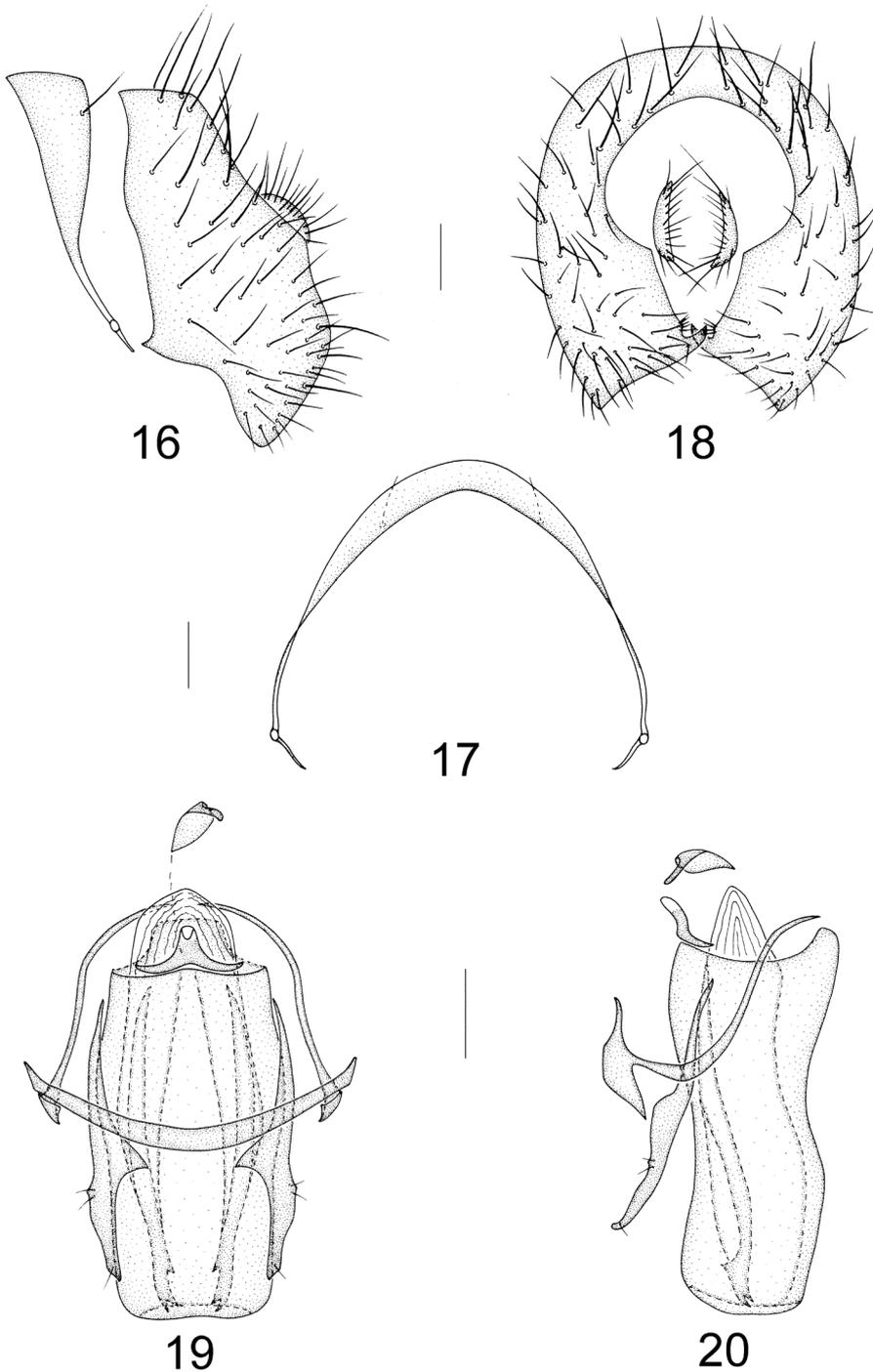
<http://zoobank.org/A3170A09-8EE1-4D2A-8C39-F33835BF1F54>

Figs 16–20, 36–38, 46

Type material. Holotype: ♂ (CAUC), CHINA, Hunan: Changde, Shimen, Hupingshan National Nature Reserve, Zhipeng River, 450 m, 6. vi. 2008, Kuiyan Zhang. Paratypes: 2 ♂♂, 3 ♀♀ (CAUC), CHINA, Hunan: data same as holotype; 5 ♀♀ (CAUC), CHINA, Hunan: Changde, Shimen, Hupingshan National Nature Reserve, Zhipeng River, 450 m, 6. vi. 2008, Li Shi

Etymology. Latin, *tri-*, meaning three, + *dentata*, meaning toothed; referring to a pair of subuliform inner sclerites of the phallus each with 3 acute apical teeth; a feminine adjective

Diagnosis. Antennal scape blackish brown, pedicel yellow, 1st flagellomere yellow except upper and apical edges black and brown on apical half; rays of arista with longest setulae shorter than 1/4 height of 1st flagellomere. Mesonotum with anterior-most dorsocentral setae slightly beyond suture. Legs black, except mid and hind tibiae dark yellow on basal 2/3 and tarsomeres 3–5 blackish brown. Male genitalia: surstylus broad triangular in lateral view, with a tiny acute process and a small triangular apical process in ventral view. Female sternite eight confluent with tergite 8, sternite 9 narrow triangular apically and slightly curved.



Figures 16–20. *Minettia (Plesiominettia) tridentata* sp. n. Male. **16** sytergosternite 7+8 and epandrium, lateral view **17** sytergosternite 7+8, anterior view **18** epandrial complex, posterior view **19** aedeagal complex, ventral view **20** aedeagal complex, lateral view. Scale bar: 0.1 mm.

Description. Male. Body length 3.8–4.3 mm, wing length 3.7–4.3 mm. Female. Body length 3.5–4.0 mm, wing length 3.9–4.4 mm.

Head blackish brown. Frons with narrow yellow margin; ocellar triangle grayish black; ocellar setulae developed, slightly shorter than anterior fronto-orbital setae, anterior fronto-orbital setae reclinate, shorter than posterior fronto-orbital setae. Face dark black and parafacial grayish black. Gena approximately 1/5 height of eye. Antennal scape blackish brown and pedicel yellow, 1st flagellomere yellow except upper and apical edges black and brown on apical half, 1st flagellomere 1.4 times longer than high; arista black, with microscopic setulae, and rays of arista with longest setulae shorter than 1/4 height of 1st flagellomere. A blackish brown spot present between eye and base of antenna. Proboscis blackish brown and palpus black.

Thorax black to blackish brown with dense brownish pollinosity. Mesonotum with 0+3 dorsocentral setae (anteriormost dorsocentral setae slightly beyond suture), acrostichal setulae in eight irregular rows, prescutellar setae as long as 1st postsutural dorsocentral setae; one anepisternal seta, two katapisternal setae. Scutellum black with dense brown pollinosity. Legs black, except mid and hind tibiae dark yellow on basal 2/3 and tarsomeres 3–5 blackish brown. Fore femur with 5–6 posteroventral setae and 10 posterodorsal setae, fore tibia with one short preapical anterodorsal seta and one short apicoventral seta. Mid femur with 5–6 anterior setae, one apical posterior seta and one apicoventral seta; mid tibia with one strong preapical anterodorsal seta and one strong apical posterior seta. Hind femur with a row of anteroventral seta on apical half and one short preapical anterodorsal seta, hind tibia with one short preapical anterodorsal seta and one short apical posterior seta. Wing (Fig. 46) slightly dark yellow; costa with 2nd (between R₁ and R₂₊₃), 3rd (between R₂₊₃ and R₄₊₅) and 4th (between R₄₊₅ and M₁) sections in proportion of 5.7:1.5:1; *r-m* at middle of discal cell; ultimate and penultimate sections of M₁ in proportion of 1:1.1; ultimate section of CuA₁ approximately 1/6 of penultimate. Halter yellow.

Abdomen blackish brown with sparse brownish pollinosity, subglossy. Male genitalia (Figs 16–20): syntergosternite 7+8 semicircular with a pair of dorsal setulae; epandrium narrow basally with long setae and broad apically; surstylus broad triangular in lateral view, with a tiny acute process and a small triangular apical process in ventral view; hypandrium slightly V-shaped, hypandrial apodeme short; postgonite slender with a triangular medial process and two subapical setulae and one apical setula in ventral view; phallus broad columnar, with a trapezoidal basal process and a pair of subuliform inner sclerites each with three acute apical teeth, and medial membranous section beyond base of phallus in ventral view; phallapodeme short, Y-shaped, slightly projecting forward. Female sternite 8 confluent with tergite 8, sternite 9 narrow triangular apically and curved slightly; spermathecae 1+2, round with short brown stem (Figs 36–38).

Remarks. The new species is very similar to *Minettia* (*P.*) *nigrantennata* **sp. n.** from China (Hunan) in the following characters: body blackish brown; frons with yellow anterior margin and face dark black; thorax black to blackish brown with dense brownish pollinosity, mesonotum with 0+3 dorsocentral setae (anteriormost dorsocentral setae

slightly beyond suture) and prescutellar setae as long as 1st postsutural dorsocentral setae; wing slightly dark yellow; abdomen blackish brown with sparse brownish pollinosity, subglossy. It can be separated from the latter by the yellow antennal 1st flagellomere, except upper and apical edges being black and brown on apical half; the legs being black, except mid and hind tibiae being dark yellow on basal 2/3. In *Minettia* (*P.*) *nigrantennata*, the antennal 1st flagellomere is blackish brown and the legs are black, except basal tip of tibia is yellow, mid and hind tarsomeres 1–2 are dark yellow.

Distribution. China (Hunan).

***Minettia* (*Plesiominettia*) *zhejiangica* sp. n.**

<http://zoobank.org/A692F0A2-DC3E-4017-8A98-15A69A4E7C67>

Figs 21–25, 39–40, 47

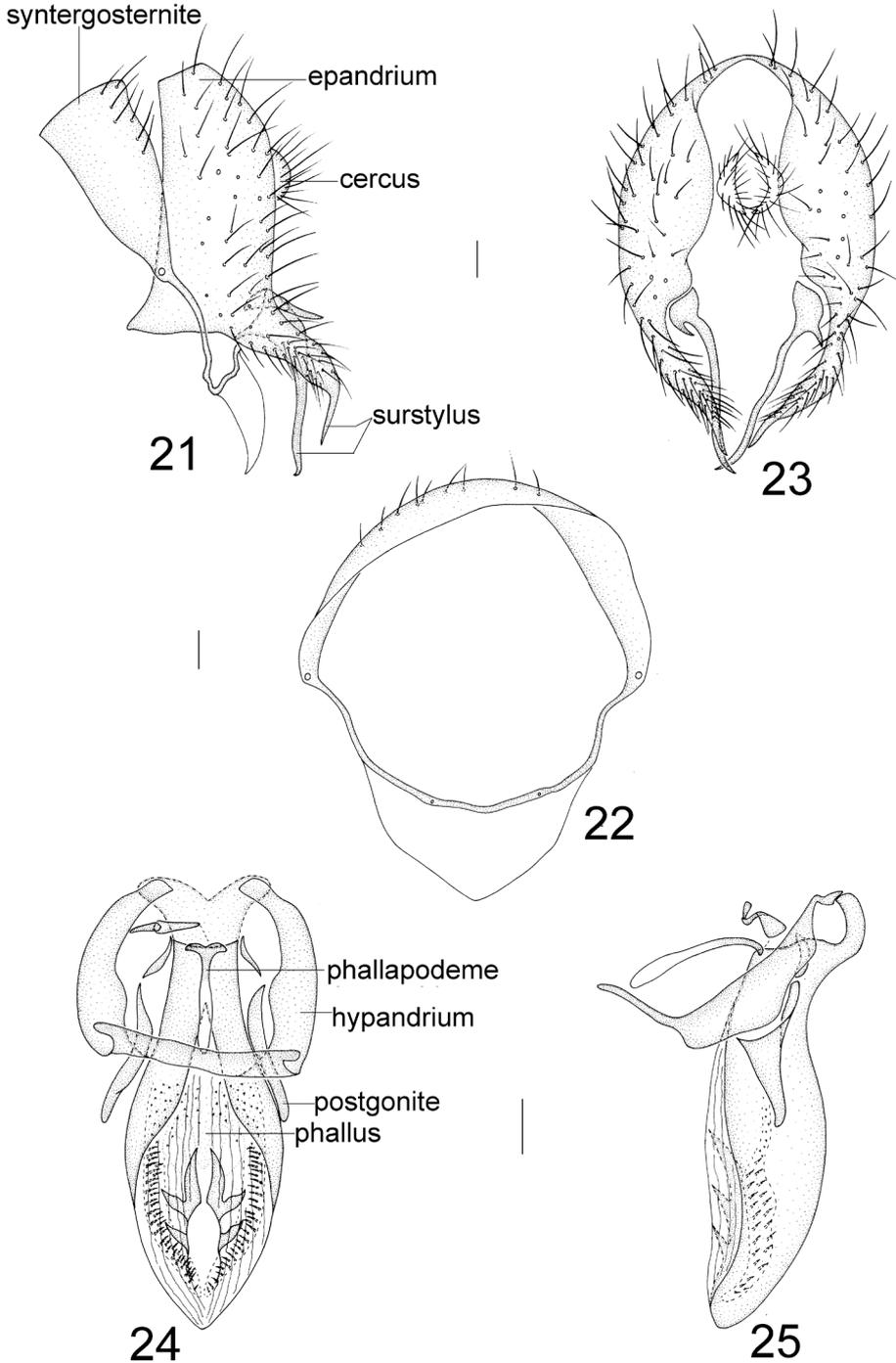
Type material. Holotype: ♂ (CAUC), CHINA, Zhejiang: Longquan, Fengyangshan National Nature Reserve, Fengyang Lake, 28. vii. 2007, Yajun Zhu. Paratypes: CHINA, Zhejiang: 1 ♂, 2 ♀♀ (CAUC), Longquan, Fengyangshan National Nature Reserve, 26. vii. 2007, Yajun Zhu; 1 ♂ (CAUC), Longquan, Fengyangshan National Nature Reserve, Huangmaojian, 27. vii. 2007, Yajun Zhu; 1 ♀ (CAUC), Longquan, Fengyangshan National Nature Reserve, Huangmaojian, 29. vii. 2007, Yajun Zhu; 1 ♀ (CAUC), Longquan, Fengyangshan National Nature Reserve, Qixingtian, 1. viii. 2007, Yajun Zhu; 1 ♂, 1 ♀ (CAUC), Lin'an, Tianmushan National Nature Reserve, 19. vii. 2007, Yajun Zhu.

Etymology. The new species is named after the type locality, Zhejiang Province.

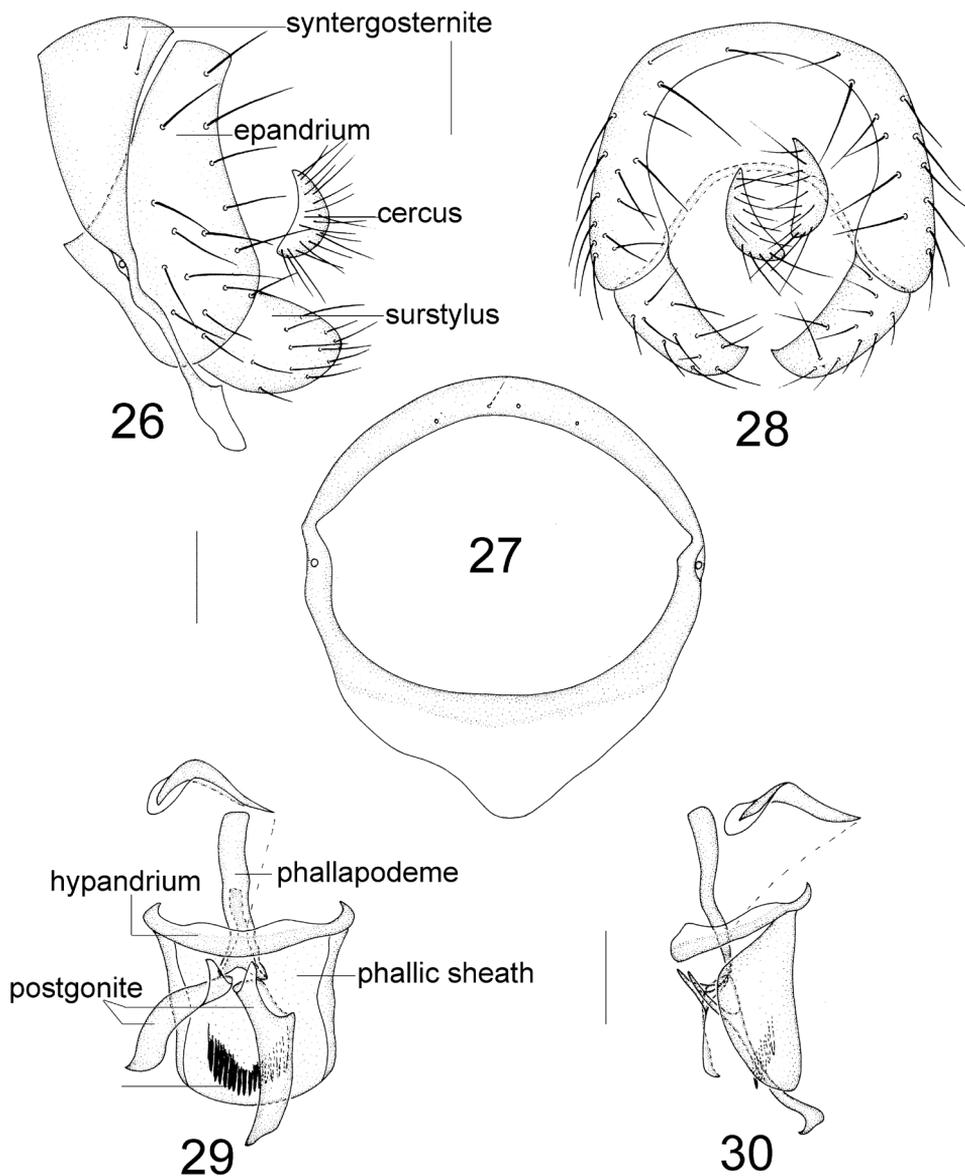
Diagnosis. Frons with sparse whitish gray pollinosity. Face pale brown on dorsal 1/2 and black on ventral 1/2, with sparse whitish gray pollinosity; parafacial yellow, inner margin blackish brown on ventral 1/2. Arista short plumose, rays of arista with longest setulae as long as 1/2 height of 1st flagellomere. Mesonotum with anteriormost dorsocentral setae situated on basal 1/3 between transverse scutal suture and scutoscutellar suture. Male genitalia: surstylus with a curved needle-like inner process and a geniculate outer process, acute apically.

Description. Male. Body length 6.0–6.2 mm, wing length 5.9–6.3 mm. Female. Body length 5.6–6.2 mm, wing length 5.6–6.7 mm.

Head pale brown. Frons with sparse whitish gray pollinosity, pale yellow on narrow anterior margin; ocellar triangle grayish black; ocellar setulae developed, longer than posterior fronto-orbital setae, anterior fronto-orbital setae reclinate, slightly shorter than posterior fronto-orbital setae. Face pale brown on dorsal 1/2 and black on ventral 1/2, with sparse whitish gray pollinosity; parafacial yellow, blackish brown on ventral 1/3, inner margin glossy black. Gena approximately 1/5 height of eye. Antenna brownish yellow, 1st flagellomere pale brown on apical 2/3, 1st flagellomere 2.0 times longer than high; arista black except yellow at base, short plumose, rays of arista with longest setulae as long as 1/2 height of 1st flagellomere. A grayish black triangular spot present between eye and base of antenna. Proboscis brown and palpus black.

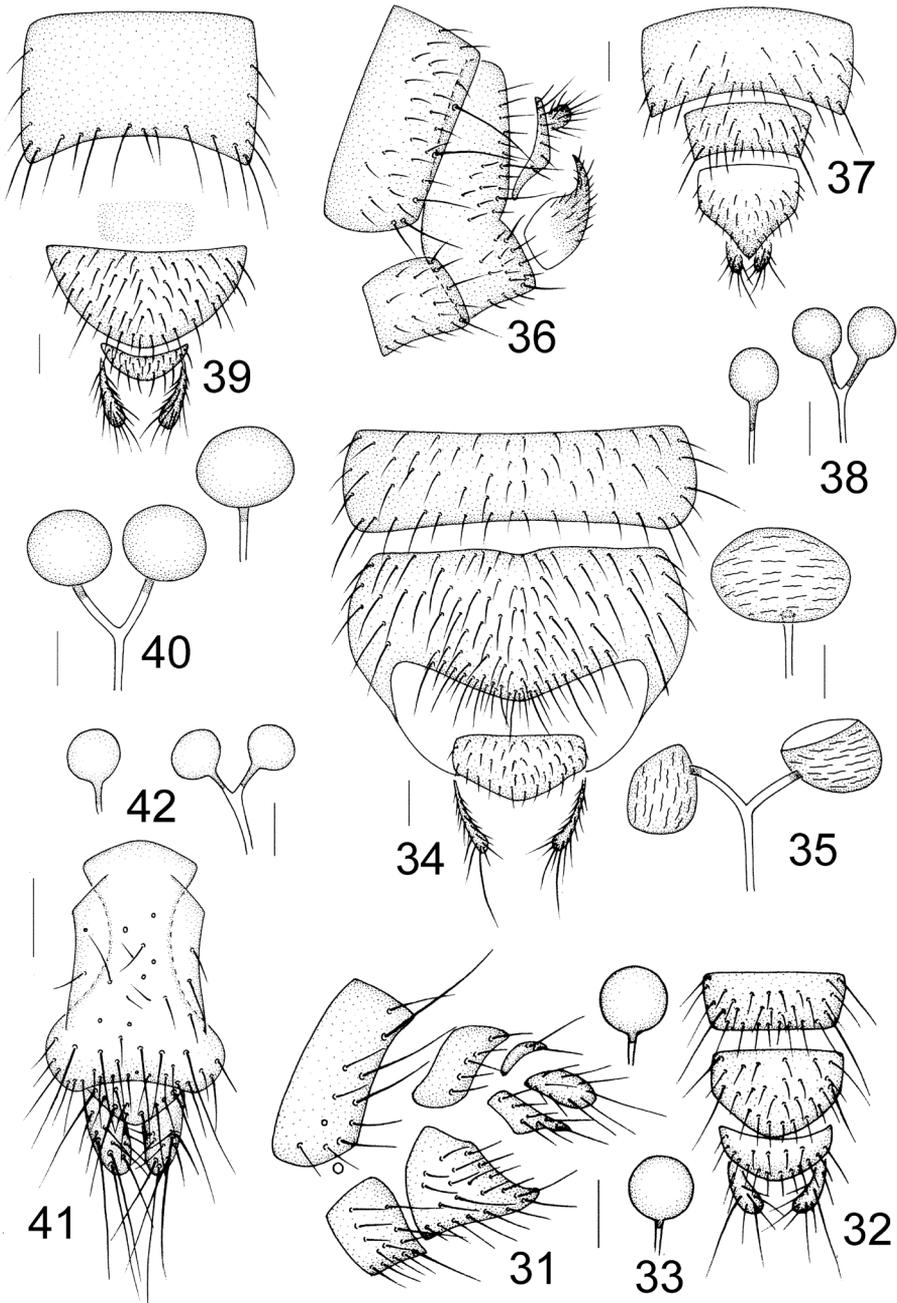


Figures 21–25. *Minettia (Plesiminettia) zhejiangica* sp. n. Male. **21** syntergosternite 7+8 and epandrium, lateral view **22** syntergosternite 7+8, anterior view **23** epandrial complex, posterior view **24** aedeagal complex, ventral view **25** aedeagal complex, lateral view. Scale bar: 0.1 mm.

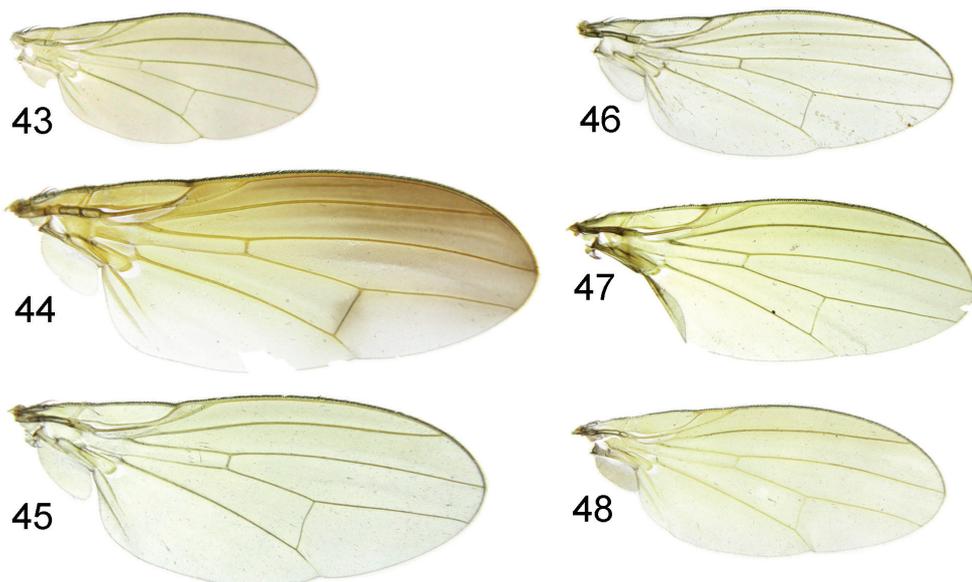


Figures 26–30. *Minettia* (*Minettia*) *lupulina* (Fabricius). Male. **26** sytergosternite 7+8 and epandrium, lateral view **27** sytergosternite 7+8, anterior view **28** epandrial complex, posterior view **29** aedeagal complex, ventral view **30** aedeagal complex, lateral view. Scale bar: 0.1 mm.

Thorax brown with grayish pollinosity, anterior half sparse and posterior half dense. Mesonotum with 0+3 dorsocentral setae (anteriormost dorsocentral setae situated on basal 1/3 of mesonotum), acrostichal setulae in eight rows; a pair of long acrostichal setulae present in front of prescutellar setae, prescutellar setae longer than



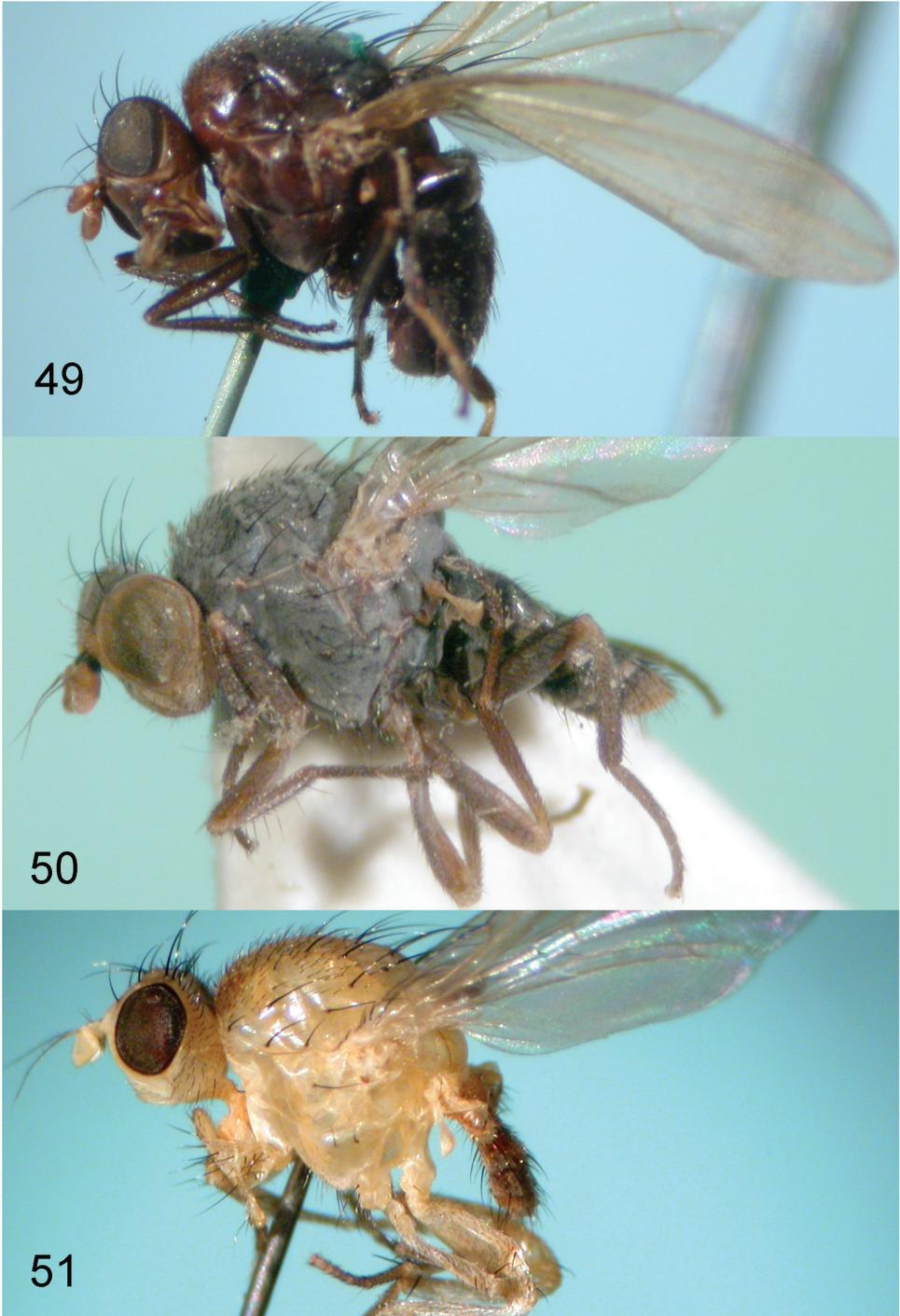
Figures 31–42. Female terminalia. *Minettia (Plesioinettia) flavoscutellata* sp. n. **31, 32** sternites 7–9, lateral and ventral view **33** spermathecae. *Minettia (Plesioinettia) longaculiformis* sp. n. **34** sternites 7–9, ventral view **35** spermathecae. *Minettia (Plesioinettia) tridentata* sp. n. **36, 37** sternites 7–9, lateral and ventral view **38** spermathecae. *Minettia (Plesioinettia) zhejiangica* sp. n. **39** sternites 7–9, ventral view **40** spermathecae. *Minettia (Minettia) lupulina* (Fabricius) **41** sternites 8–9, ventral view **42** spermathecae. Scale bar: 0.1 mm.



Figures 43–48. Wing. **43** *Minettia* (*Plesiominettia*) *flavoscutellata* sp. n. **44** *Minettia* (*Plesiominettia*) *longaciculiformis* sp. n. **45** *Minettia* (*Plesiominettia*) *nigrantennata* sp. n. **46** *Minettia* (*Plesiominettia*) *tridentata* sp. n. **47** *Minettia* (*Plesiominettia*) *zhejiangica* sp. n. **48** *Minettia* (*Minettia*) *lupulina* (Fabricius).

1st postsutural dorsocentral setae; one strong intra-alar seta. Anepisternum black and katepisternum brown, both with sparse grayish pollinosity; one prescutellar setae, two katepisternal setae. Scutellum blackish brown with grayish pollinosity. Legs with femora black; tibiae brown except yellow on basal tip; tarsi dark yellow except tarsomeres 3–5 pale brown. Fore femur with 5–6 posteroventral setae and ten posterodorsal setae; fore tibia with one short preapical anterodorsal seta and one short apicoventral seta. Mid femur with six anterior setae and one apical posterior seta; mid tibia with one strong preapical anterodorsal seta and two strong apicoventral seta. Hind femur with a weak preapical anterodorsal seta; hind tibia with one preapical anterodorsal seta and one short apicoventral seta. Wing (Fig. 47) slightly yellow, pale brown at base; costa with 2nd (between R_1 and R_{2+3}), 3rd (between R_{2+3} and R_{4+5}) and 4th (between R_{4+5} and M_1) sections in proportion of 5:1.5:1; *r-m* at middle of discal cell; ultimate and penultimate sections of M_1 in proportion of 1:1.2; ultimate section of CuA_1 approximately 1/6 of penultimate. Halter yellow.

Abdomen black with grayish white pollinosity. Male genitalia (Figs 21–25): sytergosternite 7+8 circular with a long irregular ventral process and many dorsal setulae; epandrium slender, slightly projecting at anterior ventral corner; surstylus with a curved aciculiform inner process and a geniculate outer process, acute apically; hypandrium inverted-U shape, hypandrial apodeme very short; postgonite short claviform in ventral view; phallus broad at middle, three acute processes and many spinules on membranous section in ventral view and slender and round apically in lateral view;



Figures 49–51. Habitus. **49** *Minettia* (*Plesiominettia*) *divaricata* Sasakawa, holotype male, OMNH **50** *Minettia* (*Plesiominettia*) *gemmata* Shatalkin (paratype female of junior synonym *Minettia kimi* Sasakawa & Kozanek, OMNH) **51** *Minettia* (*Plesiominettia*) *punctata* Sasakawa, holotype male, OMNH.

phallapodeme small, projecting forward. Female sternite 7 rectangular and sternite 8 semicircular; spermathecae 1+2, nearly rounded and stem brown at base (Figs 39–40).

Remarks. The new species is similar to *Minettia* (*P.*) *longistylis* Sasakawa from China (Taiwan) in size (large), mesonotum brown with grayish pollinosity, a pair of long acrostichal setulae present in front of prescutellar setae. It can be separated from the latter by the arista being short plumose, the rays of arista having longest setulae as long as 1/2 height of antennal 1st flagellomere; the mesonotum having acrostichal setulae in eight rows; the mid tibia having two posteroventral setae; the surstylus having a curved aciculiform inner process and a geniculate outer process, acute apically. In *Minettia* (*P.*) *longistylis*, the arista is pubescent and the rays of arista have longest setulae as long as 1/4 height of antennal 1st flagellomere; the mesonotum has acrostichal setulae in ten irregular rows; the mid tibia has one posteroventral seta; the surstylus is very long (Sasakawa 2002).

Distribution. China (Zhejiang).

Key to the subgenera of *Minettia* and the species of the subgenus *Plesiominettia*

[Modified from Stuckenberg 1971, Shatalkin 2000 and Shi and Yang 2014]

- 1 Frons shiny and face flat; arista pubescent; mesonotum with 0–1+2–3 dorsocentral setae and 0–1+2–4 long acrostichal setulae; katepisternum with 1 strong katepisternal seta; male genitalia: phallic sheath present subgenus ***Minettiella* Malloch**
- Frons often dull and face slightly concave; arista pubescent or plumose; mesonotum with 0–1+3 dorsocentral setae and 0+2–3 long acrostichal setulae; katepisternum with 1 strong and 1 weak katepisternal setae; male genitalia: phallic sheath absent **2**
- 2 Lower part of face with a distinct, slight and weakly round swelling on each side **3**
- Lower part of face without round swelling on each side **4**
- 3 Basal part of wing black; arista long plumose, rays of arista with longest setulae longer than height of 1st flagellomere (rarely shorter than height of 1st flagellomere); male genitalia: two pairs of ventral hypandrial appendages (including some Palearctic and Oriental species)....subgenus ***Frendelia* Collin**
- Basal part of wing yellow; arista short plumose, rays of arista with longest setulae as long as 1/2 height of 1st flagellomere; male genitalia: one pair of ventral hypandrial appendages (such as *M. eoa* Shatalkin, 1992) part of subgenus ***Scotominettia* Shatalkin**
- 4 Male genitalia: one pair of ventral hypandrial appendages (such as *Minettia austriaca* Hennig, 1951)..... part of subgenus ***Scotominettia* Shatalkin**
- Male genitalia: hypandrial appendages often transverse, bar–like, U–shaped or other shapes..... **5**

- 5 Arista with gentle setulae, rays of arista with longest setulae as long as or shorter than 1/4 height of 1st flagellomere, sometimes bare; male genitalia: phallic sheath absent (Fig. 24)subgenus ***Plesiominettia* Shatalkin...6**
- Arista pubescent or plumose, rays of arista with longest setulae longer than 1/3 height of 1st flagellomere; male genitalia: phallic sheath present (Fig. 29), square, rectangular, triangular or trapezial in shape (for example: *Minettia* (*Minettia*) *lupulina* (Fabricius, 1787), Figs 26–30, 41–42, 48) subgenus ***Minettia* Robineau-Desvoidy**
- 6 Mesonotum with presutural dorsocentral setae 7
- Mesonotum without presutural dorsocentral setae **8**
- 7 Frons with dark gray stripes through *or* rows; mesonotum and scutellum dark gray; wing with dark costal margin ***M. (P.) styriaca* (Strobl)**
- Frons without stripes; mesonotum yellowish brown and scutellum yellow; wing faintly yellow..... ***M. (P.) ishidai* (Sasakawa)**
- 8 Mesonotum without strong acrostichal setae before prescutellar setae **9**
- Mesonotum with 1–2 pairs of strong acrostichal setae before prescutellar setae..... **13**
- 9 Body entirely yellow ***M. (P.) filia* (Becker)**
- Body partly black or entirely brown to black (entire thorax with grey pruinose in *M. kimi* which is a junior synonym of *M. (P.) gemmata* Shatalkin, fig. 50)..... **10**
- 10 Acrostichal setulae in 8 irregular rows; male genitalia: epandrium and surstylus fused; phallus broad and truncate apically (Figs. 16, 19)..... ***M. (P.) tridentata* sp. n.**
- Acrostichal setulae in 6 rows; male genitalia: epandrium and surstylus articulate, blunt apically lateral view; phallus narrow or rounded apically **11**
- 11 Face yellow with a large black round median spot above ventral margin; arista with microscopic rays; abdominal tergites 2–5 each with brownish yellow posterior margin ***M. (P.) gemmata* Shatalkin**
- Face brown to black without black medial spot; rays of arista with longest setulae slightly shorter than 1/3 height of 1st flagellomere or longer than half height of 1st flagellomere; abdominal tergites 2–5 without brownish yellow posterior margin **12**
- 12 Arista pubescent, rays of arista with longest setulae slightly shorter than 1/3 height of 1st flagellomere; abdomen blackish brown with sparse brownish pollinosity, subglossy; male genitalia: surstylus with narrow double processes in lateral view (Fig. 11) ***M. (P.) nigrantennata* sp. n.**
- Arista plumose, rays of arista with longest setulae as long as 3/5–4/5 height of 1st flagellomere; abdominal tergites brownish yellow along medial line and dark brown to black on lateral margins; male genitalia: surstylus short and broad in lateral view (Shatalkin 2000: Fig. 95)..... ***(P.) gemina* Shatalkin**
- 13 Mesonotum with two pairs of strong acrostichal setae..... **14**

- Mesonotum with one pair of strong acrostichal setae **16**
- 14 Body black except frons, face, mesonotum, scutellum and metanotum yellow; male genitalia: surstylus blunt and with hairy outgrowths (Remm and Elberg 1979: Fig. 13)..... *M. (P.) loewi* (Schiner)
- Body entirely brown to black including mesonotum, scutellum and metanotum brown to black; male genitalia: surstylus short broad or in other shape..... **15**
- 15 Arista with microscopic rays, rays of arista with longest setulae as long as 1/7 height of 1st flagellomere; frons and legs entirely brown; male genitalia: surstylus widened apically and narrow subapically, with a small concavity at middle of apical edge (Shatalkin 2000: fig. 94, Remm and Elberg 1979: Fig. 12)..... *M. (P.) helvola* (Becker)
- Arista short plumose, rays of arista with longest setulae at least longer than 1/3 height of 1st flagellomere; frons yellow on ventral 1/5; legs dark brown except base of tibiae and tarsi yellowish; male genitalia: surstylus consisting of outer process narrow basally and bifurcated apically, and inner process clubbed and slender in lateral view (Sasakawa 1985: Fig. 2A–C) *M. (P.) divaricata* Sasakawa
- 16 Body entirely yellow (Fig. 51)..... **17**
- Body mostly brown to black **18**
- 17 Mesonotum with anteriormost dorsocentral setae slightly longer than length of acrostichal setulae rows, and distinctly shorter than other dorsocentral setae; scutellum without large black lateral spots on lateral margin.....
..... *M. (P.) helva* Czerny
- Mesonotum with anteriormost dorsocentral setae distinctly stronger than other dorsocentral setae; scutellum with a pair of large black lateral spots on lateral margin (Fig. 51) *M. (P.) punctata* Sasakawa
- 18 Mesonotum with acrostichal setulae in 4–6 rows **19**
- Mesonotum with acrostichal setulae in 8–10 rows **22**
- 19 Mesonotum with acrostichal setulae in 6 rows; body length 6.5 mm; male genitalia: surstylus with a pair of slender spatulate processes; phallus with 4 sharp apical processes (Shatalkin 2000: Fig. 90)..... *M. (P.) crassulata* Shatalkin
- Mesonotum with acrostichal setulae in 4 rows; body length 3.3–5.0 mm; male genitalia: surstylus without a pair of slender spatulate processes; phallus without sharp apical processes..... **20**
- 20 Arista short plumose, rays of arista with longest setulae as long as 1/2 height of 1st flagellomere; male genitalia: epandrium and surstylus articulated; surstylus with a long falcate process (Fig. 1)..... *M. (P.) flavoscutellata* sp. n.
- Rays of arista with longest setulae as long as or slightly longer than basal height of arista; male genitalia: epandrium and surstylus fused; surstylus in another shape..... **21**
- 21 Halter dark brown except stem yellowish at base; male genitalia: surstylus slender and curved, claviform, acute apically (Shatalkin 2000: Fig. 97).....
..... *M. (P.) fuscescens* Shatalkin

- Halter entirely yellow; male genitalia: surstylus short and rounded apically (Shatalkin 2000: Fig. 96) ***M. (P.) tenebrica* Shatalkin**
- 22 Body yellow to dark yellow; male genitalia: surstylus with a pair of very long aciculiform processes (Fig. 6) ***M. (P.) longaciculiformis* sp. n.**
- Body brown to black; male genitalia with short claviform process or process shaped otherwise **23**
- 23 Mid tibia with 2 strong apicoventral setae; halter dark brown except stem yellowish; male genitalia: surstylus claviform narrowing gradually, with a tiny median process and tiny setulae in lateral view (Shatalkin 2000: Fig. 91)
..... ***M. (P.) omei* Shatalkin**
- Mid tibia with 1 strong apicoventral seta; halter yellow; male genitalia: surstylus in another shape, if claviform, then without a median process in lateral view **24**
- 24 Arista pubescent, rays of arista with longest setulae as long as 1/4 length of 1st flagellomere; acr in 10 irregular rows; mid tibia with 1 apicoventral seta; male genitalia: surstylus with a long clubbed process, curved ventrally at tip (Sasakawa 2002: Fig. 6) ***M. (P.) longistylis* Sasakawa**
- Arista short plumose, rays of arista with longest setulae as long as 1/2 length of 1st flagellomere; acrostichal setulae in 8 rows; mid tibia with 2 apicoventral setae; male genitalia: surstylus with an acute geniculate outer process and a curved needle-like inner process (Fig. 21) ***M. (P.) zhejiangica* sp. n.**

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Appendix

Genus *Minettia* Robineau-Desvoidy, 1830

Subgenus *Plesiominettia* Shatalkin, 2000

1. *Minettia* (*Plesiominettia*) *crassulata* Shatalkin, 1998a: 61. Holotype male, ZMAN. Type locality: China, Sichuan, Mt. Omei. Palaearctic: Russia. Oriental: China (Sichuan).
2. *Minettia* (*Plesiominettia*) *divaricata* Sasakawa, 1985: 5. Holotype male, OMNH. Type locality: Japan, Mie Prefecture, Osugidani. Palaearctic: Japan (Hokkaido, Kyushu).
3. *Minettia* (*Plesiominettia*) *filia* (Becker, 1895: 237) (*Sapromyza*). Syntypes female, possibly HZMZ and/or ZMHB. Type locality: Croatia, Dalmatia; Poland (“Dalmatien und Polen”). Palaearctic: Britain, Croatia, Czech Republic, Finland, Germany, Hungary, Ireland, Norway, Poland, Romania, Russia, Slovakia, Spain, Switzerland. [Note: the syntypes were in the collections of Langhoffer and Schnabl; most of the Langhoffer collection is in HZMZ, but some specimens are in ZMHB; the Schnabl collection was reported by Šifner (2008) as destroyed in WWII according to A. A. Stackelberg.]. *Combination Papp*, 1978: 223.
Minettia dissimilis Collin, 1966: 144. Holotype female, RSME. Type locality: Scotland. Dumbarton, Bonhill. Synonymy Shatalkin, 1998b: 815.
4. *Minettia* (*Plesiominettia*) *flavoscutellata* **sp. n.** Holotype male, CAUC. Type Locality: China, Hubei Province, Shennongjia National Natural Reserve, Pingqian. Oriental: China (Hubei).
5. *Minettia* (*Plesiominettia*) *fuscescens* Shatalkin, 1998b: 812. Holotype male, ZMUM. Type locality: Japan, Honshu, Nagano Prefecture, Chino-Shi. Palaearctic: Japan (Honshu, Nagano-Ken, Chino-Shi).
6. *Minettia* (*Plesiominettia*) *gemina* Shatalkin, 1992: 83. Holotype male, ZMUM. Type locality: Russia, Primorsky Krai, Ussuri District, Kamenushka. Palaearctic: Russia, Korea.
Minettia tarsata Sasakawa & Kozánek, 1995: 327. Holotype male, SNMC. Type locality: North Korea, Myohyangsan Mts., 5 km SW of Hyangsan. Synonymy Shatalkin, 1998b: 815.
7. *Minettia* (*Plesiominettia*) *gemmata* Shatalkin, 1992: 83. Holotype female, ZMUM. Type locality: Russia, Primorsky Krai, Ussuri District, Kamenushka. Palaearctic: Russia, Korea.

- Minettia kimi* Sasakawa and Kozánek, 1995: 323. Holotype female, SNMC. Type locality: North Korea, Ryongaksan Mts., 10 km W of Pyongyang. Synonymy Shatalkin, 1998b: 814.
8. *Minettia (Plesiominettia) helva* Czerny, 1932. Syntypes, 3 male and 2 female, NHMW. Type locality: “Unterlaufe des Amur” (=Russian Far East). Palaeartic: Russia.
 9. *Minettia (Plesiominettia) helvola* (Becker, 1895: 220) (*Sapromyza*). Syntypes male and female, HNHM. Type locality: Hungary; Russia. Palaeartic: Austria, Czech Republic, Estonia, Hungary, Latvia, Liechtenstein, Russia, Slovakia, Switzerland. [Note: the syntypes were in the collections of Thalhammer and Schnabl; most of the Thalhammer collection is in HNHM; the Schnabl collection was reported by Šifner (2008) as destroyed in WWII according to A. A. Stackelberg.]. Combination Czerny, 1932: 25.
 10. *Minettia (Plesiominettia) ishidai* (Sasakawa, 1985: 2) (*Prorhaphochaeta*). Holotype female, OMNH. Type locality: Japan, Hokkaido, Yukomanbetsu, Mt. Daisetsu. Palaeartic: Japan (Honshu), Russia.
 11. *Minettia (Plesiominettia) loewi* (Schiner, 1864: 104) (*Sapromyza*). Replacement name for *Sapromyza bicolor* Loew, 1858. Palaeartic: Austria, Czech Republic, Finland, France, Germany, Hungary, Japan, Latvia, Lithuania, Poland, Romania, Russia, Slovakia, Switzerland. Combination Czerny, 1932: 25.
Sapromyza bicolor Loew, 1858: 12. Syntypes male and female, possibly MNHW and/or ZMHB (Scholtz collection). Type locality: “Silesia” (=region of Central Europe, mostly Poland, partly Czech Republic and Germany). Preoccupied by Macquart, 1835: 403.
 12. *Minettia (Plesiominettia) longaculiformis* **sp. n.** Holotype male, CAUC. Type locality: China, Zhejiang Province, Lin’an, Tianmushan. Oriental: China (Zhejiang).
 13. *Minettia (Plesiominettia) longistylis* Sasakawa, 2002: 45. Holotype male, BPBM. Type locality: China, Taiwan, Mt. Alishan. Oriental: China (Taiwan). New subgenus combination.
 14. *Minettia (Plesiominettia) nigrantennata* **sp. n.** Holotype male, CAUC. Type locality: Chian, Hunan Province, Changde, Shimen, Hupingshan National Nature Reserve, Zhipeng River. Oriental: China (Hunan).
 15. *Minettia (Plesiominettia) omei* Shatalkin, 1998a: 61. Holotype male, ZMAN. Type locality: China, Sichuan, Mt. Omei, (between Oingyin and Chunyang). Palaeartic: Russia. Oriental: China (Sichuan).
 16. *Minettia (Plesiominettia) punctata* Sasakawa, 1985: 5. Holotype male, OMNH. Type locality: Japan, Kyushu, Fukuoka Prefecture, Aburayama. Palaeartic: Russia, Japan (Honshu, Kyushu).
 17. *Minettia (Plesiominettia) styriaca* (Strobl, 1892) (*Sapromyza*). Holotype female, NMBA. Type locality: Austria, Natterriegel, near Admont. Palaeartic: Austria, Finland. Combination with *Prorhaphochaeta* Czerny, 1932: 31. Combination Collin 1948: 225 (although the species was not mentioned, the genus *Prorhaphochaeta* was synonymized under *Minettia*).

18. *Minettia (Plesiominettia) tenebrica* Shatalkin, 1992: 84. Holotype male, ZMUM. Type locality: Russia, Primorsky Krai, Ussuri District, Kamenushka. Palaearctic: Russia.
19. *Minettia (Plesiominettia) tridentata* **sp. n.** Holotype male, CAUC. Type locality: China, Hunan Province, Changde, Shimen, Hupingshan National Nature Reserve, Zhipeng River. Oriental: China (Hunan).
20. *Minettia (Plesiominettia) zhejiangica* **sp. n.** Holotype male, CAUC. Type locality: China, Zhejiang Province, Longquan, Fengyangshan National Nature Reserve, Fengyang Lake. Oriental: China (Zhejiang).

A new case of an Holarctic element in the Colombian Andes: first record of *Cordyla* Meigen (Diptera, Mycetophilidae) from the Neotropical region

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Abstract

Three new species of Mycetophilidae – *Cordyla monticola* **sp. n.**, *Cordyla pseudopusilla* **sp. n.** and *Cordyla reducta* **sp. n.** – are described from the Colombian Andes, representing the first described species of *Cordyla* Meigen from the Neotropical region. Colour photos of their habitus, wing and terminalia are provided. The morphological affinities of male terminalia are discussed in a worldwide context. The distributional pattern of the genus clearly indicates a case of northern elements reaching the north-western region of the Neotropics that corresponds to a secondary extension of a Holarctic clade to the south.

Keywords

Diptera, Mycetophilidae, fungus gnats, *Cordyla*, Neotropical region, taxonomy, distribution

Introduction

Members of the monophyletic genus *Cordyla* Meigen, 1803 are well distinguished among fungus gnats (Diptera: Mycetophilidae) because of their considerably small size, strongly humpbacked habitus, reduced number of flagellar segments and swollen antepenultimate segment of palpus. The latter character is unique within fungus gnats worldwide, enabling immediate recognition while working with collections on the genus level.

Further identification of the species is based on several body-characters with emphasis on the number of flagellomeres, and colour and length of the swollen palpal segment. However, as usual, the most important set of the species level morphological characters is that of the male terminalia. According to their structure, the genus has been divided into three subgeneric groups as defined by Kurina (2001). Tuomikoski (1966) transferred *Cordyla* to the tribe Exechiini within which it has a rather isolated position (Rindal et al. 2009, Ševčík and Kjærandsen 2012).

Thirty-nine species are described in the limits of the genus so far, viz. twenty-four from the Palaearctic region, ten from the Nearctic region, three from the Oriental region and two from the Australasian region (Kurina and Oliveira 2013 and references therein). From undescribed species, the genus has also been known in the Neotropical region (Oliveira et al. 2007, Vockeroth 2009, Kurina and Oliveira 2013).

Over recent years the junior author has accumulated *Cordyla* specimens collected in Colombian Andes. The aim of this paper is to describe, illustrate and discuss three new *Cordyla* species from that material representing the first named species in the Neotropical region.

Material and methods

All material was collected with Malaise traps from the Colombian Andes at an altitude greater than 1900 m a.s.l. from 2001 to 2003. The collecting was performed during “The Colombian Arthropod Project (CAP)” – a collaborative arrangement between the Humboldt Institute in Villa de Leyva, Colombia, the University of Kentucky, and the Natural History Museum of Los Angeles County (LACM) – funded by U. S. National Science Foundation (NSF DEB 9972024) and the Humboldt Institute (see also <http://www.sharkeylab.org/biodiversity/static.php?app=colombia&page=index>). The material herein studied was collected from three protected areas of Colombia (see also <http://www.parquesnacionales.gov.co/> and Fig. 38) as follows: 1) the “Parque Nacional Natural Farralones de Cali” (“PNN Farralones de Cali” within the label data) located on the West Cordillera and characterized by a great variety of climates that are reflected in a variety of ecosystems, as cold regions with paramillos and its diverse vegetation, warm areas with plants with tabular roots and reaching considerable heights, and temperate areas with oaks and black oaks; 2) the “Santuario de Flora y Fauna Otún Quimbaya” (“SFF Otún Quimbaya” within the label data) characterized by evergreen sub-Andean jungle vegetation, in which the effect of the rainy and dry seasons is masked by the usual presence of mist formations and there is gradual replacement of low synchrony in the production of fruits and leaves, and is located on the West Cordillera, and 3) the “Santuario de Flora y Fauna Iguaque” (“SFF Iguaque” within the label data) locating on the East Cordillera, in a region of paramo and Andean forest ecosystems, including a representative sample of oak forest.

The examined material was initially stored in ethyl alcohol, within which most specimens – after study under a stereomicroscope Leica S8APO – are still preserved.

In case of several specimens, for more detailed study of male terminalia, they were detached and macerated in a solution of KOH, followed by neutralization in acetic acid and washing in distilled water. The remaining chitinous parts were thereafter inserted into glycerine for study including illustrations and preserved as glycerine preparations in polyethylene microvials (cf. Kurina 2003). A few specimens including their terminalia were slide mounted in Euparal following the method described by Hippa and Kurina (2012). The preservation method of each specimen is indicated in the material section. The measurements are given as the range of measured specimens followed by the mean value, while the measurements and setosity information from the holotype are given in square brackets. The ratios of the three apical palpal segments are given as 3rd:4th:5th. All measurements are taken from specimens in alcohol. Morphological terminology follows generally that of Söli et al. (2000) and Amorim and Rindal (2007) while the interpretation by Kjærandsen (2006) and Oliveira and Amorim (2012) are used for terminalia and thorax, respectively.

The habitus photos have been made in an alcohol medium and combined by software LAS V.4.5.0. from multiple gradually focused images taken by a camera Leica DFC 450 attached to the stereomicroscope Leica M205C. The photos of terminalia were combined by the same software but the camera was attached to the compound microscope Leica DM 6000 B (see also Kurina et al. 2015). Adobe Photoshop CS5 was used for editing the figures and compiling the plates.

The distributional map was performed with the software DIVA-GIS 7.5.0 (<http://www.diva-gis.org/>) and edited with Adobe Photoshop. The shapefile maps from Colombia and South America were obtained from DIVA-GIS and Dreamstime (ID 10514087 © Michael Schmeling and Dreamstime.com) websites, respectively.

The following acronyms are used for depositories:

- IAvH** Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia.
IZBE Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences [former Institute of Zoology and Botany], Tartu, Estonia.
MZUSP Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil.

The species

Cordyla monticola sp. n.

<http://zoobank.org/7A0180A6-F306-4E8D-B566-89853152F6AA>

Figs 1–9, 38

Type material. *Holotype*. ♂, COLOMBIA, Boyacá / SFF Iguaque El Níspero / 05°38'N 73°31'W 2730 m / Malaise 2 07-21.xii.2001 / P. Reina Leg. M. 2585 [IAvH]. *Paratype*. 1♂, same as holotype [MZUSP].

Description. Male (Fig. 1). Total length 4.1–4.6, 4.4 [4.6] mm (n=2).



Figures 1–2. *Cordyla monticola* sp. n. **1** male habitus **2** head with antennae and maxillary palpi, closer view. Scale bars: 1 mm (**1**) and 0.2 mm (**2**).

Head (Fig. 2) dark yellow, mouthparts pale. Two ocelli encircled by brown areas, close to compound eyes. All three visible palpal segments (Fig. 2) setose, swollen antepenultimate segment dark brown, succeeding segments pale. 4th segment slightly widening apically, 5th segment apically tapering. Swollen palpal segment 1.6 times as long as broad medially from lateral view, and 1.1–1.3, 1.2 [1.1] times as long as height of compound eye. Ratios of three apical palpomeres 1.0: 0.9: 0.9. Antenna yellow with 2+13 segments. Scape and pedicel with brown setae, flagellum with somewhat paler setosity. Scape elongate cup-shaped, 1.7–2.2, 2.0 [2.2] times as long as wide apically. Pedicel cup-shaped, 0.9 times as long as wide apically. Flagellomeres rectangular, about twice as wide as long. Apical flagellomere conical, about 1.6 times as long as wide basally. **Thorax** yellow, mesonotum medially, laterotergite and mediotergite somewhat darker. Hind margin of laterotergite narrowly brown. Anterior part of mesepimeron with a dark brown patch leaving anteroapical margin yellow. Haltere with pale knob, and basally pale and apically yellow stem. All setosity on thorax brown. Scutum entirely covered with decumbent setae, scutellum with setae including two pairs of marginal bristles, laterals shorter than internals. Antepronotum with setae including 6–8 [8] bristles, proepisternum with setae including 6–8 [8] bristles. Anepisternum with 2–4 [4] bristles at hind margin and with ca. 50 setae on its upper two thirds. Mesepimeron and katepisternum bare. Laterotergite with 5–6 [6] bristles and ca. 20 setae on upper half. Mediotergite bare. Metepisternum with 5–7 [7] bristles and ca. 10 setae. **Wing** with yellowish tinge, cell R1 somewhat darker. Length 3.1 [3.1] mm (n=2). Ratio of length to width 2.5–2.6, 2.6 [2.6]. All veins light brown. Radial veins seem darker

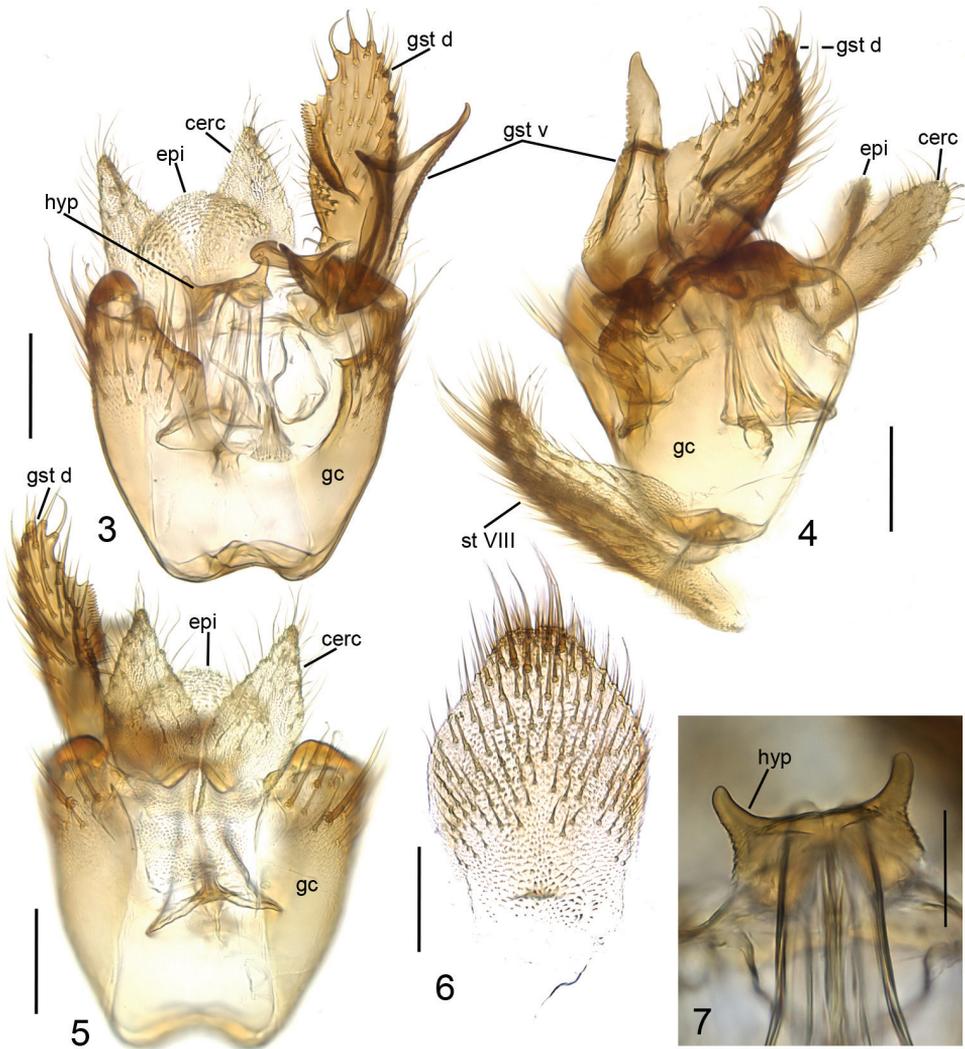
because of setae on both surfaces; other veins bare. Crossvein r-m apically disjunct. M1+2 3.5–3.7, 3.6 [3.7] times as long as r-m. M2 not reaching wing margin, broken 0.4–0.8, 0.6 [0.4] times of M1+2 length before it. Posterior fork begins clearly before anterior fork, at the middle of M1+2. **Legs** yellow, hind femora infuscated at apical fourth. Tarsi seem darker because of dense brown setae. Hind coxa with 4 [4] posterolateral bristles basally, with 0–2 [2] posterior bristles apically, and with ca. 30 weaker setae along posterolateral margin. Ratio of femur to tibia for fore-, mid- and hind legs: 1.5; 1.1; 1.0. Ratio tibia to first tarsomere for fore-, mid- and hind legs: 1.0; 1.2; 1.4. Fore-tibia with a spur about 0.5 of fore basitarsus; mid-tibia with anterior spur about 0.3–0.4, 0.4 [0.3] and with posterior spur about 0.6–0.7, 0.7 [0.6] of mid basitarsus; hind tibia with anterior spur about 0.5–0.6, 0.6 [0.5] and with posterior spur about 0.6–0.7, 0.7 [0.6] of hind basitarsus. **Abdomen** with first segment dorsally and laterally light brown and ventrally yellow. 2–4 segments dorsally brown with anterior and posterior margins yellowish, and laterally and ventrally yellowish; succeeding segments brownish. **Terminalia** (Figs 3–9) with gonocoxite basally yellow and apically brownish; gonostylus brownish; sternite 8 seems brownish because of dense setosity. Basal two thirds of sternite 8 cylindrical, apical third tapering, apex truncated. Basal third of sternite 8 membranous and bare, apical setae stronger than other setae. Gonocoxite slightly oblong, with broad ventral incision more than half of gonocoxite height. Ventral incision of gonocoxite with apically pointed basal projection about one third height of incision. Ventral medial margin of gonocoxite angular. Dorsal medial margin of gonocoxite simple. Cerci setose, clearly separated, basally wide, well tapering apically and protruding over gonocoxite. Basal half of gonocoxite bare, apical half with strong bristles. Dorsal branch of gonostylus rectangular, apically rounded, with a medially situated sclerotized comb of about half height of branch on its ventral surface. Apical setae somewhat stronger, deviating from other setosity of the branch. Dorsal branch of gonostylus with an indistinct basal tubercle on its ventral surface, close to base of medial branch. Ventral branch of gonostylus bare, subequal to dorsal branch, with serrated lateral margin and medially drawn out to a distinct lobe. The apical third of ventral branch is well tapering in ventral view. Medial branch of gonostylus divided at apical two thirds into two subequal lobes: ventral lobe rectangular, apically truncated, bearing 9–10 setae on its ventral part; dorsal lobe beak-shaped with two setae subapically on its ventral margin. Epiproct rounded with small setulae that arise in lines of 4 to 8 from small ridges. Hypoproct bowl-shaped with well-outlined lateral shoulders.

Female. Unknown.

Biology. Unknown.

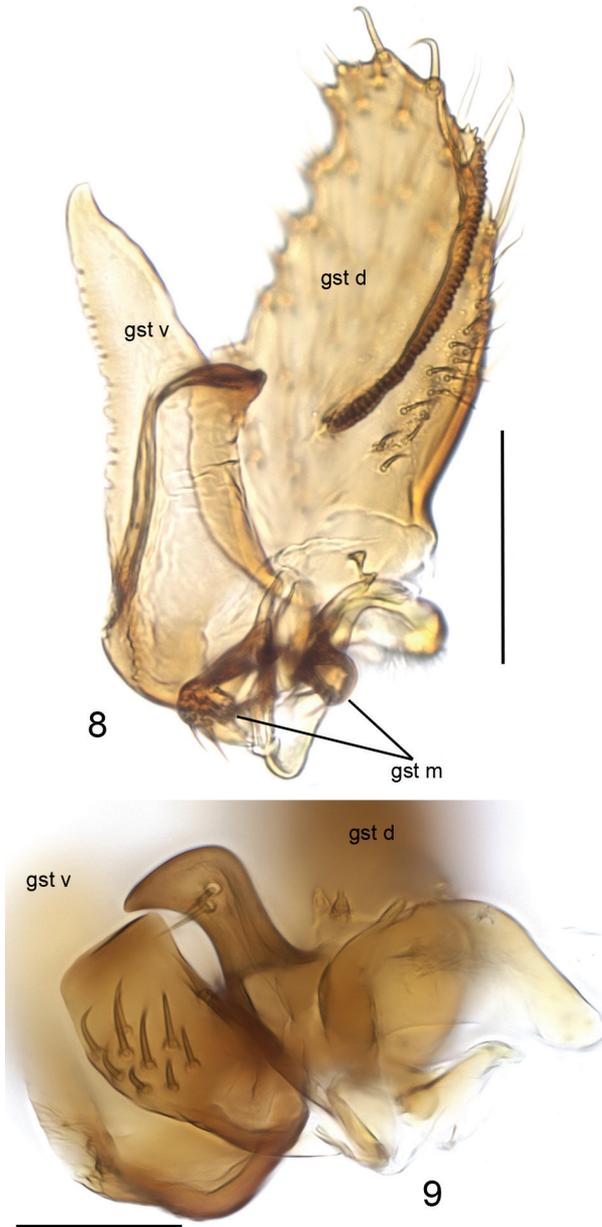
Etymology. The species is named to indicate its occurrence at high altitude (2730 m a.s.l.); Latin *monticola* means “mountain dweller”. The specific epithet is noun in apposition.

Comments. The paratype has seemingly 14 flagellar segments at one side, caused by an aberrantly divided apical one. This, as well as partial fusion of some flagellar segments unilaterally, is common and frequently observed in the Palaearctic specimens of the genus (OK *pers. obs.*). According to the structure of male terminalia, especially in



Figures 3–7. *Cordyla monticola* sp. n., male terminalia. **3** ventral view **4** lateral view **5** dorsal view **6** sternite VIII, ventral view **7** hypoproct, ventral view. Scale bars: 0.1 mm (**3**, **4**, **5**, **6**) and 0.05 mm (**7**). Abbreviations: cerc = cercus; epi = epiproct; gc = gonocoxite; gst d = dorsal branch of gonostylus; gst v = ventral branch of gonostylus; hyp = hypoproct; st VIII = sternite VIII.

having the medial branch of the gonostylus divided into two subequal lobes, the species belongs to the *C. murina* species-group as defined by Kurina (2001). Within the group, *C. monticola* sp. n. shares a 13-segmented flagellum and brown to dark brown swollen antepenultimate palpal segment with two Palearctic (viz. *C. semiflava* Staeger, 1840 and *C. borealis* Wu in Wu & Zheng, 2000) and three Nearctic (*C. manca* Johannsen, 1912, *C. scita* Johannsen, 1912 and *C. gracilis* Fisher, 1938) species. *Cordyla semiflava*, *C. borealis* and *C. manca* have the sternite VIII subapically remarkably



Figures 8–9. *Cordyla monticola* sp. n., gonostylus. **8** internal view **9** lobes of medial branch of gonostylus. Scale bars: 0.1 mm (**8**) and 0.05 (**9**). Abbreviations: gst d = dorsal branch of gonostylus; gst m = medial branch of gonostylus; gst v = ventral branch of gonostylus.

constricted, while it is smoothly tapering in *C. monticola* sp. n. The shape of the lobes of medial branch of the gonostylus and the hypoproct are different from those in all species of the group.

***Cordyla pseudopusilla* sp. n.**

<http://zoobank.org/1EF47BC4-2CCE-449D-B9D7-95165BBC79FA>

Figs 10–23, 38

Type material. *Holotype*. ♂, COLOMBIA Boyacá / SFF Iguaque El Níspero / 05°38'N 73°31'W 2730 m / Malaise 1 13-28.x.2001 / P. Reina Leg. M. 2475 [IAvH]. *Paratypes*. 1♂, same as holotype except 28.x-14.xi.2001, M. 2482 [IAvH]; 1♂ 2♀♀, same as holotype except 2 07-21.xii.2001, M. 2585 [1♂ 1♀ at MZUSP, 1♀ at IAvH]; 1♂ 1♀, same as holotype except 19.i-03.ii.2002, M. 3067 [IZBE]; 1♂, same as holotype except 3-18.ii.2002, M. 3068 [IAvH].

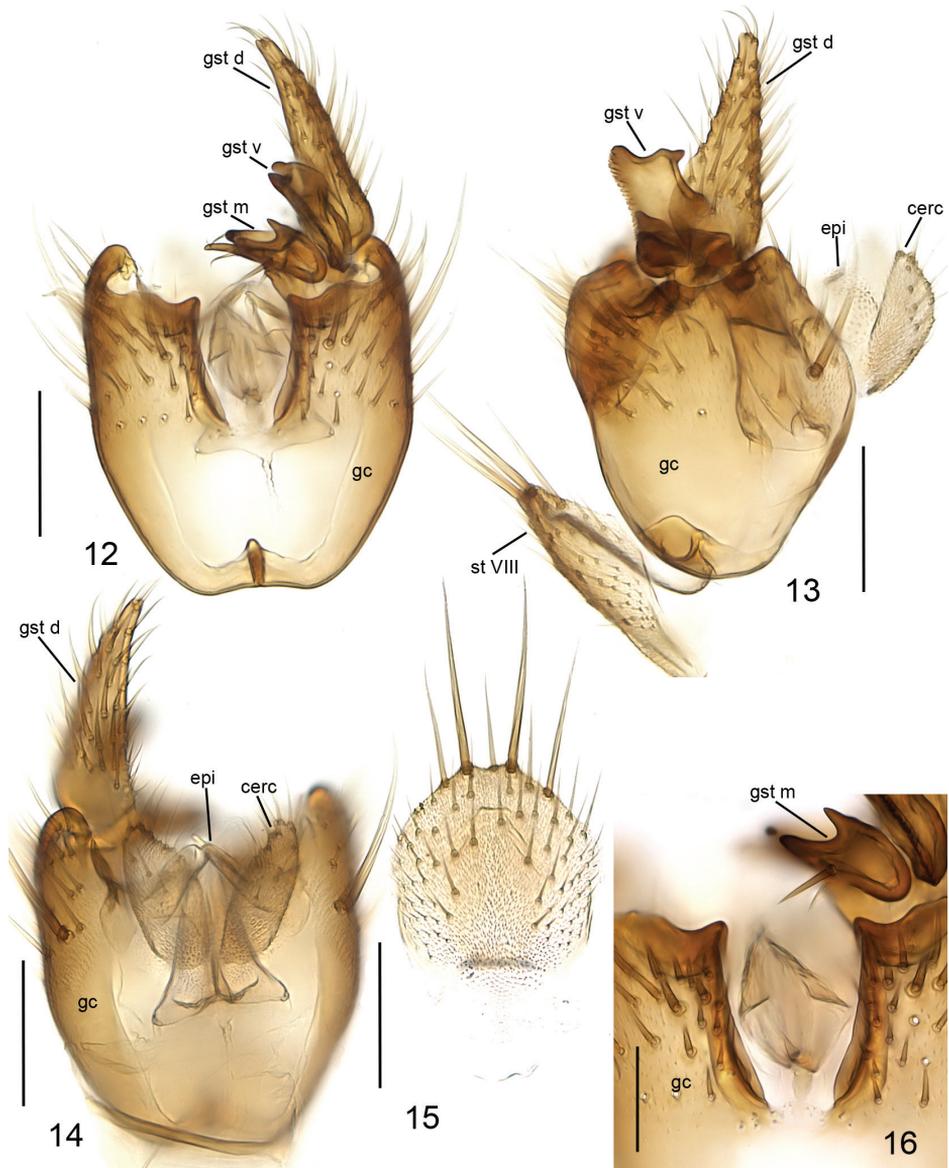
Description. Male (Fig. 10). Total length 2.9–3.4, 3.2 [3.4] mm (n=5).

Head (Fig. 11) dark yellow, mouthparts somewhat paler. Two ocelli encircled by brown areas, close to compound eyes. All three visible palpal segments (Fig. 11) setose, swollen antepenultimate segment light brown to brown, succeeding segments pale. 4th segment slightly widening apically, 5th segment apically tapering. Swollen palpal segment 1.8–2.2, 2.0 [1.8] times as long as broad medially from lateral view, and as long as height of compound eye. Ratios of three apical palpomeres 1.0: 0.8: 1.0. Antenna with 2+10 segments. Scape and pedicel yellow, flagellum somewhat darker, all with brown setosity. Scape elongate cup-shaped, 1.9–2.3, 2.1 [2.0] times as long as wide apically. Pedicel cup-shaped, 0.6–0.8, 0.7 [0.6] times as long as wide apically. Flagellomeres rectangular, about twice as wide as long. Apical flagellomere conical, about 2.2 times as long as wide basally. **Thorax** yellow, mesonotum medially somewhat darker. Hind margin of laterotergite narrowly brown. Katepisternum lighter. Anterior part of mesepimeron with a brown patch leaving anteroapical corner yellow. Haltere with a pale knob, stem basally pale and darker. All setosity on thorax brown. Scutum entirely covered with decumbent setae, scutellum with setae including two pairs of marginal bristles, laterals considerably shorter than internals. Anteprenotum with setae including 5–7 [7] bristles, proepisternum with setae including 4–7 [7] bristles. Anepisternum with 4 bristles at hind margin and with ca. 40 setae on its upper two thirds. Mesepimeron and katepisternum bare. Laterotergite with 2–3 [3] bristles medially and ca. 10 setae along upper margin. Mediotergite bare. Metepisternum with 5–6 [5] bristles and ca. 10 setae. **Wing** with yellowish tinge, otherwise clear. Length 2.2–2.5, 2.4 [2.4] mm (n=10). Ratio of length to width 2.4–2.7, 2.6 [2.4]. All veins light brown. Radial veins seem darker because of setae on both surfaces; other veins bare. Crossvein r-m apically disjunct. M1+2 5–6 times as long as r-m. M2 not reaching wing margin, broken 0.5–0.7, 0.6 [0.5] times of M1+2 length before it. M1 and M4 apically very faint. Posterior fork begins clearly before anterior fork. **Legs** yellow, hind femora slightly infuscated at apical fifth. Tarsi seem darker because of dense brown setae. Hind coxa with 2–3 [3] posterolateral bristles basally, with one posterior bristle apically, and with ca. 10 weaker setae along posterolateral margin. Ratio of femur to tibia for fore-, mid- and hind legs: 1.5–1.6, 1.6 [1.5]; 1.0–1.1, 1.1 [1.1]; 1.0, 1.0 [1.0]. Ratio tibia to first tarsomere for fore-, mid- and hind legs: 1.0–1.1, 1.0 [1.0]; 1.1–1.3, 1.2 [1.1]; 1.4–1.5, 1.5 [1.4]. Fore-tibia with a spur about 0.5–0.6, 0.5 [0.6]



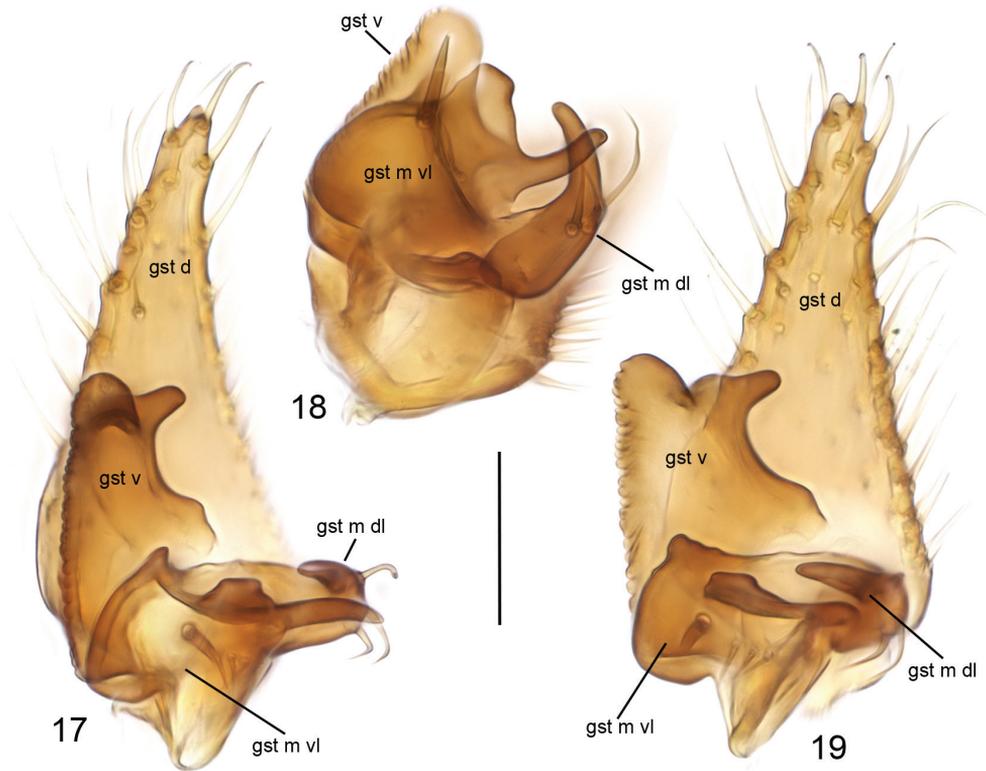
Figures 10–11. *Cordyla pseudopusilla* sp. n. **10** male habitus **11** head with antennae and maxillary palpi, closer view. Scale bars: 1 mm (**10**) and 0.2 mm (**11**).

of fore basitarsus; mid-tibia with anterior spur about 0.3, 0.3 [0.3] and with posterior spur about 0.6–0.7, 0.7 [0.7] of mid basitarsus; hind tibia with anterior spur about 0.5 and with posterior spur about 0.6 of hind basitarsus. **Abdomen** with first segment light brown, and 2–4 segments dorsally brownish, laterally yellow and ventrally pale yellow; succeeding segments entirely brown. **Terminalia** (Figs 12–19) with basal part of gonocoxite and cerci yellow; apical part of gonocoxite slightly darker; dorsal branch of gonostylus yellow; ventral and medial branches of gonostylus brown. Sternite 8 oblong, apical fourth conical, apex truncated, basal half membranous and bare, setae on apical quarter somewhat stronger than other setae, two apical setae well deviating from other setosity. Gonocoxite subquadrate, with narrow ventral incision about half height of gonocoxite. Ventral medial margin of gonocoxite apically angular and with membranous formations dorsad from the ventral surface of gonocoxite. Dorsal medial margin of gonocoxite simple. Cerci setose, setae on medial margin slightly stronger, deviating from other setosity; clearly separated, prolonged, subapically somewhat constricted, not protruding over gonocoxite. Basal half of gonocoxite bare, apical half with strong bristles. Dorsal branch of gonostylus elongated, tapering, without sclerotized comb, dorsal surface with homogeneous setosity. Ventral branch of gonostylus bare, about half as long as dorsal branch, with serrated lateral margin. Apical corners of ventral branch of gonostylus drawn out to small lobes: ventral wider and rounded, dorsal



Figures 12–16. *Cordyla pseudopusilla* sp. n., male terminalia. **12** ventral view **13** lateral view **14** dorsal view **15** sternite VIII, ventral view **16** ventromedial incision of gonocoxite, ventral view. Scale bars: 0.1 mm (**12**, **13**, **14**, **15**) and 0.05 mm (**16**). Abbreviations: cerc = cercus; epi = epiproct; gc = gonocoxite; gst d = dorsal branch of gonostylus; gst m = medial branch of gonostylus; gst v = ventral branch of gonostylus; st VIII = sternite VIII.

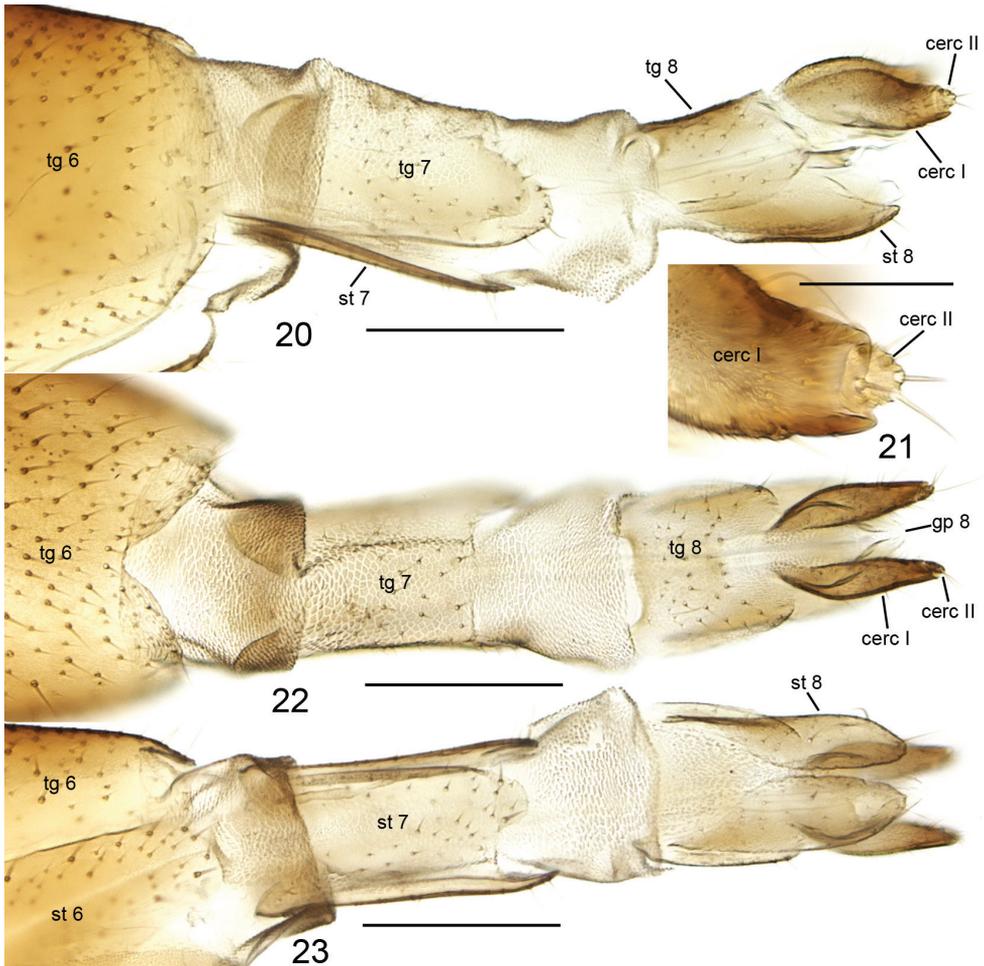
narrow. Dorsal margin of ventral branch of gonostylus with wide and shallow incision. Medial branch of gonostylus divided into two lobes: 1) ventral lobe with three apical protrusions separated by concavities (ventral finger-like protrusion well discernible at



Figures 17–19. *Cordyla pseudopusilla* sp. n., gonostylus **17** ventral view **18** anterior view of medial branch **19** internal view. Scale bars: 0.05 mm. Abbreviations: gst d = dorsal branch of gonostylus; gst m dl = dorsal lobe of medial branch of gonostylus; gst m vl = ventral lobe of medial branch of gonostylus; gst v = ventral branch of gonostylus.

internal view), internal surface with one strong and three weaker setae, and 2) dorsal lobe curved, tapering, as long as the medial protrusion of ventral lobe and with three medial setae. Epiproct apically rounded, covered with small setulae. Hypoproct indiscernible.

Female. Total length 3.6–3.7, 3.6 mm (n=3). Wing length 2.4–2.5, 2.4 mm. Ratio of length to width 2.4–2.6, 2.5. Antennae 2+9 segments. In setosity and coloration similar to male. Terminalia (Figs 20–23) light brown. Cercus two-segmented: apical segment small, sunken into basal segment, with 2-3 long setae deviating from other setosity; basal segment long ovate, slightly sinusoidal and considerably wider than apical segment. Gonapophysis VIII membranous, visible in dorsal view. Tergite VIII rectangular, subequal to length of basal segment of cercus, apically angular in lateral view, basally and apically well emarginated in dorsal view. Sternite VIII tapering lateroapically, with deep medial cleft in ventral view. Tergite VII longer than tergite VIII, basally and apically well emarginated in dorsal view. Sternite VII apically conical, subequal to length of tergite VII. Tergite VI apically rounded in lateral view and with apicodorsal incision.



Figures 20–23. *Cordyla pseudopusilla* sp. n. female terminalia. **20** lateral view **21** cerci, closer view **22** dorsal view **23** ventral view. Scale bars: 0.2 mm (**20**, **22**, **23**) and 0.05 mm (**21**). Abbreviations: cerc = cercus, gp = gonapophysis, st = sternite, tg = tergite.

Biology. Unknown.

Etymology. The specific name is derived by the Greek prefix *pseudo-* from the Palearctic species *Cordyla pusilla* Edwards, 1925 to indicate their morphological similarity.

Comments. Belonging to the *C. fusca* species-group (cf. Kurina 2001), *C. pseudopusilla* sp. n. shares a 10-segmented flagellum and brown to dark brown swollen antepenultimate segment of palpus with five Palearctic (viz. *C. bomloensis* Kjørandsen & Kurina, 2004, *C. brevicornis* Staeger, 1840, *C. geminata* Sasakawa, 2005, *C. pusilla* and *C. triloba* Sasakawa, 2008) and three Nearctic (viz. *C. neglecta* Johannsen, 1912, *C. recens* Johannsen, 1912, *C. scutellata* Garrett, 1925) species. Within these, the new species is remarkably similar to *C. pusilla* and *C. neglecta*. All three species have the

ventral branch of gonostylus subquadrate with emarginated dorsal margin. However, the emargination is more pronounced in *C. pseudopusilla* sp. n. The new species has the medial branch of the gonostylus with a curved dorsal lobe (straight in the other two species) and with deep concavities on the ventral lobe apically (more shallow in the other two species).

***Cordyla reducta* sp. n.**

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Figs 24–38

Type material. *Holotype.* ♂, COLOMBIA, Risaralda SFF / Otún Quimbaya Robledal / 04°44'N 75°35'W 1980 m / Malaise 04–20.iii.2003 / G. López Leg. M. 3686 [IAvH]. *Paratypes.* 1♂ 2♀♀, same as holotype [IAvH]; 1♂, Boyacá / SFF Iguaque El Nispero / 05°38'N 73°31'W 2730 m / Malaise 2 07–21.xii.2001 / P. Reina Leg. M. 2585 [IAvH]; 4♂♂ 2♀♀, Valle del Cauca / PNN Farallones de Cali / Cgto. La Meseta / 03°34'N 76°40'W 1960 m / Malaise 09–26.x.2003 / S. Sarria & M. Losso Leg. M. 4548 [IZBE]; 1♂, Valle del Cauca / PNN Farallones de Cali / Cgto. La Meseta / 03°34'N 76°40'W 1960 m / Malaise 27.viii–10.ix.2003 / S. Sarria & M. Losso Leg. M. 4549 [IAvH]; 1♂ 2♀♀, Valle de Cauca / PNN Farallones de Cali Cgto. / La Meseta 03°34'N 76°40'W 2200 m / Malaise 26.xi–10.xii.2003 / S. Sarria & M. Losso Leg. M. 4562 [MZUSP]; 2♂♂, COLOMBIA, Risaralda SFF / Otún Quimbaya Robledal / 04°44'N 75°35'W 1980 m Malaise 20.iii–04.iv.2003 / G. López Leg. M. 3682 [MZUSP]; 1♀, Risaralda SFF / Otún Quimbaya Urapanera / 04°44'N 75°35'W 1960 m Malaise 20.iii–04.iv.2003 / G. López Leg. M. 3688 [IAvH]; 1♂ 3♀♀, Risaralda SFF / Otún Quimbaya Robledal / 04°44'N 75°35'W 1980 m Malaise 18.ii–04.iii.2003 / G. López Leg. M. 3699 [IAvH]; 1♀, Risaralda SFF / Otún Quimbaya Robledal / 04°44'N 75°35'W 1980 m Malaise 04–19.iv.2003 / G. López Leg. M. 3710 [IAvH]; 5♂♂ 3♀♀ COLOMBIA, Risaralda SFF / Otún Quimbaya Robledal / 04°44'N 75°35'W 1980 m Malaise 11–27.x.2003 / G. López Leg. M. 4182 [3♂♂ 2♀♀ MZUSP, 2♂♂ 1♀ IZBE].

Description. Male (Fig. 24). Total length 2.5–3.2, 2.9 [3.2] mm (n=10).

Head (Fig. 25) dark yellow, mouthparts somewhat paler. Two ocelli encircled by brown areas, close to compound eyes. All three visible palpal segments (Fig. 25) setose, swollen antepenultimate segment dark yellow, succeeding segments pale. 4th segment slightly widening apically, 5th segment apically tapering. Swollen palpal segment 2.3–2.6, 2.4 [2.5] times as long as broad medially from lateral view, and 1.1–1.3, 1.2 [1.1] times as long as height of compound eye. Ratios of three apical palpomeres 1.0: 0.7: 1.0–1.1, 1.0 [1.1]. Antenna yellow with 2+12 segments. Scape and pedicel with brown setae, flagellum with somewhat paler setosity. Scape elongate cup-shaped, 1.8–2.2, 2.0 [1.9] times as long as wide apically. Pedicel cup-shaped, 0.7–0.9, 0.8 [0.9] times as long as wide apically. Flagellomeres rectangular, about twice as wide as long. Apical flagellomere conical, about 1.7 times as long as wide basally. **Thorax**



Figures 24–25. *Cordyla reducta* sp. n. **24** male habitus **25** head with antennae and maxillary palpi, closer view. Scale bars: 1 mm (**24**) and 0.2 mm (**25**).

yellow, mesonotum medially somewhat darker. Hind margin of laterotergite narrowly brown. Anterior part of mesepimeron with a brown patch. Haltere with knob apically pale and basally brownish, and pale stem. All setosity on thorax brown. Scutum entirely covered with decumbent setae, scutellum with setae including two pairs of marginal bristles, laterals considerably shorter than internals. Anteprepronotum with setae including 2–3 [3] bristles, proepisternum with setae including 3–5 [5] bristles. Anepisternum with 3–4 [4] bristles at hind margin and with ca. 45 setae on its upper two thirds. Mesepimeron and katepisternum bare. Laterotergite with 2 bristles and ca. 15 setae on upper half. Mediotergite bare. Metepisternum with 2–4 [4] bristles and ca. 10 setae. **Wing** with yellowish tinge, otherwise clear. Length 2.0–2.3, 2.2 [2.3] mm (n=10). Ratio of length to width 2.6–2.9, 2.7 [2.9]. All veins light brown. Radial veins seem darker because of setae on both surfaces; other veins bare, except about 5 setae on dorsal surface of M4. Crossvein r-m apically disjunct. M1+2 3–4 times as long as r-m. R5 slightly sinusoid. M2 not reaching wing margin, broken 0.7–1.0, 0.8 [0.9] times of M1+2 length before it. Posterior fork begins clearly beyond anterior fork. **Legs** yellow, hind femora slightly infuscated at apical fifth. Tarsi seem darker because of dense brown setae. Hind coxa with 2–4 [4] posterolateral bristles basally, with one posterior bristle apically, and with ca. 25 weaker setae along posterolateral margin. Ratio of femur to tibia for fore-, mid- and hind legs: 1.3–1.6, 1.4 [1.4]; 0.8–1.0, 0.9 [1.0]; 1.0, 1.0 [1.0]. Ratio tibia to first tarsomere for fore-, mid- and hind legs: 1.0–1.1, 1.0 [1.0]; 1.1–1.3, 1.2 [1.2]; 1.4, 1.4 [1.4]. Fore-tibia with a spur about 0.5–0.6, 0.5 [0.6] of fore basitarsus; mid-tibia with anterior spur about 0.3 and with posterior

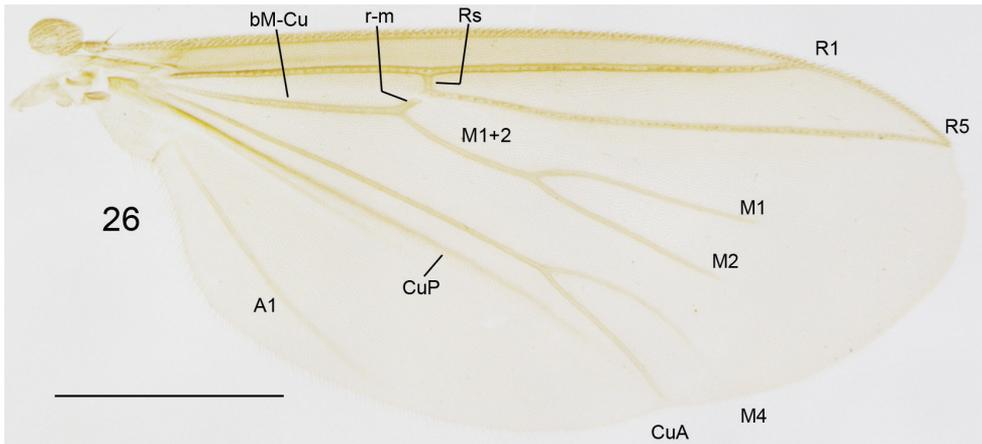
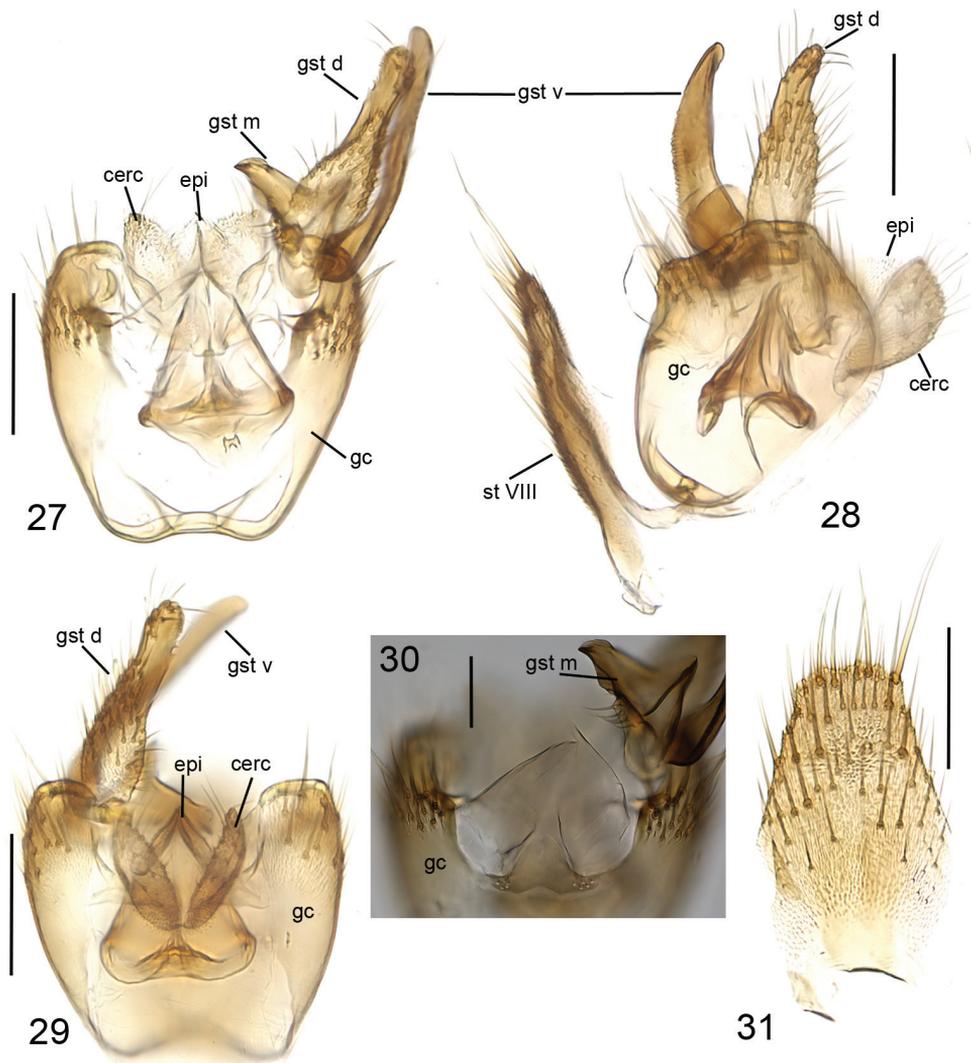


Figure 26. *Cordyla reducta* sp. n. **26** female wing. Scale bars: 0.5 mm.

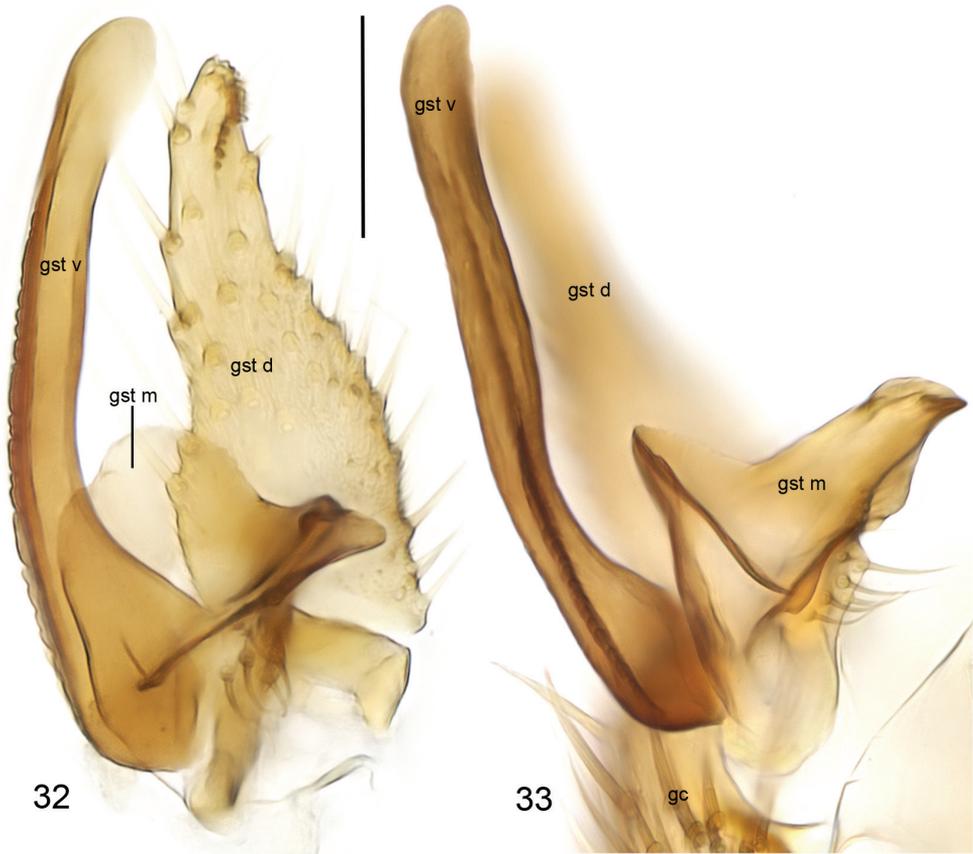
spur about 0.6–0.7, 0.7 [0.6] of mid basitarsus; hind tibia with anterior spur about 0.6 and with posterior spur about 0.6–0.7, 0.7 [0.6] of hind basitarsus. **Abdomen** with first 3 segments dorsally brownish, laterally yellow and ventrally pale yellow; succeeding segments brown, only slightly lighter laterally and ventrally. **Terminalia** (Figs 27–33) with gonocoxite and cerci yellow; gonostylus brownish; sternite 8 seems brownish because of dense setosity. Sternite 8 oblong with truncated apex, basal third membranous and bare, setae on apical quarter somewhat stronger than rest of them. Gonocoxite subquadrate, with broad ventral incision about half of gonocoxite height. Ventral incision of gonocoxite with apically rounded basal hump about one third height of incision. Ventral medial margin of gonocoxite angular with apically pointed membranous formations dorsad from the ventral surface of gonocoxite. Dorsal medial margin of gonocoxite bulging. Cerci setose, setae on medial margin stronger, deviating from other setosity; clearly separated, prolonged, subapically somewhat constricted, not protruding over gonocoxite. Basal half of gonocoxite bare, apical half with strong bristles. Dorsal branch of gonostylus oblong, basally wider, apically slightly tapering, with a sclerotized comb of about one fifth branch height on its ventral surface. Setosity on basal two thirds more dense, leaving subapical area almost bare; apical setae slightly stronger than other setosity of the branch. Ventral branch of gonostylus bare, basally bulbous and apically rounded, slightly curved, longer than dorsal branch, with serrated lateral margin. Medial branch of gonostylus hump-backed, not divided into lobes but medially extended, and with 5–6 strong setae at medial margin ventrobasally. Epiproct abruptly narrowing subapically, with pointed apex, covered with small setulae. Hypoproct indiscernible.

Female. Total length 2.5–3.4, 2.9 mm. Wing length 1.7–2.5, 2.1 mm. Ratio of length to width 2.4–2.7, 2.6. Antennae 2+9 segments. In setosity and coloration similar to male, except for entirely pale second abdominal segment. Both M1 and M2 not reaching wing margin, M4 extremely faint at its distal part (Fig. 26). Terminalia



Figures 27–31. *Cordyla reducta* sp. n., male terminalia. **27** ventral view **28** lateral view **29** dorsal view **30** ventromedial incision of gonocoxite, ventral view **31** sternite VIII, ventral view. Scale bars: 0.1 mm (**27**, **28**, **29**, **31**) and 0.05 mm (**30**). Abbreviations: cerc = cercus; epi = epiproct; gc = gonocoxite; gst d = dorsal branch of gonostylus; gst m = medial branch of gonostylus; gst v = ventral branch of gonostylus; st VIII = sternite VIII.

(Figs 34–37) light brown. Cercus two-segmented: apical segment small, with a few long setae deviating from other setosity, obliquely connected with basal segment; basal segment long ovate, sinusoidal and wider than apical segment, ventroapical corner drawn out to a setose small lobe. Gonapophysis VIII membranous, visible in ventral view, apically rounded. Tergite VIII rectangular, subequal to length of basal segment



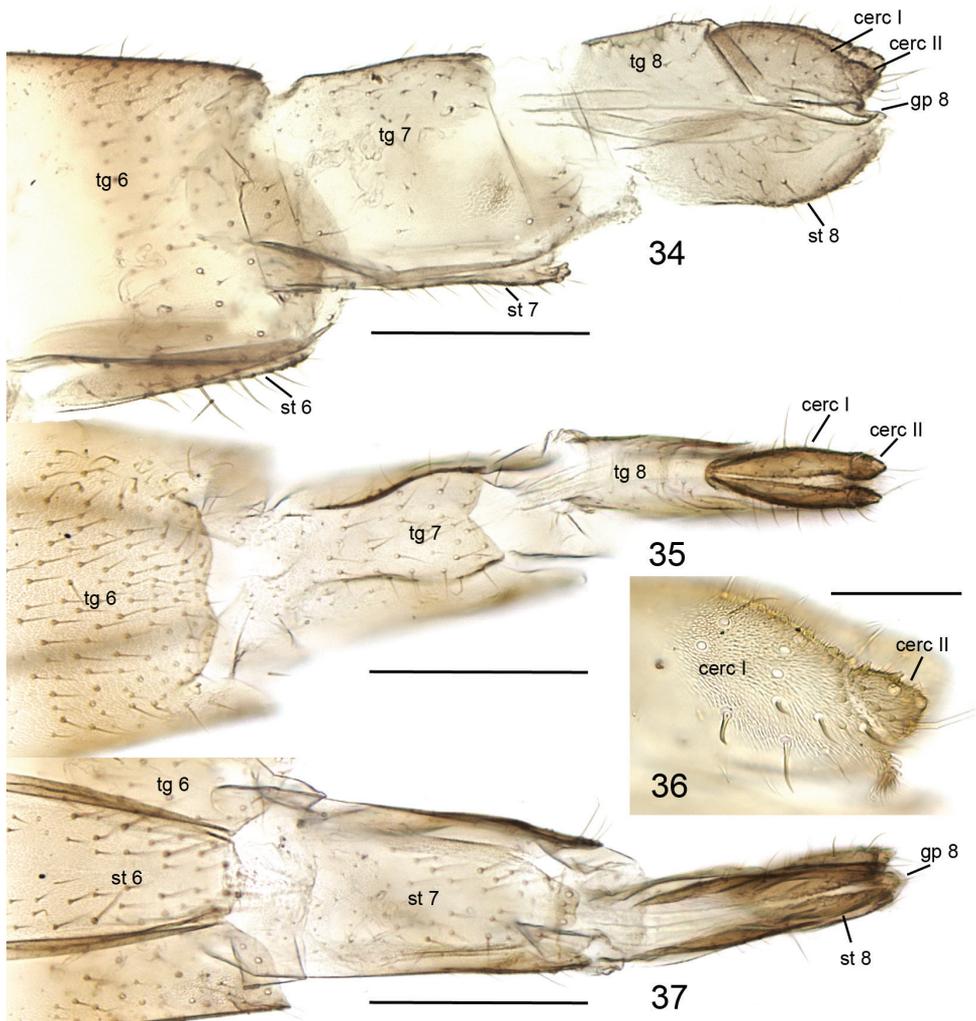
Figures 32–33. *Cordyla reducta* sp. n., gonostylus **32** internal view **33** ventral view. Scale bars: 0.05 mm. Abbreviations: gc = gonocoxite; gst d = dorsal branch of gonostylus; gst m = medial branch of gonostylus; gst v = ventral branch of gonostylus.

of cercus, apically angular, basally and apically emarginated in dorsal view. Sternite VIII tapering lateroapically, with deep medial cleft in ventral view. Tergite VII about twice as long as tergite VIII, apically widening and with apical incision in dorsal view. Sternite VII apically conical, subequal to length of tergite VII. Tergite VI apically emarginated in dorsal view.

Biology. Unknown.

Etymology. The specific name refers to the distally extremely reduced medial veins of the female wing: from Latin *reducta* meaning “distant”. An adjective.

Comments. By the structure of male terminalia, *C. reducta* sp. n. belongs to the *C. fusca* species-group (cf. Kurina 2001). In its 12-segmented flagellum and yellow to light brown swollen antepenultimate segment of palpus, the new species is similar to the Palearctic *C. flaviceps* (Staeger, 1840) and Oriental *C. borneoensis* Kurina, 2005.



Figures 34–37. *Cordyla reducta* sp. n. female terminalia. **34** lateral view **35** dorsal view **36** cerci, closer view **37** ventral view. Scale bars: 0.2 mm (**34**, **35**, **37**) and 0.05 mm (**36**). Abbreviations: cerc = cercus, gp = gonapophysis, st = sternite, tg = tergite.

The male terminalia of *C. reducta* sp. n. are remarkably different from these two species but otherwise resemble the Palearctic *C. fasciata* Meigen, 1818 in having a similar outline to the ventral and dorsal branches of the gonostylus. However, the medial branch of the gonostylus is hump-backed and medially extended in *C. reducta* sp. n., while it has two small medial lobes in *C. fasciata* (cf. Zaitzev 2003: fig. 21–27). All studied female specimens have both medial veins of the wing distally extraordinarily reduced (Fig. 26), unique among the species worldwide.



Figure 38. Collecting localities of *Cordyla* species in Colombian Andes. **1** “Parque Nacional Natural Farallones de Cali” **2** “Santuario de Flora y Fauna Otún Quimbaya” **3** “Santuario de Flora y Fauna Iguaque”.

Discussion

Herein we present the occurrence of the genus *Cordyla* from the Colombian Andes (Fig. 38), representing the first described species in the Neotropical region. However, according to Vockeroth (2009: 276), unidentified specimens of *Cordyla* were previously known from Mexico, Belize, Guatemala, and Costa Rica. We were able to study the material housed at the Instituto Nacional de la Biodiversidad (INBio), San Jose, Costa

Rica, that includes eight females from the provinces of Guanacaste, San José, Heredia, Puntarenas, and Limón (Vockeroth 2009; SSO pers. obs.). Because of the absence of associated male specimens, the formal description of species from that material has not been performed at the moment.

The presence of *Cordyla* in the north-western part of Colombia, in the Neotropical region, clearly corresponds to a secondary extension of a Nearctic clade to the south. The range of this extension is, however, restricted to the mountains of Colombian Andes. In spite of having studied a vast fungus gnat material from the Amazonian basin, the genus has not been recorded there (SSO, OK pers. obs.). This distribution pattern is actually not restricted only to *Cordyla* within the Mycetophilidae. Also *Docosia adusta* Oliveira & Amorim, 2011 belongs to a group of Nearctic mycetophilid species reaching Colombia. According to regionalization of the Neotropical region by Morrone (2014), two of the three localities (“PNN Farralones de Cali” and “SFF Otún Quimbaya”) lie in the Pacific Dominion and “SFF Iguaque” is located in the South American transition zone called Paramo province. *Cordyla reducta* sp. n. was recorded from all three localities while *C. monticola* sp. n. and *C. pseudopusilla* sp. n. are in the present stage of knowledge endemic to the Paramo province only (Fig. 38).

The complexity of the overlap of different biogeographical elements in Colombia was highlighted by Oliveira et al. (2007) and Oliveira and Amorim (2011). The Colombian fauna encompasses elements of Nearctic origin (p. ex. *Docosia adusta*), tropical origin, and a number of typical circum-antarctic elements that reach the north-western part of Neotropical region following the Andes final uplift to the north. The genera *Paraleia* Tonnoir, of which six new species were recently described from the Colombian Andes (Oliveira and Amorim 2012), and *Procycloneura* Edwards, of which at least ten species are being described by Oliveira and Amorim (in prep.) from Colombia and Costa Rica, are examples of amphinotic elements in Colombian fauna. Further studies of the diversity of Mycetophilidae and *Cordyla* in the Andes would contribute to a better understanding of the distributional patterns into the family and, hence, the biogeographical evolution of the region.

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Male secondary sexual structures and the systematics of the *Thereus oppia* species group (Lepidoptera, Lycaenidae, Eumaeini)

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Abstract

The *Thereus oppia* species group includes species with and without a scent pad, which is a histologically and morphologically characterized male secondary sexual structure on the dorsal surface of the forewing. To assess the hypothesis that these structures are lost evolutionarily, but not regained (Dollo's Law), the taxonomy of this species group is revised. *Thereus lomalarga* **sp. n.**, and *Thereus brocki* **sp. n.**, are described. Diagnostic traits, especially male secondary structures, within the *T. oppia* species group are illustrated. Distributional and biological information is summarized for each species. Three species have been reared, and the caterpillars eat Loranthaceae. An inferred phylogeny is consistent with the hypothesis that scent pads in the *T. oppia* species group have been lost evolutionarily twice (in allopatry), and not re-gained.

Keywords

Dollo's Law, Loranthaceae, Scent pads, *Thereus brocki*, *Thereus lomalarga*, *Thereus orasus*

Introduction

Evolutionary “losses” and “gains” of male secondary sexual structures are being actively documented in the Eumaeini. Evolutionary losses appear to occur when a species is allopatric with its closest relative (Robbins et al. 2012), a result that had been predicted by theory (Phelan and Baker 1987). Evolutionary gains are rare, as appears to be true for most animals (Wiens 2001), especially when the structure was lost previously (Quental 2008). In the three documented lineages in which a new male secondary sexual structure evolved, each clade diversified into more extant species than its sister clade (Robbins and Busby 2015). The Neotropical *Thereus* Hübner (Lycaenidae: Eumaeini) possesses a variety of male secondary sexual structures, for which reason we are beginning to revise the genus systematically. The four primary secondary sexual structures in *Thereus* are forewing scent patches, hindwing scent patches, forewing scent pads, and abdominal brush organs (terminology from Robbins 1991, where these structures are characterized).

Thereus was characterized morphologically for 27 species (Robbins 1991). About a third of the species have been reared. With one exception, all were reared from plants in the Santalales, which includes the mistletoes (Robbins 2000, Heredia and Robbins, in prep.). *Rekoa* Kaye and *Arawacus* Kaye were proposed as the closest relatives of *Thereus* based on morphology, and *Rekoa* was later confirmed as its sister genus based on molecular sequences (Quental 2008). *Thereus* contains species with a diverse set of wing patterns and shapes and, as noted, a variety of male secondary sexual traits, which may be the reason that *Thereus* has five junior synonyms (Robbins 2004). Another reason for this lengthy synonymy may be that only one of the three proposed synapomorphies for *Thereus* has been illustrated (Robbins 2000).

The *Thereus oppia* species group, consisting of *T. orasus* (Godman & Salvin) and *T. oppia* (Godman & Salvin), is distinguished from the remainder of the genus by the presence of scent patches near the costa of the dorsal hindwing and on the inner margin of the ventral forewing (Figs 9–14). *Thereus oppia* also has a “brush” of piliform androconia on the ventral surface of the forewing (Fig. 14), a structure that has not been previously reported in the Eumaeini.

Another two *Thereus* species have been discovered with scent patches similar to those of the *T. oppia* species group (Figs 10, 12, 13). One of these species has a ventral forewing androconial “brush”, and both share virtually indistinguishable male and female genitalic structures with *T. orasus* and *T. oppia* (Figs 15–24). For these reasons, we add them to the *T. oppia* species group. We have reared one of the newly discovered species in Colombia and are documenting its life history (Heredia and Robbins, in prep.).

Two species of the *T. oppia* species group lack scent pads on the dorsal surface of the forewing and two possess scent pads (Figs 5–8). Given previous results (Wiens 2001, Quental 2008), we predict that the scent pad was lost evolutionarily once or twice in the *T. oppia* species group. However, if the two newly discovered species that possess scent pads were phylogenetic sisters (cf. Discussion), this result would be consistent with the re-evolution of a scent pad, which would represent the first such documented case in the Eumaeini.

One purpose of this paper is to illustrate the proposed traits that characterize *Thereus* and to provide a brief overview of the biology of the genus. Another is to delimit the *T. oppia* species group and to provide names for the two unnamed species in this group. These names are needed for the phylogenetic analysis and for the publications of the life history of one of them. The third purpose is to propose a preliminary phylogenetic hypothesis for the *T. oppia* species group to assess whether male scent pad re-evolution is likely to have occurred in this species group.

Materials and methods

The species level taxonomy of the *Thereus oppia* species group is based on an analysis of variation among 121 pinned specimens from various museum and private collections, as noted below. Species accounts include notes, when relevant, on nomenclature, history, morphological variation, elevation, seasonality, and behavior. Males and females were associated by similarity of ventral wing pattern and distribution. In one species, rearing confirmed the association in a species with sexually dimorphic wing patterns. Geographic distributions of the new species are mapped. Labels on holotypes are recorded verbatim with brackets used for information not explicitly noted on the labels and for descriptions of the labels. Otherwise, months are abbreviated by their first three letters in English. Citations for original descriptions can be found in Lamas et al. (1995). Biogeographic zones follow Brown (1982), who partitioned the forested lowland continental Neotropics into the Transandean Region, Amazonian Region, and Atlantic Region. Many male eumaeines perch in “territories” at certain times of day to wait for receptive females to fly through and “defend” these areas by flying at other males that enter the territory (e.g., Powell 1968; Alcock and O’Neill 1987; Cordero et al. 2000). Recorded times from our fieldwork for “territorial” behavior are standard time at that locality.

Morphological characters for the phylogenetic analyses are utilized because DNA sequences are currently available only for *T. oppia*. Standard references for morphological terminology are Comstock (1918) for insect wing veins; Klots (1970) and Robbins (1991) for lepidopteran genitalia as modified for the Eumaeini; Robbins (1991) and Robbins et al. (2012) for male secondary sexual structures; and Snodgrass (1935) for all other morphological structures. To quantify nudum extent, the number of segments from the antennal tip to the first segment with a complete ventral row of scales was counted (Robbins 1991).

Four species are recognized in the *T. oppia* species group (Table 1), two being newly described. The first outgroup for the phylogenetic analysis is *Thereus cithonius* (Godart), which shares small teeth on the dorsal tip of the penis with two species in the *T. oppia* species group (Figs 16, 17). The second outgroup is *Thereus ortalus* (Godman & Salvin), which shares a relatively straight vinculum strut in lateral aspect and a slender “crescent” shaped dorsal cornutus with the *T. oppia* species group (Figs 15–18).

Seventeen characters were coded (Table 1), and their states for each ingroup and outgroup species were recorded (Table 2). We searched exhaustively for shortest trees

Table 1. Morphological characters and their states in the *Thereus oppia* species group.

1. Male shape of forewing inner margin (0) straight, (1) convex.
2. Male ventral forewing iridescent blue (0) absent, (1) present.
3. Male dorsal forewing dark border (0) a marginal line, (1) present.
4. Ventral forewing postmedian line shape (0) relatively straight, (1) gently curved.
5. Orange spot on dorsal hindwing anal lobe (0) present, (1) absent.
6. Male dorsal hindwing costa with an iridescent sheen (0) absent, (1) present.
7. Female hindwing tail length (0) same length as male, (1) longer than male.
8. Basal edge of ventral hindwing postmedian line (0) a dark line, (1) a broad orange-brown band.
9. Male ventral forewing scent patch (0) absent, (1) present.
10. Male ventral forewing brush of erect scales (0) absent, (1) present.
11. Male dorsal hindwing with gray-charcoal androconia (0) absent, (1) present and iridescent, (2) present, but not iridescent.
12. Male dorsal forewing scent pad at vein udc (0) present, (1) absent.
13. Male dorsal hindwing scent patch (0) without piliform setae, (1) with piliform setae.
14. Shape of ventral cornutus in male genitalia penis (0) shaped like a crescent moon, (1) posteriorly thickened.
15. Length of male 8 th abdominal tergum (0) about 1.1 mm, (1) about 1.7 mm or longer.
16. Vento-lateral processes of male genitalia vinculum (0) present, (1) absent.
17. Teeth on subterminal dorsal penis of male genitalia (0) absent, (1) present.

Table 2. Character matrix for the *Thereus oppia* species group.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<i>T. cithonius</i> outgroup	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1
<i>T. ortalus</i> outgroup	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>T. oppia</i>	1	0	1	0	0	0	0	0	1	1	2	1	1	0	0	1	1
<i>T. brocki</i>	1	0	1	0	1	0	0	0	1	1	2	0	0	0	0	1	0
<i>T. lomalaria</i>	1	1	1	0	1	1	1	0	1	0	0	0	0	0	0	1	1
<i>T. orasus</i>	1	1	0	1	1	1	1	1	1	0	1	1	0	0	0	1	0

using the implicit enumeration option of TNT software (Goloboff et al. 2008) to derive a most parsimonious cladogram. To test the assumption of equally weighted characters, implied weighting was performed over a range of values for the parameter K (1, 10, 50, 250, and 1000).

To assess scent pad evolution in the *T. oppia* species group and to avoid potential circular reasoning, we repeated the analyses with Character 12 (presence or absence of a dorsal forewing scent pad) omitted. Characters were then mapped on trees with WinClada software (Nixon 2002) with the unambiguous changes option.

Specimens cited in this study are deposited in the following collections (abbreviations where available from Evenhuis (2013)).

BMNH The Natural History Museum [formerly British Museum (Natural History)], London, United Kingdom.

IAVH	Instituto Alexander von Humboldt, Villa de Leyva, Boyacá, Colombia.
MUSENUV	Museo de Entomología de la Universidad del Valle, Cali, Colombia.
MUSM	Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima, Peru.
RCB	Private Collection of Robert C. Busby, Andover, MA, USA.
UCRC	Entomology Research Museum, Department of Entomology, University of California, Riverside, California, USA.
USNM	National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

Systematics

Thereus Hübner, [1819]

Type species. *Papilio lausus* Cramer

Diagnosis. Robbins (1991) characterized *Thereus* by (1) a pair of sclerotized invaginations on the membrane attached to the ventro-lateral sides of the papillae anales (Figs 23–24, figured in Robbins 2000), (2) a pair of ventro-lateral brush organs (often inconspicuous) in addition to the pair of dorsal ones (Figs 15–18), and (3) the number of antennal nudum segments (as defined in the methods section) is sexually dimorphic, being greater in females than that in males by five or more segments (Fig. 25). The second trait is lacking in *T. pseudarcula* (Giacomelli), suggesting that this species is the phylogenetic sister to the remainder of the genus. Interestingly, it is the only *Thereus* species that appears to be a subtropical endemic. During this study, we found an instance in which sexual dimorphism of antennal nudum length was four segments—not five or greater—so the generic diagnosis is modified accordingly.

Robbins (1991) noted that *Thereus* contained 27 species, but more than a decade later, 31 were listed, of which nine were undescribed and two had questionable generic placement (Robbins 2004). Bálint (2005) proposed a new specific name, and we now recognize 35 species, of which eleven are undescribed and two have questionable generic placement. This paper begins the task of recognizing species groups, which facilitates the description of new species, and of determining the generic placement of the species with questionable generic placement.

Nomenclature. Robbins (1991) accorded *Thereus* Hübner priority over *Molus* Hübner and synonymized *Noreena* K. Johnson, MacPherson & Ingraham. Subsequently proposed names *Solanorum* Johnson, *Timokla* Johnson, Kruse & Kroenlein, and *Pedusa* d'Abrera were synonymized later (Robbins 2004).

Distribution. *Thereus* occurs throughout the Neotropics from northern Mexico to Uruguay and Argentina. *Thereus lausus*, *T. cithonius*, and *T. ortalus* range widely from Mexico to southern Brazil, but distributions of species are otherwise more restricted. Approximately 2/3 of the species in the genus occur in the Amazon Region, as demarcated by Brown (1982).

Habitat. Most species inhabit wet lowland forest, with only a few exceptions. *Thereus gabathana* (Strand), *T. wojtusiaki* Bálint, *T. orasus* (Godman & Salvin), and an undescribed species are montane endemics, and *T. pseudarculara* is subtropical. The widespread *T. cithonius* occurs in a great variety of habitats, from wet forest to very dry deciduous forest and from sea level to 2,000 m elevation.

Biology. Heredia and Robbins (in prep.) summarize the food plant records for the genus, almost all of which belong to the plant family Loranthaceae (mistletoe). As noted in this paper, males of some species set up mating territories in the morning before 09:30 hours while others set up territories in the early afternoon.

Thereus oppia species group

Diagnosis. The four members of the *T. oppia* species group possess the proposed synapomorphies of *Thereus* and are distinguished by a convex inner margin of the forewing (Figs 9–12), a scent patch on the dorsal forewing covering the basal part of cell r_5 - M_1 (Figs 9–12), and a scent patch on the ventral surface of the forewing (Figs 13–14). No other *Thereus* species possesses any of these traits. They also have genitalic structures that are indistinguishable, or nearly so (Figs 15–24).

Male secondary sexual organs (Figs 5–18). There are four distinct kinds of male secondary sexual organs in the *T. oppia* species group.

(1) Two of the four species have a scent pad on the dorsal surface of the forewing located at the basal origin of veins r_3 and M_1 (Figs 5–8). Scent pad histology and morphology have been detailed (Thomas 1893, Robbins 1991, Robbins et al. 2012). Scent pads occur only in the Eumaeini and Tomarini, but no case is known in which a scent pad was lost evolutionarily and then regained.

(2) A scent patch on the dorsal surface of the hindwing centered at the base of cell r_5 - M_1 occurs in all four species of the *T. oppia* group (Figs 9–12), but in no other *Thereus* species. The blue and gray (in *T. orasus*) androconia are iridescent in *T. orasus* and *T. lomalarga* (Figs 9–10). The androconia are gray to black in *T. oppia* and *T. brocki* (Figs 11–12). There are also piliform shaped androconia in *T. oppia* (Fig. 11, noted in Godman and Salvin 1887–1901). Superficially similar kinds of scent patches occur widely in the Eumaeini, such as *Allosmaitia* (Clench 1964) and *Lathecla* (Robbins and Busby 2015), but not in other *Thereus* or *Rekoa*.

(3) A scent patch on the ventral surface of the forewing located between the inner margin and the cubital vein (Figs 13–14). Again, superficially similar scent patches occur widely in the Eumaeini, but not in other *Thereus* or *Rekoa*. In *T. oppia* and *T. brocki*, there are also erect piliform setae that attach to the inner margin (Fig. 14). The tips of these setae are evident in *T. oppia* (Fig. 3, underside of male) and can be seen on the left underside of the male in *T. brocki* (Fig. 4). Superficially similar setae occur in the tribe Deudorigini (Eliot 1973: 403), where they are almost universal, but are unreported in other Eumaeini or in any other Theclinae, so far as we are aware. Further, the erect setae in Deudorigini are not associated with a scent

patch on the ventral forewing, as in *T. oppia* (the light tan scales under the tips of the setae – Fig. 14).

(4) All *Thereus* species have a pair of dorsal and a pair of ventral brush organs (Figs 15–18). Brush organs are bundles of hollow setae attached to the membrane connecting the male genitalia vinculum to the posterior 8th abdominal segment (Eliot 1973). They have a chamber at the anterior end, presumably containing a secretory cell (Robbins 1991). The only other Eumaeini with four brush organs are two species of *Chalybs* Hübner, a genus that is unrelated to *Thereus* (Quental 2008).

Male genitalia (Figs 15–18). There is little interspecific variation in the male genitalic structures in the *T. oppia* species group. All males of the *T. oppia* group possess minute teeth on the vesica inside the penis, but there are also minute teeth on the external ventral tip of the penis of *T. lomalarga* and *T. oppia* (Figs 16–17). Otherwise, we cannot distinguish the species based on genitalia.

Female genitalia (Figs 19–22). The ductus seminalis arises dorsally from the posterior end of the ductus bursae. Signa are absent, but occasionally vestigial remnants can be observed. Although shape and size of the ductus bursae are variable, as illustrated, this variation does not distinguish species.

Distribution and habitat (Fig. 26). Members of the species group occupy the Transandean and Amazon Regions, as outlined in Brown (1982). *Thereus orasus* is a montane endemic, but the others occur in lowland and lower montane forest.

Biology. Three of the four species have been reared from Loranthaceae (see below). Male behavior is recorded for *T. lomalarga* and *T. oppia*.

Thereus orasus (Godman & Salvin, 1887)

Figs 1, 5, 9, 15, 19

Diagnosis. *Thereus orasus* differs from other members of the *T. oppia* group by having a gray ventral ground color, not brown (Figs 1–4). The male is also unique in having no dorsal forewing brown border (except for some marginal black scales) and the gray part of the dorsal hindwing scent patch is restricted to the basal part of cell r_s-M_1 (Fig. 9).

Nomenclature. Robbins (2004) synonymized *Thecla echinita* Schaus (Fig. 1, female type in USNM) with *Thecla orasus* (male holotype in BMNH) because they share a similar ventral wing pattern and occur in the same habitats and have the same distribution. We have examined both types.

Distribution and habitat. *Thereus orasus* is an uncommon species that is recorded from montane habitats from central Mexico (Colima and Veracruz) to those of western Panama (Chiriquí) at elevations from 1100 to 1800 m.

Caterpillar food plant. Greg Ballmer collected a larva on 30 Aug 1988 at El Jabalí, 13 mi NE Comala, Colima, Mexico, at 1100–1200 m. The caterpillar was eating *Struthanthus condensatus* Kuijt (Loranthaceae). An eclosed adult female and its pupal case are deposited in UCRC. The mistletoe plant was growing on coffee and was identified by Kuijt.



Figures 1–4. Adults of the *Thereus oppia* species group. Male (left, dorsal wing surface on left) and female (right). **1** *T. orasus* ♂ Panama, ♀ Mexico (holotype of *Thecla echinita* Schaus) **2** *T. lomalarga* ♂ Colombia (holotype), ♀ Colombia (paratype) **3** *T. oppia* ♂ Nicaragua, ♀ Nicaragua **4** *T. brocki* ♂ Ecuador (holotype), ♀ Ecuador (paratype). Scale bars: 1.0 cm.

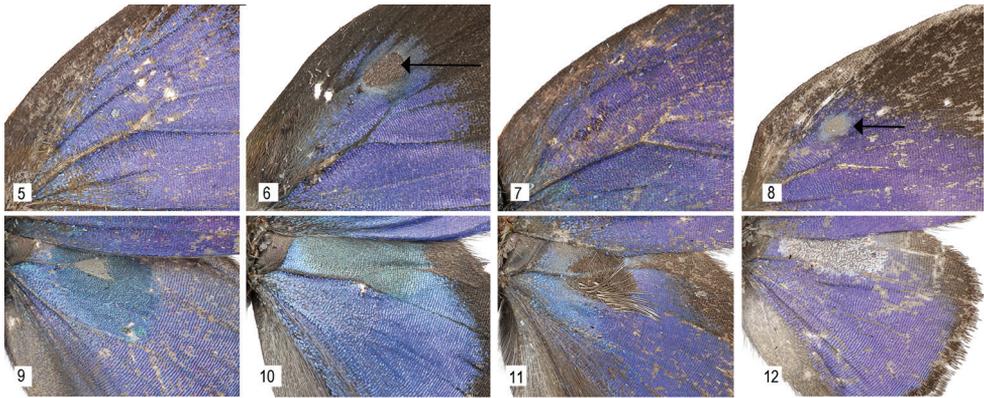
***Thereus lomalarga* Robbins, Heredia & Busby, sp. n.**

<http://zoobank.org/39501F5C-16C9-437D-874F-74722CEF1AB6>

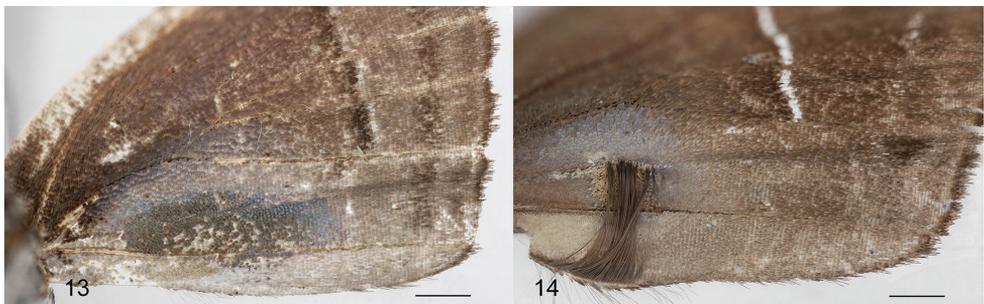
Figs 2, 6, 10, 13, 16, 20, 23, 25

Type material. Holotype: ♂ (Fig. 2). [printed on white paper] COLOMBIA: Valle del Cauca/Cali, Pance, Loma Larga/1200m, 3°19'N/76°34'W/1 April 2011/Leg. M.D. Heredia. [printed label on red paper] Holotype/*Thereus lomalarga*/Robbins, Busby, & Heredia. [printed white barcode label] Instituto Humboldt/Colombia/IA-vH-E-146988. Deposited IAVH.

Paratypes (32♂, 41♀). **Costa Rica.** 1♀ Turrialba, 2,000 ft, 13 Jul 1965 (USNM). **Panama.** Canal Area. Paraíso, Cerro Luisa, 4 Feb 1979 (2♂ USNM), 16 Feb 1979 (1♂ USNM), 1 Mar 1979 (1♂ USNM), 4 Mar 1979 (1♂ USNM), 10 Mar 1979 (1♂ USNM). Pedro Miguel, Chiva Chiva Road, 14 Jan 1979 (1♀



Figures 5–12. 5–8 Scent pads on the dorsal forewing. 5 *T. orasus* (absent) 6 *T. lomalarga* (arrow) 7 *T. oppia* (absent) 8 *T. brocki* (arrow) 9–12 Scent patches on the dorsal hindwing, also showing the convex forewing inner margin. 9 *T. orasus* 10 *T. lomalarga* 11 *T. oppia* 12 *T. brocki*.



Figures 13–14. Scent patches on the ventral forewing. 13 *T. lomalarga* 14 *T. oppia*, showing the erect androconia attached to the inner margin (also in *T. brocki*), which occurs in no other Eumaeini. Superficially similar androconia are widespread in tribe Deudorigini. Scale bars: 1.0 mm.

USNM). Panama Province. Cerro Campana. 1500 ft/500m. 3 Jan 1965 (1♀), 26 Jan 1966 (1♀ USNM), 28 Jan 1980 (1♀ USNM). 850 m. 23 Feb 1979 (1♀ USNM). Chiriquí Province. Potrerillos. 3600 ft. 27 Dec 1965 (1♀ USNM), 28 Dec 1965 (2♀ USNM), 27 Dec 1965 (1♀ USNM), 1 Jan 1966 (2♀ USNM), 28 Jan 1966 (2♀ USNM), 29 Jan 1966 (1♀ USNM), 2 Feb 1966 (1♀ USNM), 19 Feb 1966 (1♀ USNM), 5 Mar 1966 (1♀ USNM). **Colombia.** Valle del Cauca, Cali. Pance, 3000 ft, 14 Jan 1985 (1♀ USNM)). Loma Larga, 1200m. 3°19'N/76°34'W. 15 May 2009 (1♂ MUSENUV). 18 Nov 2010 (1♂ USNM, IAvH-E-112219). 5 Dec 2010 (1♂ MUSENUV). 10 Dec 2010 (1♀ MUSENUV). 13 Jan 2011 (1♀ USNM, IAvH-E-112207). 15 Mar 2011 (1♀ MUSENUV). 17 Mar 2011 (1♀ MUSENUV). 19 Mar 2011 (1♀ MUSENUV). 31 Mar 2011 (1♀ MUSENUV). 1 Apr 2011 (1♂ & 1♀ MUSENUV). 2 Apr 2011 (1♂ MUSENUV). 5 Apr 2011 (1♀ MUSENUV). 6 Apr 2011 (1♀ IAVH, IAvH-E-146990). 24 Apr 2011 (1♂

IAVH, IAvH-E-146982). 21 May 2011 (1♂ USNM, IAvH-E-146983). 29 May 2011 (1♂ MUSENUV). 30 May 2011 (1♂ IAVH, IAvH-E-146987). 1 Jun 2011 (1♂ USNM, IAvH-E-146985). 15 Jun 2011 (1♂ MUSENUV). 17 Jun 2011 (1♂ MUSENUV). 21 Jun 2011 (1♀ IAVH, IAvH-E-146986). 23 Jun 2011 (1♂ MUSENUV). 27 Jun 2011 (1♀ MUSENUV). 28 Jun 2011 (1♀ IAVH, IAvH-E-146984). 14 Jul 11 (1♂ MUSENUV). 23 Jul 2011 (1♂ MUSENUV). 17 Dec 2011 (1♀ MUSENUV). 31 Dec 2011 (1♀ USNM, IAvH-E-146989). 20 Feb 2012 (1♂ MUSENUV). 23 Feb 2012 (1♀ MUSENUV). 3 Mar 2012 (1♂ MUSENUV). 4 Mar 2012 (1♂ MUSENUV). 6 Mar 2012 (1♂ MUSENUV). 12 Mar 2012 (1♂ MUSENUV). 3 Apr 2012 (1♂ MUSENUV). 15 Apr 2012 (1♂ MUSENUV). 19 Apr 2012 (1♂ MUSENUV). 22 Apr 2012 (1♀ MUSENUV). 29 Aug 2012 (1♂ MUSENUV). 25 Jun 2014 (1♀ MUSENUV). 4 Jul 2014 (1♂ MUSENUV). 3 Jul 2014 (1♂ MUSENUV). **Ecuador.** Pichincha, 10 km Celica-Sardinas Road, 0°11.6'N, 79°00.8'W, 550–775 m, 27 May 2008, (1♀ RCB); 7 km Pacto-Guayabillas Road, 0°09.0'N, 78°48.9'W, 1600m, 18 Jun 2014, (2♀ RCB); 5 km Nanegal- García Moreno Road, 0°09.2'N, 78°39.4'W, 1375–1700m 21 Jan 2015, (1♀ RCB); 24 May 2008, (1♀ RCB).

Etymology. This species is named for Loma Larga, a housing development on the outskirts of Parque Nacional Natural Farallones de Cali. Loma Larga has had an ecological and conservation policy for about 15 years that has designated a substantial plot of land for natural forest regeneration (Fig. 27) in contrast to cow pasture. The name is a noun in apposition.

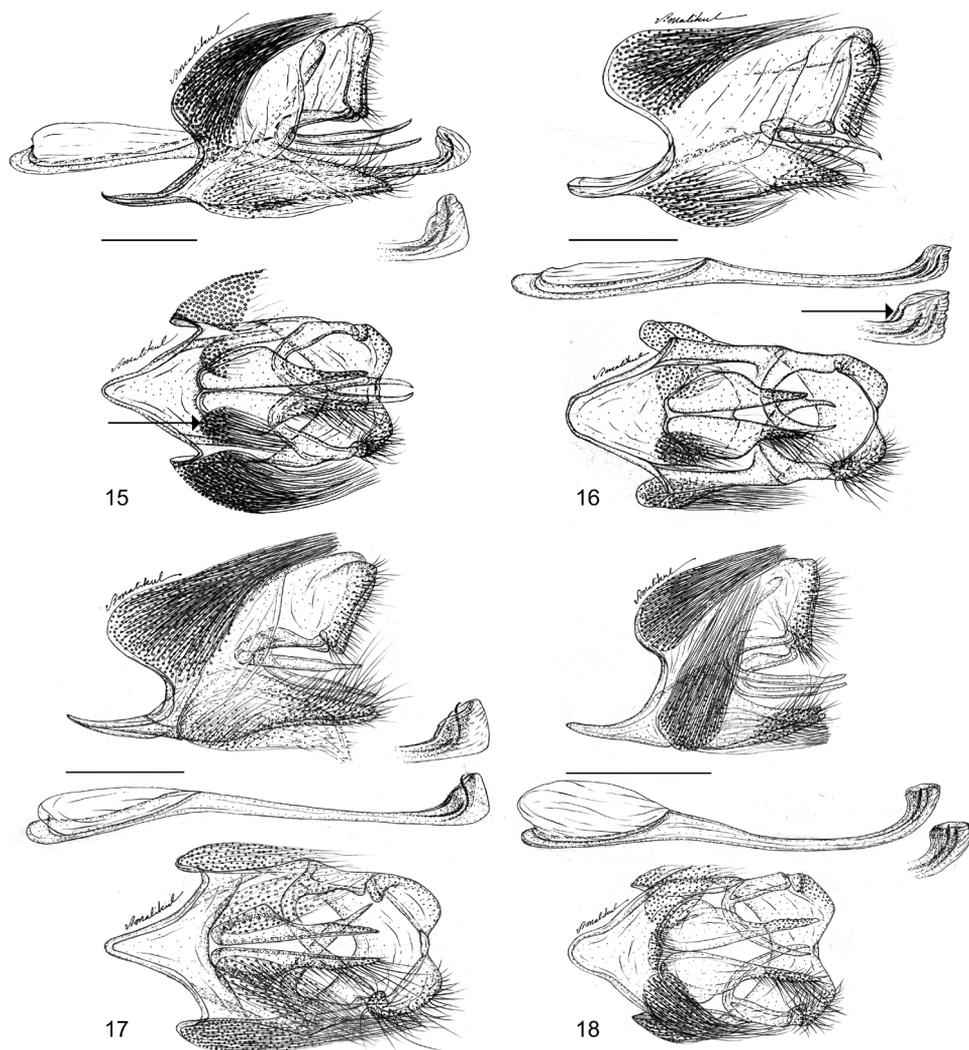
Type locality (Fig. 27). The type locality is naturally regenerated forest in Loma Larga.

Diagnosis and description. *Thereus lomalarga* belongs to *Thereus* because it possesses the synapomorphies of the genus (Figs 16, 23, 25). It belongs to the *T. oppia* species group (Figs 2, 10, 13). It shares a dorsal forewing scent pad with *T. brocki* (Figs 6, 8), but differs in having an iridescent scent patch on the dorsal hindwing and in lacking erect piliform setae on the inner margin of the ventral forewing (Figs 2, 10, 13). The male has evident teeth on the dorsal tip of the penis (Fig. 16), which distinguishes it from *T. orasus* and *T. brocki*. The wing pattern, androconia, genitalia, and antennae are illustrated (Figs 2, 6, 10, 13, 16, 20, 23, 25). Mean forewing size of males is 11.67 mm (sd = 0.52, N = 23) and of females is 11.34 mm (sd = 0.65, N = 16).

Variation. Expression of the female orange-red spot at the anal lobe of the ventral hindwing between vein Cu_2 and the inner margin varies from a fused double spot, as in Fig. 2, to completely absent. The ventral ground color varies from gray to brown. The postmedian line on the ventral hindwing varies slightly in shape from that in Fig. 2 to that of *T. brocki* in Fig. 4.

Distribution (Fig. 26). Costa Rica to the western slope of the Andes in Ecuador. It is allopatric with *T. orasus*, its hypothesized phylogenetic sister (Fig. 28). Their ranges overlap in Costa Rica and Panama, but in these countries, *T. lomalarga* is recorded below 1100 m and *T. orasus* at 1800 m.

Habitat. *Thereus lomalarga* occurs in the great variety of forested habitats. In Central America, it occurs from sea level to lower montane humid forest at 1100 m elevation.



Figures 15–18. Male genitalia of the *Thereus oppia* species group. Lateral view of capsule and penis (top) with penis tip enlarged and ventral view (bottom). Posterior of insect to the right **15** *T. orasus* (arrow points to ventral brush organ) **16** *T. lomalarga* (arrow points to position of small teeth) **17** *T. oppia* **18** *T. brocki*. Scale bars: 0.5 mm.

In South America, it also occurs at elevations up to 1600 m. Although two females have labels with the elevation range 1375–1700 m, we have since learned from the collectors that they were found in the lower half of this range.

Phenology. Adults in Panama were collected during the dry season without exception, suggesting adult seasonality. However, caterpillars in Colombia were found throughout the year and reared to the adult stage. Perhaps adults of this species are more apparent to collectors during the dry season.

Male behavior. Six males displayed territorial hilltopping behavior from 09:00–09:30 hours during the dry season (February, March 1979) at the top of a small tree on the southwest edge of the summit of Cerro Luisa, Paraíso (9°02'N, 79°37'W), Canal Area, Panama (vouchers in USNM). The longitude on one specimen is incorrectly labeled 79°38'W.

Caterpillar food plant. *Oryctanthus alveolatus* (H.B.K.) Kuijt (Loranthaceae) growing on *Miconia minutiflora* (Bonpl.) DC. Details of the life history will be published elsewhere (Heredia and Robbins in prep.).

Remarks. *Thereus lomalarga* is a peculiar butterfly in that adult females are far more frequently encountered—at least by butterfly collectors—than are adult males. For example, all collected adults from Costa Rica, Colombia, and Ecuador are females. Among collected adults in Panama, females have been found from sea level in the Canal Area to Cerro Campana (at about 850 m along the trail to the summit, Panama Province) to Potrerillos at 1,100 m (Chiriquí Province). In contrast, adult males have been collected only at the top of one small tree on Cerro Luisa in the Canal Area in the dry season in 1979. All other males, including the holotype, were reared from caterpillars. Among 44 reared individuals at the type locality, 27 are males, so the sex ratio among immatures is not biased towards females.

No museum specimens other than those in the type series have been seen by the authors. However, females of *T. lomalarga* are “non-descript small gray hairstreaks”, and other specimens may be found in museum collections.

***Thereus oppia* (Godman & Salvin, 1887)**

Figs 3, 7, 11, 14, 17, 21

Diagnosis. The male of *T. oppia* is distinguished from *T. lomalarga* and *T. brocki* by lacking a scent pad on the dorsal forewing (Fig. 3) and from *T. orasus* by having a darker gray/brown ventral ground color (Fig. 3). Both sexes differ from the other members of the species complex by lacking dark scaling along the basal edge of the postmedian line on the ventral wings (Fig. 3). *Thereus oppia* has small teeth on the ventral tip of the penis (Fig. 17), in contrast to *T. brocki* and *T. orasus*.

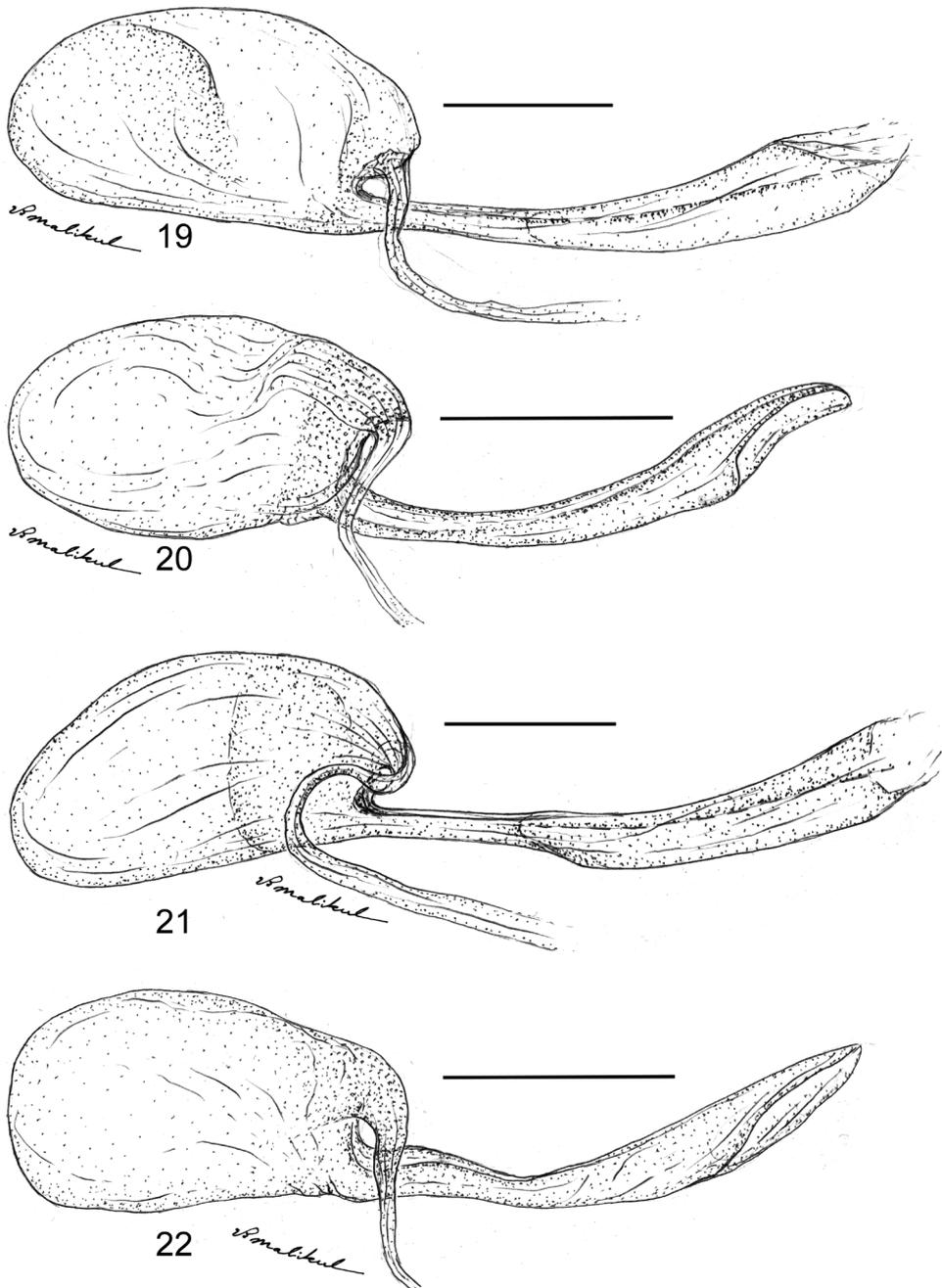
Nomenclature. We examined a syntype of this species in the BMNH.

Distribution and habitat. *Thereus oppia* occurs from Mexico to Costa Rica at a variety of elevations. Most localities where it occurs appear to be deciduous dry forest. It is allopatric with its sister species, *T. brocki*.

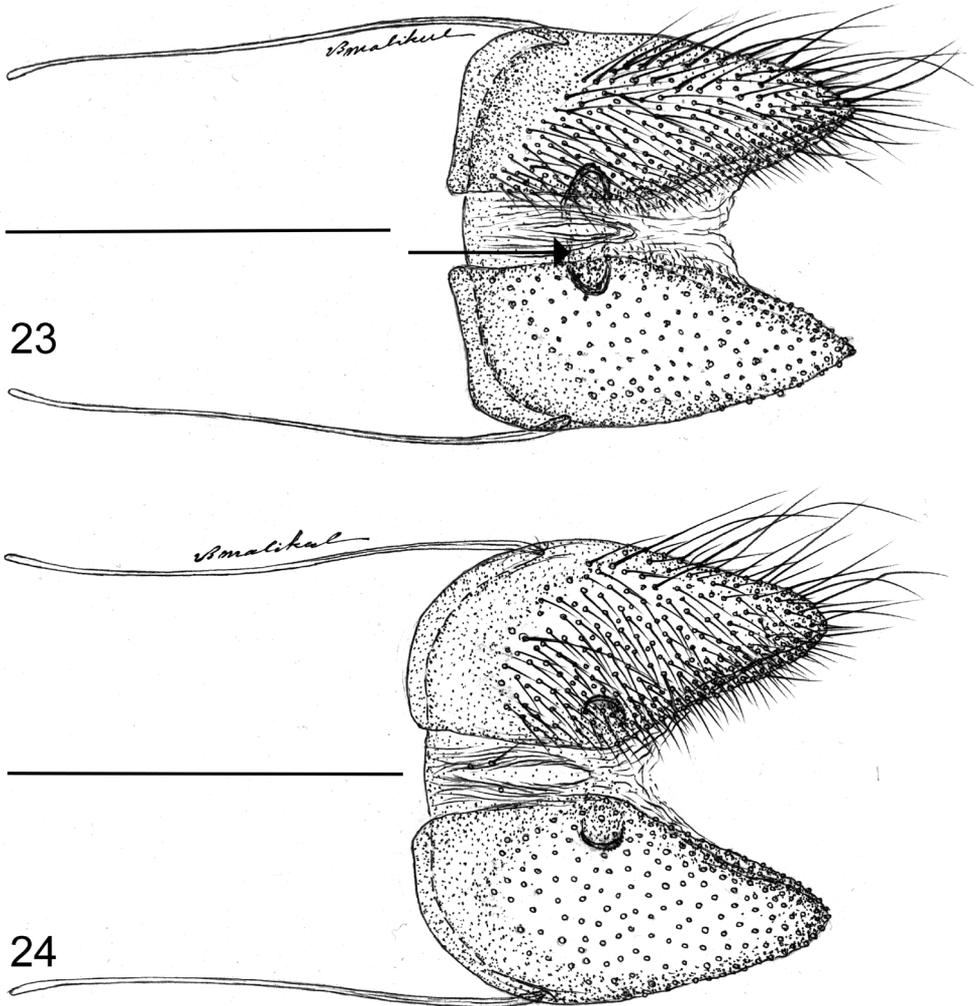
Male behavior. Territorial male behavior was observed at Ciudad Valles, SLP, Mexico in the early afternoon (vouchers in RCB), in contrast to the early morning territorial behavior of *T. lomalarga*.

Adult flower feeding. Adults of *T. oppia* were found nectaring on *Cordia* (Boraginaceae) flowers at two localities in Veracruz, Mexico (vouchers in RCB).

Caterpillar food plant. From Janzen and Hallwachs (2015), a pupa was found 30 April 1993 on *Struthanthus orbicularis* (Kunth) Blume (Loranthaceae) at Send-



Figures 19–22. Female bursa copulatrix of the *Thereus oppia* species group. Dorsal (top) and lateral view of the ductus copulatrix. Posterior of insect to the right. **19** *T. orasus* **20** *T. lomalarga* **21** *T. oppia* **22** *T. brocki*. Scale bars: 0.5 mm.



Figures 23–24. Female papillae anales in ventral aspect showing sclerites that characterize *Thereus* (arrow). Posterior of insect to the right. **23** *T. lomalarga* **24** *T. brocki*. Scale bars: 0.5 mm.

ero Carobonal, Santa Rosa, Area de Conservación Guanacaste, Guanacaste, Costa Rica, latitude 10.77594, longitude -85.65799. An adult male (voucher 93-SRNP-30, deposited USNM) emerged 16 May 1993. As an associated comment on the web site, “red-brown pupa with white markings laterally so that it looks just like a bird turd, sitting on the top of a mistletoe leaf in middle of large plant (this species is a sprawler, vine/shrub); host tree was leafless.” The leafless host tree is the reason that we consider the mistletoe plant on which the pupa was found to be the caterpillar food plant.

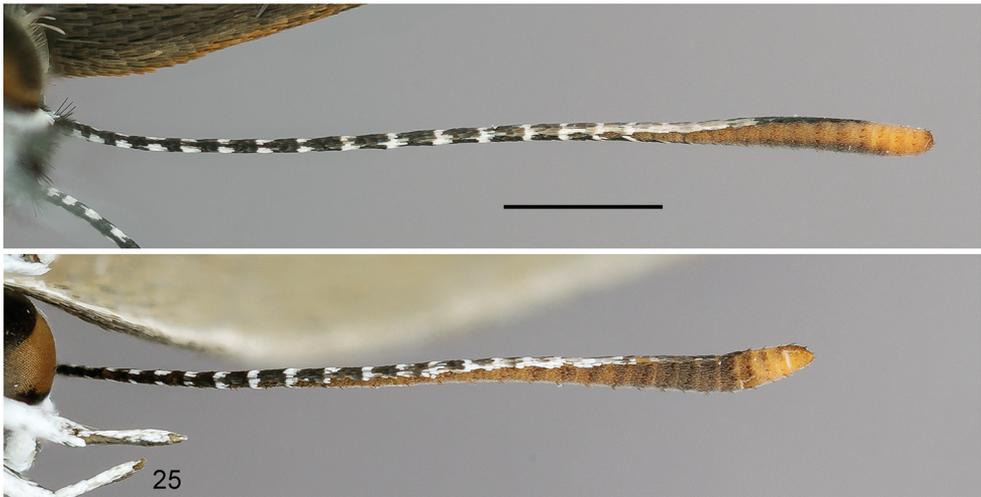


Figure 25. Male (top) and female antennae of *T. lomalarga* in ventral aspect showing nudum extent. The male has 14 nudum segments in contrast to 21 nudum segments in the female. Scale bar: 2 mm.

***Thereus brocki* Robbins, Heredia & Busby, sp. n.**

<http://zoobank.org/458AA4B0-A519-40D3-90BA-9FC7F286DBCE>

Figs 4, 8, 12, 18, 22, 24

Type material. Holotype: ♂ (Fig 4). [printed and handwritten on white paper] 28 June 1980/25 km. n. e. of Puyo,/Prov. Pastaza, Ecuador/leg. Jim P. Brock [printed on green paper] GENITALIA No./2013: 56♂/R. K. ROBBINS [printed white barcode label] USNM ENT 00181942 [printed label on red paper] Holotype/*Thereus brockii* Robbins, Busby, & Heredia. Deposited USNM.

Paratype (1♀). **Ecuador.** 1♀ (Fig. 4). Napo, 14 km Tena-Puyo Road, Apuya, 01°06.7'S, 77°46.9'W, 600 m, 10 Sep 2010, (RCB).

Other specimens (1♂, 2♀). **Colombia.** 1♀. Vaupés, Mitú, 28 Jun 1972 (USNM). **Peru.** 1♂ San Martin, Juanjuí, 7°11'S, 77°44'W, 300–400 m, Nov 2011 (MUSM, examined from an image). 1♀. Huánuco, Tingo María, 800 m, 24 Jun 1982 (USNM).

Etymology. This species is named for James Brock of Tucson, Arizona. He collected the holotype and has made numerous contributions to the knowledge and enjoyment of butterflies. The name is a masculine noun in the genitive case.

Type locality. The type locality has been a well-known collecting site for 40 years at about 975 m elevation (noted in Brown 1979 with coordinates 01°20'S, 77°55'W, but incorrectly placed in Napo Province). The entrance to this locality is approximately 25 km (measured by a car odometer) from Puyo on the western side of the Puyo-Tena Rd. New metal road markers have been erected which place the entrance between km 26 and km 27. The trees in this location have been selectively logged for decades, and we do not believe much forest remains.



26

Figure 26. Geographic distribution of *T. lomalarga* (circles) and *T. brocki* (squares).

Diagnosis and description. *Thereus brocki* belongs to *Thereus* because it possesses the synapomorphies of the genus (Figs 18, 24). It belongs to the *T. oppia* species group (Figs 4, 12). It shares a dorsal forewing scent pad with *T. lomalarga* (Figs 6, 8), but



Figure 27. Natural succession forest in Loma Larga, the type locality of *T. lomalarga*, with flowering *Miconia minutiflora* (Bonpl.) DC.

differs in having a gray-brown scent patch on the dorsal hindwing without iridescence and in possessing erect piliform setae on the inner margin of the ventral forewing (Fig. 4). The male lacks evident teeth on the dorsal tip of the penis (Fig. 18), which distinguishes it from *T. lomalarga* and *T. oppia*. The female of *T. brocki* is very similar to that of *T. lomalarga*, but differs in having more orange-red scales on the ventral hindwing at the anal lobe (Fig. 4). The wing pattern, androconia, and genitalia are illustrated (Figs 4, 8, 12, 18, 22, 24). Forewing size of the holotype male is 0.9 cm and of two females is 1.1 cm and 1.2 cm.

Female. The female paratype of *T. brocki* is associated with the male by the shape similarity of the ventral hindwing postmedian line and by their capture approximately 30 km apart. Although the females of *T. brocki* and *T. lomalarga* illustrated in Figs 2 & 4 would seem to be distinguishable phenotypes, wing pattern variation in the extensive type series of the latter encompasses both phenotypes. For this reason, we restricted the paratype series. We unsuccessfully tried to extract DNA sequences from *T. brocki* to confirm the identification of the females.

Sexual dimorphism. Forewing discal cell length in the male of *T. brocki* (Fig. 12) is shorter than in the female.

Distribution (Fig. 26). Eastern Colombia to eastern Peru. It is allopatric with its phylogenetic sister, *T. oppia*.

Habitat. *Thereus brocki* has been recorded only from wet forest up to about 1,000 m elevation.

Remarks. The holotype and the Peruvian male are the only males in collections, so far as we are aware. The type locality has been a “famous” collecting locality for decades, as noted, so it is somewhat unexpected that the holotype remains the only known Ecuadorian male. It would appear that adult males of *T. brocki*, like those of *T. lomalarga*, are

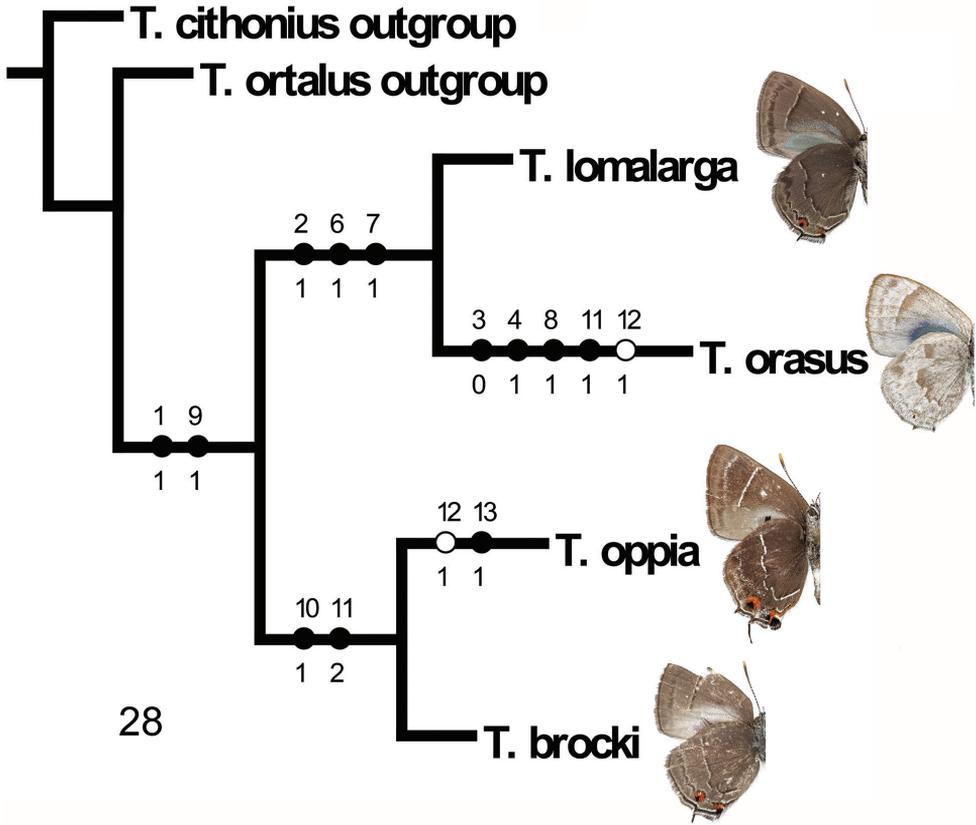


Figure 28. Most parsimonious cladogram for of the *Thereus oppia* species group with unambiguous character state changes (22 steps, CI = 81, RI = 66). Hollow circles are homoplastic changes. Numbers to right of nodes in brackets are bootstrap values. The dorsal forewing scent pad (Character 12) was unambiguously lost twice. See text for further explanation.

rarely encountered by insect collectors. Although we have an image of the Peruvian male (discovered late in the publication process), we have not had an opportunity to examine it. It is identified as *T. brocki* because it has the shortened forewing discal cell of the holotype and the same male wing secondary sexual traits, except that the erect piliform setae are not visible in the image. For this reason, we exclude it from the type series.

Nomenclatural checklist – *T. oppia* species group

Thereus orasus (Godman & Salvin, 1887) (*Thecla*)

type locality: Guatemala

= *echinita* (Schaus, 1902) (*Thecla*)

type locality: Mexico (VER)

Thereus lomalarga Robbins, Heredia & Busby, sp. n.

type locality: Colombia

Thereus oppia (Godman & Salvin, 1887) (*Thecla*)

type locality: Mexico (VER)

Thereus brocki Robbins, Heredia & Busby, sp. n.

type locality: Ecuador

Phylogenetic analyses

Based on morphological characters (Table 1) coded in a matrix (Table 2), there was one most parsimonious 22-step equal weight tree (CI = 81 and RI = 66). Furthermore, each implied weight most parsimonious tree with different values for the parameter K resulted in one tree, also with the same topology as the most parsimonious equal weight tree. When the data were analyzed with the scent pad omitted (Character 12), the tree topology was the same. In accord with Quental's (2008) results, unambiguous optimization suggests that the scent pad was lost twice and not regained (Character 12, Fig. 28).

Discussion

Systematics. The genus *Thereus* has been described six times, probably because the genus contains species that possess a variety of androconial organs, wing patterns, wing shapes, and wing sizes. However, *Thereus* is clearly characterized by synapomorphies of the male genitalia, female genitalia, and antennae, as illustrated in this paper. Further, a third of the species have been reared, and with the exception of an undescribed, morphologically aberrant species, all use Loranthaceae as a caterpillar food plant. A genus that is morphologically and biologically homogenous, such as *Thereus*, conveys information, which makes it a useful and predictive classification. For example, it allows us to hypothesize that all *Thereus* species that have not yet been reared, including *T. brocki*, eat Loranthaceae.

The wing patterns of the newly described *T. lomalarga* and *T. brocki* are very similar (Figs 2, 4), for which reason these species might well be thought of as likely sister species. However, the phylogenetic analysis is consistent with the hypothesis that the wing pattern similarities are symplesiomorphies. The opposite situation occurs with the wing patterns of *T. orasus* and *T. oppia* (Figs 1, 3). Historically, these species were not considered to be close relatives (i.e., Draudt 1919–1920), presumably because their wing patterns are quite different (Figs 1, 3). The phylogenetic analysis makes clear that the wing pattern of *T. orasus* is divergent within the *T. oppia* species group because of evolutionary wing pattern changes in the ancestor of *T. orasus*, not because of a lack of relationship.

Biology. Males of the newly described *T. lomalarga* and *T. brocki* are exceedingly rare, at least in museum collections. For example, adult males of *T. lomalarga* have been collected at only one site, despite more than a century of butterfly collecting in Costa Rica, Panama, western Colombia, and western Ecuador. Alternately, immatures of three of the four species in the *T. oppia* group are associated with Loranthaceae. Searching for caterpillars on Loranthaceae may be a more efficient way to find males (and females) of these and related species. More generally, it may be the best way to document and study the biology of *Thereus* species.

Erect piliform setae on the ventral forewing that attach to the forewing inner margin (Fig. 14) evolved in the ancestor of *T. oppia* and *T. brocki* (Fig. 28, Character 10). They occur in no other Eumaeini and are presumably a newly evolved male secondary sexual organ. This evolutionary gain neither increased nor decreased diversification. These piliform setae are superficially similar to those that occur almost universally in the related tribe Deudorigini (Eliot 1973). The evident difference is that the erect setae in *Thereus* are associated with a scent patch on the ventral forewing while those in the Deudorigini are not. It remains an open question whether the genetic pathway by which these erect setae develop is the same in *Thereus* and the Deudorigini.

Quental (2008) postulated that the eumaeine scent pad has been lost evolutionarily many times without being regained, following Dollo's law. In the *T. oppia* species group, two species possess a scent pad and two lack it, and it is reasonable to ask whether the scent pad could have been re-gained evolutionarily in this group. Indeed, cladograms such as (*T. oppia* + (*T. orasus* + (*T. lomalarga* + *T. brocki*))) or (*T. orasus* + (*T. oppia* + (*T. lomalarga* + *T. brocki*))) would be consistent with the evolutionary loss and re-gain of the scent pad. However, according to the phylogenetic results in this paper, in which the scent pad character was omitted, the scent pad was unambiguously lost twice evolutionarily and not regained. In each case, the species that lost the scent pad is allopatric with its sister species. This result is consistent with previous findings (Quental's 2008, Robbins et al. 2012) and more generally, with Wiens' (2001) observations on the evolution of male secondary sexual traits in animals.

Acknowledgements

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A new marine cyclopoid copepod of the genus *Neocyclops* (Cyclopidae, Halicyclopinæ) from Korea

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Abstract

A new cyclopoid species of the genus *Neocyclops* Gurney, 1927 is described. Type specimens were collected from a beach on south-western coast of the Korean Peninsula by rinsing intertidal coarse sandy sediments. *Neocyclops hoonsooi* **sp. n.** is most characteristic in showing the conspicuous chitinized transverse ridges originating from the medial margins of the coxae of all swimming legs. The new species is most similar to *N. vicinus*, described from the Brazilian coast, and *N. petkovskii*, from Australia. All three species share a large body size (more than 750 µm long), the presence of an exopodal seta on the antenna, two setae on the mandibular palp, the same seta/spine armature on the third endopodal segment of leg 3 (3 setae + 3 spines), and the fairly long inner distal spine on the third endopodal segment of the female leg 4. However, *N. hoonsooi* **sp. n.** differs from both species by the much shorter caudal rami (less than 1.7 times as long as wide) and the shorter dorsal caudal seta VII. Furthermore, *N. hoonsooi* is clearly distinguished from *N. vicinus* by the 10-segmented antennule (vs 12 segments in *N. vicinus*), and from *N. petkovskii* by the elongate inner distal spine on leg 5 exopod and the 3-segmented leg 5 in male (vs 4-segmented in *N. petkovskii*). A tabular comparison of characters separating *N. hoonsooi* from its closest allies and a key to *Neocyclops* species from the Indo-Pacific Ocean are provided. This is the first record of the genus *Neocyclops* from the northern Pacific.

Keywords

Beach, description, interstitial, meiofauna, northwest Pacific, taxonomy

Introduction

Members of the genus *Neocyclops* Gurney, 1927 (Cyclopidae, Halicyclopinae) typically inhabit marine epibenthic or interstitial environments. The genus is widely distributed in coastal, surface and subterranean (anchialine) habitats of the Northeast and Tropical Atlantic, the Mediterranean, the Black and Red Seas and the Indo-Pacific (West Australia, Papua New Guinea), with an endemism index of about 95% (Pesce 2015). Karanovic (2008) recently revised the genus and added 11 new species, including eight interstitial species from Australia. However, notwithstanding its wide distribution and potentially high diversity, the taxonomy of the genus is in a state of disarray. The primary reasons for this state of affairs are the paucity of useful diagnostic characters to differentiate most species (due to the very conservative morphology within the genus), the significant variability displayed by some of them and the inadequate descriptions of several previously described species (Karanovic 2008). The genus currently accommodates 24 nominal species but it is known that many as yet unnamed species await description (Karanovic 2008, Walter and Boxshall 2015, Pesce 2015).

Although cyclopoid copepods constitute an important component of the marine epibenthic and interstitial fauna, our knowledge of their taxonomy and diversity is relatively very poor in comparison with freshwater cyclopoids, especially in the northwest Pacific region (Chang 2011, Karanovic 2014). Chang (2011) recorded a new species of the genus *Cyclopinoides* Lindberg, 1953 (Smirnovipinidae) from the Korea Strait (Tsu-shima Island, Japan and Busan, Korea), and recently Karanovic (2014) described a new species of *Euryte* Philippi, 1843 (Cyclopidae) from the East Sea (Sea of Japan). During field surveys of the marine interstitial cyclopoids from Korea, a new species of the genus *Neocyclops* was found in a beach on the south-western coast of the Korean Peninsula, representing the first record of the genus from the North Pacific. In this paper we provide a detailed illustrated description of both sexes, including a tabular comparison of the salient characters distinguishing the new species from its closest congeners.

Materials and methods

Collections were made at Holtong beach, located along the south-western coast of the Korean Peninsula, in shallow littoral (about 0.5–1 m deep) by scooping the surface layer of a coarse sand bottom with a long-handled dipper. Sediment samples were gathered into a bucket, subjected to freshwater shock and filtered through a conical plankton net or plankton hand-nets (mesh size 64 μm). Samples were immediately fixed in the field by adding a few drops of 35% formaldehyde. Copepods were sorted in the laboratory, using a micropipette under a zoom-stereomicroscope (Zeiss SV-11, Germany), and transferred to 80% ethanol or to 4% buffered formaldehyde for long-term preservation.

Methods for dissection, double-coverglass preparation using H-S slides (see Shirayama et al. 1993), drawing and measurements followed those outlined in Chang (2013, 2014).

Type specimens are deposited in The Natural History Museum, London (NHMUK) and the specimen room of the Department of Biological Science, Daegu University (DB), Korea.

General terminology for the description of the new species follows Huys and Boxshall (1991). Abbreviations used in the text, table and figure legends follow the conventional ones frequently used in the taxonomy of copepods: enp-1 to enp-3 or exp-1 to exp-3, the first to third endopodal or exopodal segments of each leg. Sewell's (1949) system is adopted for seta/spine armature of legs 1–4, where setae are denoted by Arabic numerals, and spines by Roman numerals (Tran and Chang 2013, for details cf. Huys and Boxshall 1991, fig. 1.5.7).

Systematic accounts

Family Cyclopidae Rafinesque, 1815

Genus *Neocyclops* Gurney, 1927

Neocyclops hoonsooi sp. n.

<http://zoobank.org/451980E9-A08A-4365-8240-7A185C4877C6>

Figs 1–4

Type locality. Holtong beach (35°03.68'N, 126°19.87'E), South Korea, Jeollanam-do Province, Muan-gun County, Hyeongyeon-myeon, Oryu-ri; western coast of the Dadohae Oceanic National Park, South Korea.

Material examined. Holotype ♀ (DB20046), allotype ♂ (DB20047), both dissected on slides. Paratypes: 1 ♀ (NHMUK reg. no. 2015. 3056), 1 ♂ (NHMUK reg. no. 2015. 3057), both undissected, ethanol-preserved; 2 ♀♀ (DB20048, 20049), 1 ♂ (DB20050), dissected on slides; 2 ♀♀ (DB20051), 2 ♂♂ (DB20052), in ethanol. All specimens were collected from the type locality by J. Lee on 21 August 2008.

Diagnosis. Female habitus large, about 830 µm long. Genital double-somite with lateral expansions in anterior quarter. Caudal rami about 1.7 times as long as wide, with 7 caudal setae, including vestigial ventrolateral seta I; inner caudal seta VI well developed, about 1.7 times longer than outer caudal seta III; dorsal seta VII slightly shorter than caudal ramus. Antennule 10-segmented. Antenna with exopodal seta. Exp-3 of legs 1–4 with setal formula 5,5,5,5 and spine formula 3,4,4,3; enp-3 of leg 3 bearing 3 spines and 3 setae; inner distal spine on enp-3 of leg 4 distinctly longer than enp-3 and outer distal spine. Leg 5 exopod subpyriform, about twice as long as wide; inner distal spine 1.2 times longer than outer spine, about 1.4 times as long as lateral spine, about 0.9 times as long as exopod. Male caudal rami 1.36 times longer than wide, with 7 caudal setae. Male leg 5 3-segmented, comprising coxa, basis and exopod.

Description. Female (Holotype). Body (Fig. 1A) large and robust, 830 µm long, (mean 826 µm, standard deviation 12, $n = 6$), excluding rostrum and caudal setae.

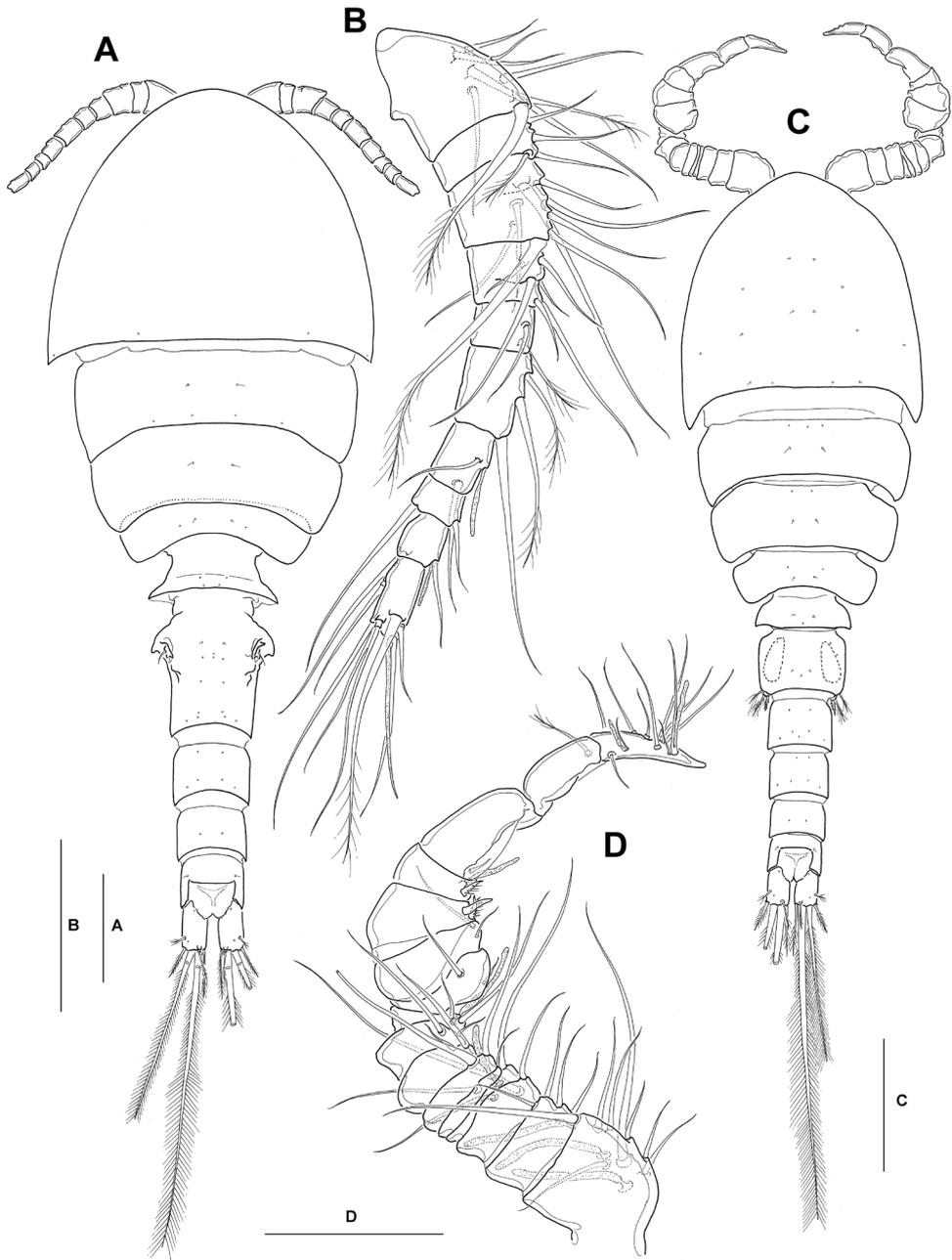


Figure 1. *Neocyclops hoonsooi* sp. n. **A–B** female: **A** habitus, dorsal **B** right antennule, dorsal. **C–D** male: **C** habitus, dorsal **D** left antennule, dorsal. Scale bars: 100 μ m (**A**, **C**), 50 μ m (**B**, **D**).

Body width 303 μm , greatest width at posterior margin of cephalothorax; body length/width ratio about 2.7. Color of preserved specimens a milky white tinge.

Prosome oval, about 1.2 times longer than urosome, a little protruding anteriorly. Rostrum reflexed downwards, not discernible in dorsal view, with blunt apex in ventral aspect. Nauplius eye not discernible. Cephalothorax not strongly protruding anteriorly, slightly longer than 4 free thoracic somites combined; first pedigerous somite completely incorporated into cephalosome. Prosomites not showing pronounced lateral expansions, with narrow and nearly smooth hyaline fringe along posterior; ornamented with one pair of minute sensilla medially on dorsal surface of second to fourth pedigerous somites, and a few integumental pores near posterior margin of each prosomite.

Urosomites (Figs 1A, 2B) length ratios, beginning with fifth pedigerous somite, 36: 100: 43: 39: 43; with hyaline membrane along posterior margins both dorsally and ventrally; spinule rows lacking, except for anal somite with about 20 minute spinules along ventral posterior margin; arrangement of cuticular pores as shown in Figs 1A, 2A and 2B. Fifth pedigerous somite slightly narrower than genital double-somite, ornamented with paired middorsal sensilla; posterolateral corner pronounced. Genital double-somite slightly longer than broad, with paired backwardly directed spinous processes in anterior quarter; leg 6 represented by one seta and one small cuticular projection surrounded by cuticular wrinkles dorsolaterally. Copulatory pore small, located midventrally in about proximal quarter of genital double-somite; seminal receptacle fully fused medially; both lateral sides transversely undulating, as shown in Fig. 2B. Anal somite much shorter than wide, about $3/4$ times longer than caudal rami; 1 pair of dorsal sensilla just anterior to lateral corners of anal operculum. Anal operculum (Fig. 2A) situated at halfway the anal somite length, not strongly convex with smooth posterior margin.

Caudal rami (Fig. 2A, B) nearly parallel, with 7 setae; ramus 1.68 times (ranging from 1.64 to 1.72, standard deviation 0.04, $n = 6$) longer than wide, in ventral view, slightly shorter than anal somite; dorsal and medial surfaces of rami smooth, without hairs along inner (medial) margin; outer margin nearly smooth, not interrupted by indentations or spinules. Anterolateral seta I vestigial, represented by minute setule, situated in anterior part of ventral surface (Fig. 2B). Lateral seta II located slightly dorsally, issuing from about distal quarter of outer margin of ramus. Outer seta III short, spiniform and bipinnate, about 0.7 times as long as ramus, a little less than $2/3$ length of inner seta VI, surrounded by 3–5 minute spinules at base. Terminal setae IV–V with fracture planes, bipinnate. Inner seta VI well developed, plumose, about 1.2 times as long as caudal rami, about 1.7 times longer than outer seta III. Dorsal seta VII slender, plumose, about $2/3$ times as long as inner seta VI, and slightly shorter (0.9 times) than caudal ramus.

Antennule (Fig. 1B) short, reaching to about middle of cephalothorax; 10-segmented; segments 3 and 5 with incomplete ventral and dorsal sutures, respectively, indicating original subdivision. Setal formula: 1-[8], 2-[4], 3-[2+6], 4-[4+2], 5-[2], 6-[3], 7-[2+1 aesthetasc], 8-[2], 9-[2+1 aesthetasc], 10-[7+1 aesthetasc].

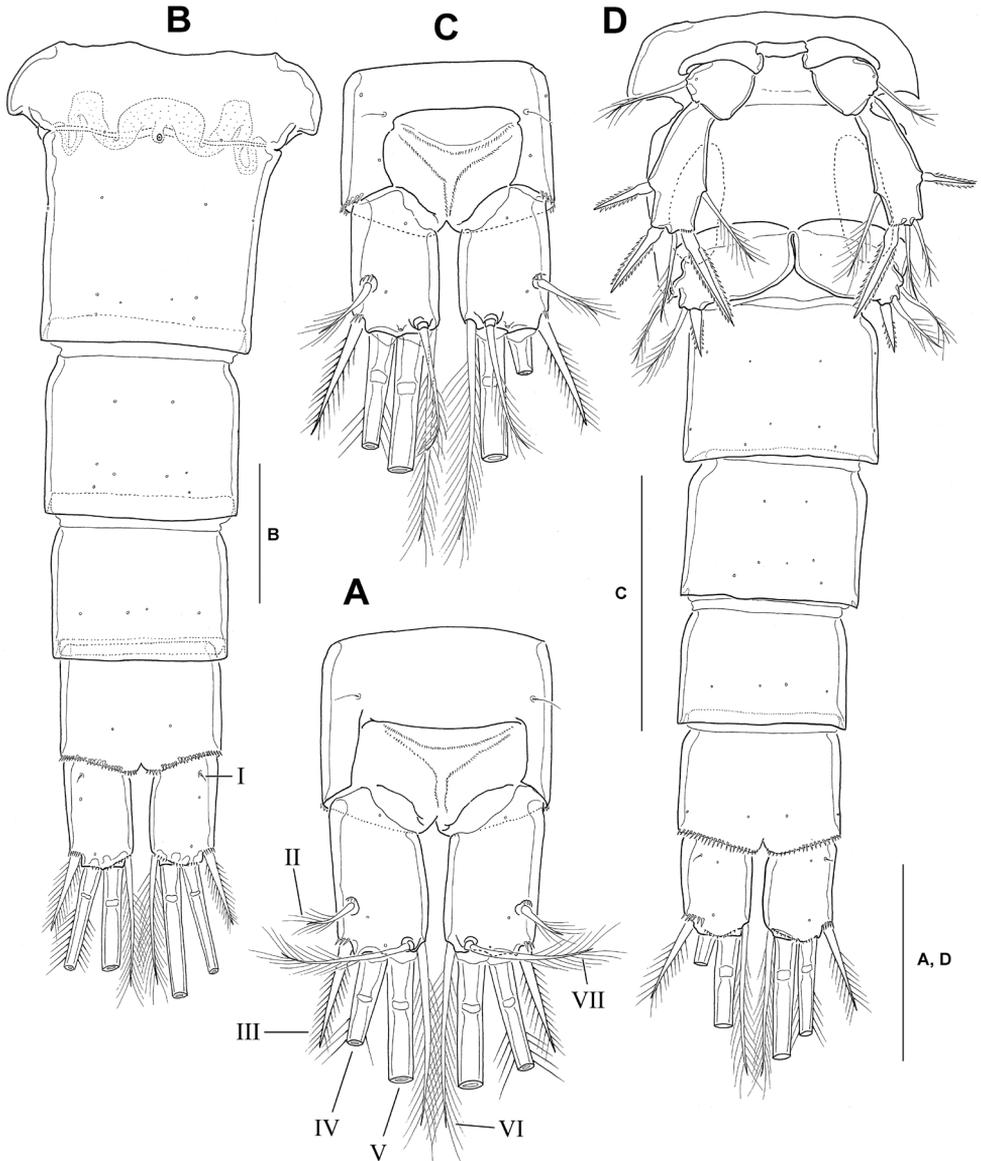


Figure 2. *Neocyclops boonsooi* sp. n. **A–B** female: **A** urosome, ventral **B** anal somite and caudal rami, dorsal. **C–D** male: **C** anal somite and caudal rami, dorsal **D** urosome and leg 5, ventral. Scale bars: 50 μ m.

Antenna (Fig. 3A) slender, distinctly 4-segmented, comprising coxobasis and 3-segmented endopod. Coxobasis about 2.1 times as long as wide, with 1 long outer seta distally (unipinnate proximally and plumose distally), representing exopod, and 2 unipinnate setae at inner distal corner. First endopodal segment about 1.7 times as

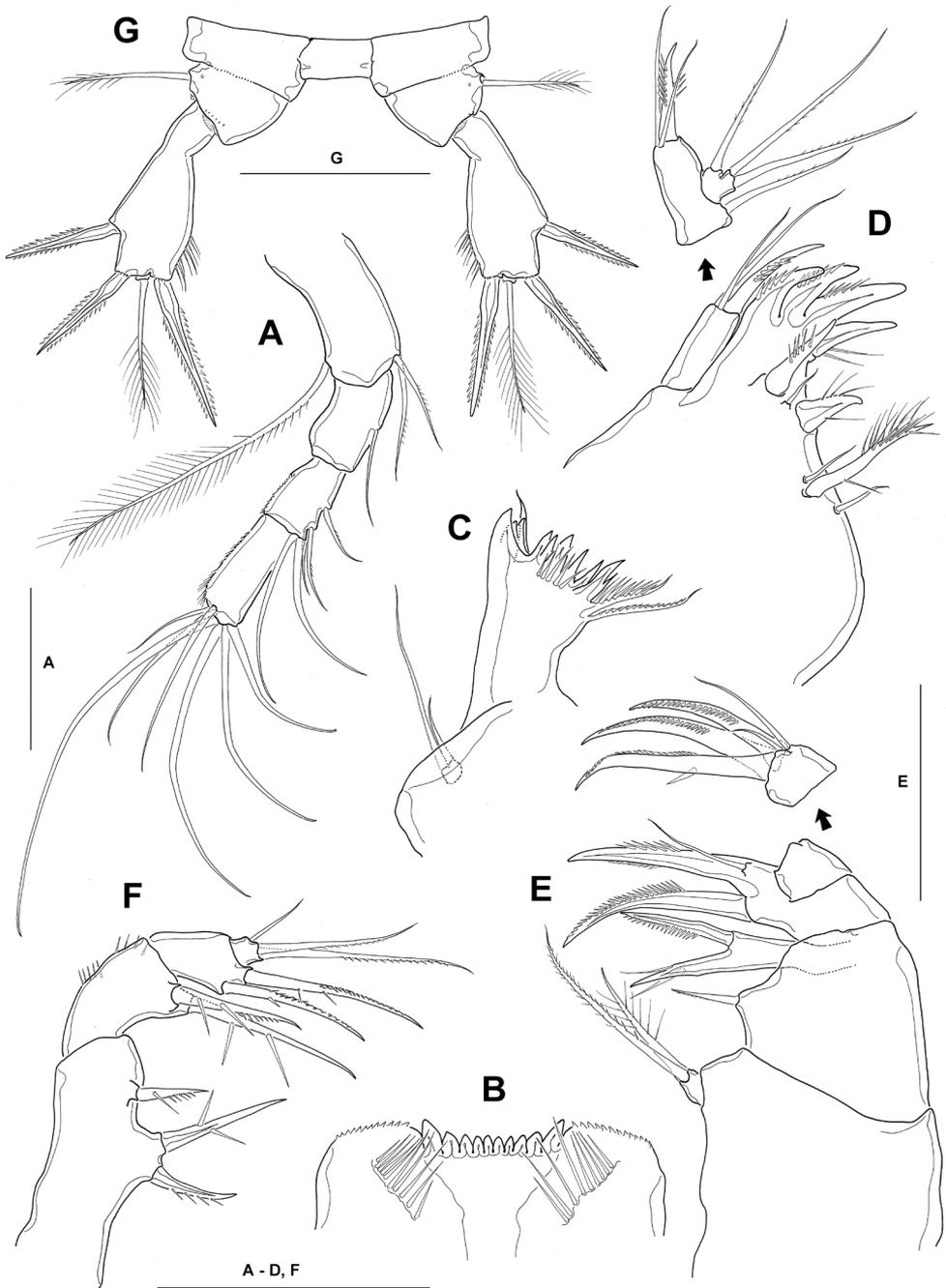


Figure 3. *Neocyclops boonsooi* sp. n., female. **A** antenna **B** labrum **C** mandible **D** maxillule **E** maxilla **F** maxilliped **G** leg 5. All scale bars: 50 μ m.

long as wide, with 1 naked seta at halfway the inner margin. Second endopodal segment small, about 1.5 times as long as wide, with minute spinules along outer margin; armed with 1 short medial, 2 short subapical and 2 long apical setae along inner margin. Third endopodal segment elongate, about 2.5 times as long as wide, ornamented with 1 spinular row along outer margin, bearing 7 apical setae including 4 geniculate and 3 slender setae.

Labrum (Fig. 3B) trapezoidal, armed with 10 strong teeth on broad, slightly concave cutting edge; serrated along distolateral margins; posterior surface with 2 oblique rows of 9–10 long, slender spinules.

Mandible (Fig. 3C), palp reduced to small protuberance, bearing 2 slender, naked setae apically; longest seta not reaching to gnathobasal teeth, about 3 times as long as shorter one. Coxal gnathobase well-developed; cutting edge armed with innermost complex of 3 stout teeth and 1 spinous element, middle group of 6 teeth and 5 sharp spinules, and outer group of 1 unipinnate spine and 1 outer subapical unipinnate seta.

Maxillule (Fig. 3D) comprising well developed praecoxa and 2-segmented palp. Praecoxa armed with 4 strong dentate spines inner distally, composed of 3 unipinnate spines basally fused together, and 1 separated posteriormost spine; 6 elements situated along inner face, consisting of 2 strong spinous setae, 1 longest pinnate seta and 3 small, naked setae. Maxillular palp completely divided, about 1.8 times as long as its greatest width, bearing 1 strong bipinnate spine and 2 slender, naked setae distally; endopod small, bearing 1 lateral and 2 apical setae, flanked by 1 proximal seta representing exopod.

Maxilla (Fig. 3E) 4-segmented (praecoxa and coxa fused on posterior surface). Praecoxa with distal endite bearing 1 pinnate and 1 plumose setae apically; proximal endite reduced and unarmed. Coxa, proximal endite represented by 1 short, minutely pinnate seta; distal endite highly mobile, armed with 1 strong, basally fused, spinous element, bearing 2 setules distally, and 1 strong, unipectinate, spinous element. Basis forming a bipinnate claw, with one 1 slender, naked seta at base; 1 strong, unipectinate, spinous element curved, slightly longer than claw. Endopod slightly tapering distally, armed with 3 long, curved, unipinnate, spinous elements and 2 naked setae.

Maxilliped (Fig. 3F) slender, 4-segmented, comprising syncoxa, basis and 2-segmented endopod. Syncoxa, about 2.2 times as long as broad, unornamented; medial margin with 2 endites, bearing 2 and 1 strong, spinous setae, respectively. Basis about 1.7 times as long as broad, with group of spinules halfway outer margin and near outer distal corner; bearing 2 spinous setae inner distally, each with 2 long secondary spinules on posterior margin. First endopodal segment unornamented, with 2 pinnate inner setae; second endopodal segment small and subquadrate, with 1 short, subapical and 2 long, apical setae.

Legs 1–4 (Fig. 4A–D) biramous, both rami 3-segmented. Intercostal sclerites of legs 1–4 with smooth distal margin, each with 2 lateral lobes, those of leg 1 most pronounced, unarmed with smooth distal margins, not ornamented with any transverse setule or spinule row on both frontal and caudal surfaces. Praecoxal sclerites not expressed. Coxae unornamented, except for spinule row on posterior margin; with

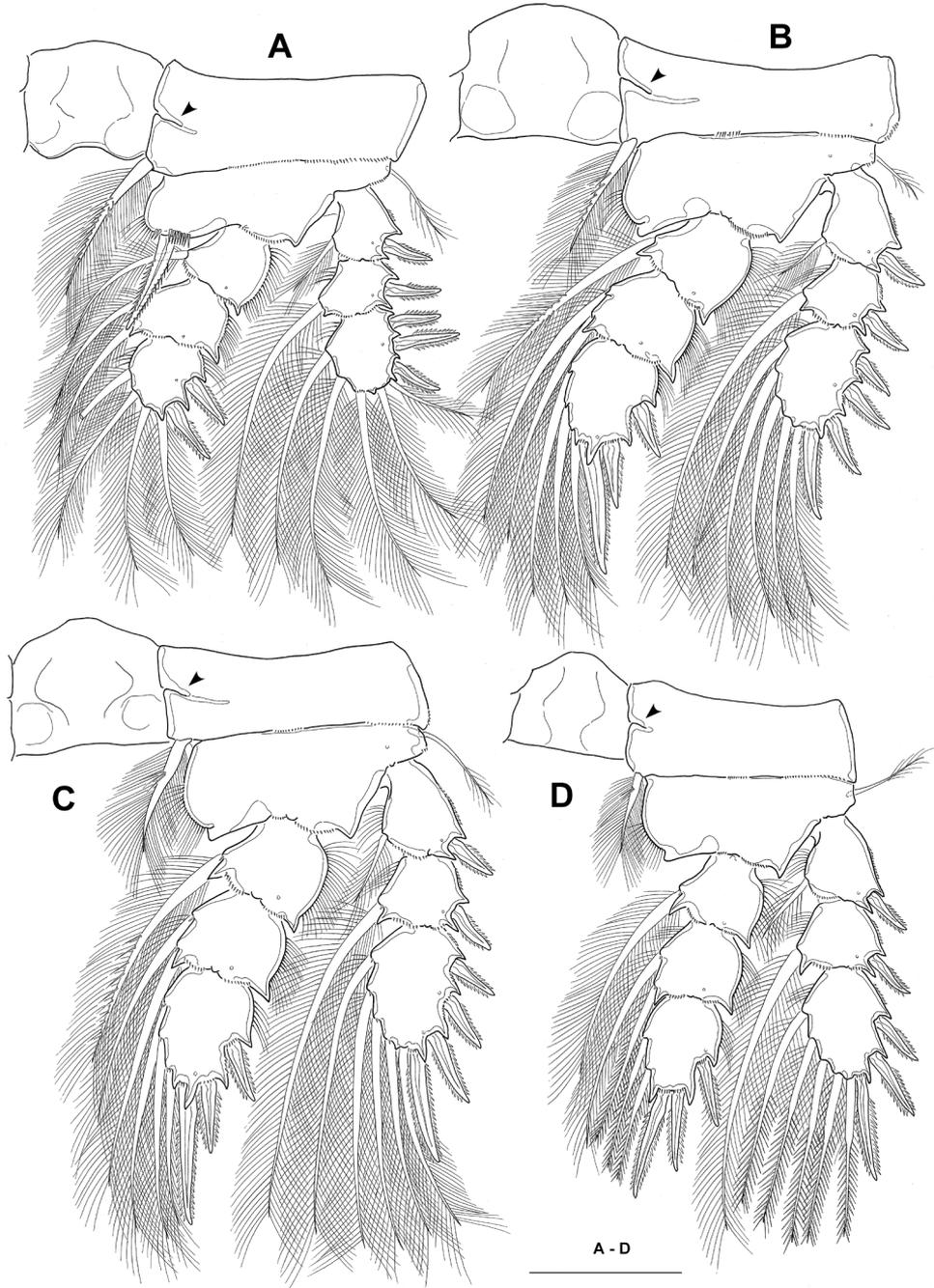


Figure 4. *Neocyclops hoonsooi* sp. n., female. **A** leg 1 **B** leg 2 **C** leg 3 **D** leg 4. All scale bars: 50 μ m.

transverse internal chitinous ridges originating from medial margins; inner distal plumose seta well developed, but that of leg 4 conspicuously shorter. Exp-3 of legs 1–4 with setal formula 5,5,5,5 and spine formula 3,4,4,3; each leg bearing 2 inner setae on enp-2, and 1 inner seta on enp-1 and exp-1. Leg 1 (Fig. 4A), intercoxal sclerite not broad, its free margin concave; inner distal seta of basis remarkably stout, bipinnate, its tip nearly reaching to distal margin of enp-2. Leg 4 (Fig. 4D), free margin of intercoxal sclerite smooth and nearly straight; enp-3 1.24 times longer than wide; inner distal spine 1.14 times longer than enp-3, 1.36 times longer than outer distal spine; inner setae on exp-3 and enp-3 with swollen proximal half and slender distal half. Seta/spine armature of legs 1–4 as follows:

	coxa	basis	exopod	endopod
Leg 1	0-1	1-1	I-1, I-1, III,2,3	0-1, 0-2, I,I+1,3
Leg 2	0-1	1-0	I-1, I-1, III,I+1,4	0-1, 0-2, II,I,3
Leg 3	0-1	1-0	I-1, I-1, III,I+1,3	0-1, 0-2, II,I,3
Leg 4	0-1	1-0	I-1, I-1, II,I+1,4	0-1, 0-2, I,II,2

Leg 5 (Fig. 3G) 3-segmented; intercoxal sclerite quadrangular, about twice as long as wide, with nearly straight posterior margin, lacking spinule ornamentation. Coxa clearly defined from fifth pedigerous somite; about twice as wide as greatest length (measured along inner margin); armed with 1 row of minute spinules along distal margin. Basis subtriangular, about 1.5 times wider than long, with 1 plumose seta laterally; 1 cuticular pore present near base of lateral seta. Exopod subpyriform, about twice as long as wide; inner margin tapering abruptly in proximal 1/5, and gradually broadening distally, then slightly narrowing with inner setule row in distal quarter; bearing 2 apical, bipectinate spines flanking 1 long plumose seta apically and 1 subapical spine in distal third of outer margin; inner distal spine 1.2 times longer than outer spine, about 1.4 times as long as lateral spine, about 0.86 times as long as exopod.

Male (allotype): Body (Fig. 1C) 564 μm long (mean 572 μm , standard deviation 10, $n = 5$). Caudal rami (Fig. 2C, D) 1.36 times longer than wide (conspicuously shorter than in female), with similar setal armature as in female.

Antennule (Fig. 1D) 16-segmented; strongly modified, digeniculate, with major geniculation between segments 14 and 15 and secondary geniculation between segments 8 and 9; segments 14–15 cuticular folds along anterior margin; segment 16 claw-like and curved, with 2 short aesthetascs along posterior margin. Aesthetasc formula: 3,0,0,1,0,0,0,0,1,0,0,0,1,0,0,2. Two elements on anterior margins of segments 12–13 short and spiniform; 1 ventral seta on segment 15 plumose; other setae naked, slender.

Leg 5 (Fig. 2D) 3-segmented, with small intercoxal sclerite; coxa unarmed; basis with slightly swollen inner margin, bearing 1 outer plumose seta, with 1 cuticular pore near base of lateral seta. Exopod about 2.3 times as long as wide; seta/spine armature similar to that in female, except for additional seta on inner margin; allotype showing aberrant asymmetrical spine armature on left side, with outer apical spine being replaced

by 1 short plumose seta (see Fig. 2D). Leg 6 reduced to operculum with 1 short inner bipinnate spine and 2 plumose setae distally; outer seta slightly longer than inner seta.

Etymology. The proposed specific name is dedicated to the late Professor Hoon Soo Kim in honor of his contribution to the development of invertebrate taxonomy in Korea.

Ecology. This species was found at Holtong beach along the western coast of the Dadohae Oceanic National Park, which is located along south-western coast of the Korean Peninsula. The beach is exposed and fringed with rocks on both sides. The intertidal, coarse to medium sandy sediments contained a little mud. Salinity: 27–32 ‰. The new species co-occurred with other interstitial ones: *Cerconeotes japonicus* (Itô, 1968), *Cyclopinna* spp. (Copepoda), *Xenotrichula* sp. (Gastrotricha), and *Echinoderes* sp. (Kinorhyncha).

Remarks. The genus *Neocyclops* currently accommodates 24 nominal species (Karanovic 2008, Pesce 2015, Walter and Boxshall 2015). Petkovski (1986) proposed a division into two subgenera according to the number of segments in the male leg 5, *i.e.* 4 segments in the subgenus *Protoneocyclops* Petkovski, 1986 and 3 segments in the subgenus *Neocyclops* Gurney, 1927. This classification has not been universally accepted yet (for details, see Karanovic 2008). Based on the presence of a 3-segmented leg 5 in the male, *Neocyclops hoonsooi* sp. n. might be allocated to the nominotypical subgenus *Neocyclops* which includes 11 species at present. Nine *Neocyclops* species which show the 4-segmented condition in the male are currently assigned to the subgenus *Protoneocyclops*, while four other species are known from females only and can as yet not be attributed to either subgenus: *N. parvus* (Sewell, 1949), *N. magnus* (Sewell, 1949), *N. improvisus* Pleša, 1973 and *N. sharkbayensis* Karanovic, 2008.

Neocyclops species typically possess 12-segmented antennules in the female but a few members are known to show fewer segments: 11-segmented in *N. improvisus* and *N. geltrudeae* Pesce & Galassi, 1993, 10-segmented in *N. petkovskii* De Laurentiis, Pesce & Halse, 1997, and only 8-segmented in *N. salinarum* (Gurney, 1927). *Neocyclops hoonsooi* sp. n. shares the 10-segmented condition with *N. petkovskii*, showing traces of subdivision in compound segments 3 and 4. *Neocyclops hoonsooi* sp. n. also shares with *N. petkovskii* an exopodal seta on the antenna, which is absent in *N. parvus*, *N. medius* Herbst, 1955, *N. affinis* (Pleša, 1961), *N. improvisus*, *N. australiensis* Karanovic, 2008, and *N. dussarti* Karanovic, 2008 [= *nomen novum* for *N. affinis* Dussart, 1974, a junior homonym of *N. affinis* (Pleša, 1961), for details, see Karanovic 2008: 262].

The new species is most characteristic in having large scar-like integumental ridges originating from the medial margins of the coxae in all swimming legs. The transverse chitinized reinforcements are very conspicuous, and consistently occurred in all specimens examined. Similar structures have been illustrated for three species that were recently described from Australia by Karanovic (2008): figure 54A and D for leg 1 and leg 4 of *N. australiensis*, figures 58D and 59B for leg 1 and leg 4 of *N. sharkbayensis*, and figure 61C for leg 3 of *N. trajani* Karanovic, 2008. However, all of them are less pronounced, and illustrated as small open-circles or ovals in close connection to the medial margin of the coxae, which showed quite different patterns from those of the new species.

The new species is also unusual in bearing a small setule on the anteroventral surface of the caudal ramus in both sexes. This setule is here identified as the anterolateral

accessory seta I. As far as we can ascertain, it was recorded only once before in the genus *Neocyclops*, i.e. in the description of the female caudal ramus of *N. pilbarensis* Karanovic, 2008, where it was interpreted as a “sensillum at anterior part ventrally”. While the caudal seta I is rarely expressed and usually lacking in members of the Cyclopoida, it can sometimes be quite conspicuous in some marine, and especially ancestral, genera, such as *Heterocyclopina* Pleša, 1968. Karanovic (2008, fig. 49A, B) interpreted a similar structure as the “lateral sensillum” in his description of *Abrsia misophrioides* but did not consider the possibility of it being one of the caudal setae. Based on positional homology we believe that the “sensillum” observed in *N. pilbarensis* and *A. misophrioides* represents the vestigial caudal seta I and is homologous with the minute seta described in *N. hoonsooi* sp. n.

Another unusual characteristic of *N. hoonsooi* sp. n. is the very short caudal ramus, being slightly less than 1.7 times as long as wide. Caudal rami of *Neocyclops* species are generally more than twice as long as wide, being about 2.0–2.5 times in *N. affinis*, *N. parvus*, *N. australiensis* and *N. ferrarii* Rocha, 1995, 2.7–3.0 times in *N. magnus* and *N. vicinus* (Herbst, 1955), and even reaching to 3.5–4.0 times in *N. remanei* (Herbst, 1952). However, in a few species the caudal ramus is much shorter, and less than twice as long as wide, being about 1.8–2.0 times in *N. medius* and *N. dussarti*, and slightly less than 1.7 times in *N. hoonsooi* sp. n. Two genuinely interstitial species from beaches in southern Australia, have extremely short caudal rami (1.5 times in *N. tropicus* Karanovic, 2008, and 1.3 times in *N. trajani*), however, these species differ clearly from *N. hoonsooi* sp. n. by the much smaller body size (546–565 µm long), the 12-segmented antennule, the presence of 3 setae on the mandibular palp, and the very long dorsal caudal seta (1.5–2.4 times longer than caudal rami). The caudal seta VII in *N. hoonsooi* sp. n. is slightly shorter or nearly as long as the caudal ramus. This condition is shared with *N. ferrarii*, *N. improvisus*, *N. magnus*, *N. mediterraneus* (Kiefer, 1960), *N. remanei* and *N. vicinus*, while most other species have a much longer dorsal seta (more than twice longer than the caudal ramus): *N. geltrudeae*, *N. pilbarensis*, *N. sharkbayensis* and *N. tropicus*. *Neocyclops papuensis* Fiers, 1986 clearly differs from all its congeners, including the present new species, by bearing an extremely short dorsal seta (0.4 times as long as the caudal ramus). Seta VI (innermost caudal seta) of *N. hoonsooi* sp. n. is much longer than seta III (outermost caudal seta), and thus differs from those species that display the reverse condition (seta III longer than seta VI) such as *N. affinis*, *N. vicinus*, *N. improvisus*, *N. monchenkoi* Karanovic, 2008 and *N. australiensis*.

Neocyclops hoonsooi sp. n. displays the typical seta/spine armature pattern on legs 1–4 found in the majority of species in the genus *Neocyclops*. The setal formula of the third exopodal segments of the new species is 5,5,5,5, which differs from the 5,5,5,4 pattern in *N. herbsti* Petkovski, 1986 and the 4,5,5,5 condition in *N. wellsi* Petkovski, 1986. The spine formula of the third exopodal segments of *N. hoonsooi* sp. n. is 3,4,4,3, and differs only from the 2,4,4,3 pattern of *N. sharkbayensis*. The setal formula on the distal endopodal segments of the new species is 4,3,3,2, and differs from the 4,3,4,2 pattern displayed by *N. affinis*, *N. dussarti* and *N. improvisus* (Pleša 1961, 1973, Dus-

sart 1974). The spine formula of the third endopodal segments of *N. hoonsooi* sp. n. is 2,3,3,3, and differs from the 2,3,2,3 condition observed in *N. monchenkoi*. *Neocyclops* species, including the new species, typically bear two setae along the inner margin of the second endopodal segment of all swimming legs; the only exception to this rule is *N. sharkbayensis* which displays a single seta only on legs 1–2.

Taking into consideration the characters mentioned above, *N. hoonsooi* sp. n. appears to be most similar to *N. vicinus*, described from the Brazilian coast, and *N. petkovskii*, from Australia. All three species share a large body size (more than 750 μm long), the presence of an exopodal seta on the antenna, two setae on the mandibular palp, the same seta/spine armature on the third endopodal segment of leg 3 (3 setae + 3 spines), and the fairly long inner distal spine on the third endopodal segment of the female leg 4. However, *N. hoonsooi* sp. n. clearly differs from *N. vicinus* by the follow-

Table 1. Character comparison among the allied species of *N. hoonsooi* sp. n.

	<i>affinis</i>	<i>australiensis</i>	<i>improvisus</i>	<i>monchenkoi</i>	<i>petkovskii</i>	<i>vicinus</i>	<i>hoonsooi</i> sp. n.
♀, body length (μm)	390–439	731	396–488	720–1,110	765	750	830
♀, antennule, no. of segments	12	12	11	12	10	12	10
Antenna, exp seta	absent	absent	absent	present	present	present	present
Mandible, no. of setae on palp	1	3	1	2	2	2	2
Caudal rami, L/W ratio, ♀	2.2–2.6	2.5	1.8–2.0	2.7–3.5	2.4	~ 3	1.6–1.7
Length ratio, caudal setae VI/III	~ 1	0.7	0.9	~ 0.9	~ 1	0.9–1.0	1.5–1.6
Length ratio, caudal setae VII/VI	~ 3	~ 3.5	~ 2.2	~ 3	≥ 1	1.4	~ 2/3
Length ratio, caudal seta V/ramus	~ 1.6	~ 1.4	~ 1	~ 1.3	~ 0.7	~ 0.9	~ 0.9
Leg 3 enp-3 armature formula	4,III	3,III	4,III	3,II	3,III	3,III	3,III
Leg 4, length ratio of inner spine/enp-3	1.5–1.8	~ 1.3	1.6	1.6–1.7	1.2	~ 1.4	~ 1.2
♀ leg 5 exp, length ratio of inner/outer spines	1.2–1.5	~ 1.1	1.2	~ 1.2	0.9	1.1	1.2
♀ leg 5 exp, length ratio of inner/lateral spines	1.3	1.5	1.3	~ 1.3	0.9	1.3	1.4
♀ leg 5, length ratio of inner distal spine/exp	0.9–1	~ 1.2	0.7	0.7–0.8	0.5	0.86	0.86
♂ leg 5, no. of segments	3	3/4 [†]	-	3	4	3	3
Distribution	Ghana ¹ ; West Indies ²	Australia ³	Cuba ⁴	Black Sea ⁵	Australia ⁶	Brazil ⁷	Korea ⁸

[†] incomplete division

References: ¹Pleša (1961), ²Pesce (1985), ³Karanovic (2008), ⁴Pleša (1973), ⁵Pleša (1964) & Monchenko (1974), ⁶De Laurentiis et al. (1997) & Karanovic (2008), ⁷Herbst (1955) & Lotufo and Rocha (1993), ⁸present study.

ing characters: (1) 10-segmented antennule (vs 12 segments in *N. vicinus*); (2) shorter caudal rami (less than 1.7 times as long as wide, while about three times longer in *N. vicinus*); and (3) much shorter dorsal caudal seta VII (about 2/3 times shorter than inner caudal seta VI, while 1.4 times longer in *N. vicinus*), and much longer inner caudal seta VI (more than 1.5 times longer than outer caudal seta III, while slightly shorter than outer one in *N. vicinus*). Furthermore, *N. hoonsooi* sp. n. also clearly differs from *N. petkovskii* by the much shorter caudal rami (vs 2.4 times as long as wide in *N. petkovskii*), the shorter inner distal spine on the female leg 5 (vs slightly shorter than the outer distal and lateral spines, and about half the length of the exopod in *N. petkovskii*), and the 3-segmented leg 5 in male (vs 4-segmented in *N. petkovskii*). Table 1 shows the character comparison between the new species from South Korea and its closest allies.

Geographical records and a key to *Neocyclops* species from the Indo-Pacific Ocean

Pacific Ocean: from Papua New Guinea, *N. papuensis* Fiers, 1986 by Fiers (1986).

Indian Ocean: from Australia, *N. australiensis* Karanovic, 2008, *N. sharkbayensis* Karanovic, 2008, *N. trajani* Karanovic, 2008 and *N. tropicus* Karanovic, 2008 by Karanovic (2008); from West Australia, *N. petkovskii* De Laurentiis, Pesce & Halse, 1997 by De Laurentiis et al. (1997); from Maldive Archipelago, *N. parvus* (Sewell, 1949) and *N. magnus* (Sewell, 1949) by Sewell (1949); from Red Sea, *N. herbsti* Petkovski, 1986, and from Mozambique, *N. wellsii* Petkovski, 1986 by Petkovski (1986); from Egypt, *N. salinarum* (Gurney, 1927) by RBINS (2015).

1	Female antennule 8-segmented.....	<i>N. salinarum</i>
–	Female antennule 10-segmented.....	2
–	Female antennule 12-segmented.....	3
2	Caudal rami not more than 1.7 times as long as wide in female; male leg 5 3-segmented	<i>N. hoonsooi</i> sp. n.
–	Caudal rami about 2.4 times as long as wide in female; male leg 5 4-segmented	<i>N. petkovskii</i>
3	Caudal rami less than 1.5 times as long as wide in female	4
–	Caudal rami about 2–2.7 times as long as wide in female	5
–	Caudal rami 3–3.5 times as long as wide in female	<i>N. papuensis</i>
4	Dorsal caudal seta VII less than twice as long as ramus	<i>N. trajani</i>
–	Dorsal caudal seta VII more than twice as long as ramus	<i>N. tropicus</i>
5	Exopodal seta on antenna lacking	6
–	Exopodal seta on antenna present	8
6	Inner caudal seta VI longer than outer caudal seta III	<i>N. parvus</i>
–	Inner caudal seta VI shorter than outer caudal seta III	7
7	Enp-2 of legs 1–3 with single inner seta; exp-3 of leg 1 with 2 spines	<i>N. sharkbayensis</i>

- Enp-2 of legs 1–3 with 2 inner setae; exp-3 of leg 1 with 3 spines.....*N. australiensis*
- 8 Setal formula of exp-3 of legs 1–4 5,5,5,5.....*N. magnus*
- Setal formula of exp-3 of legs 1–4 5,5,5,4.....*N. herbsti*
- Setal formula of exp-3 of legs 1–4 4,5,5,5.....*N. wellsi*

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Atlas of Iberian water beetles (ESACIB database)

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Abstract

The ESACIB (‘EScarabajos ACuáticos IBéricos’) database is provided, including all available distributional data of Iberian and Balearic water beetles from the literature up to 2013, as well as from museum and private collections, PhD theses, and other unpublished sources. The database contains 62,015 records with associated geographic data (10×10 km UTM squares) for 488 species and subspecies of water beetles, 120 of them endemic to the Iberian Peninsula and eight to the Balearic Islands. This database was used for the elaboration of the “Atlas de los Coleópteros Acuáticos de España Peninsular”. In this dataset data of 15 additional species has been added: 11 that occur in the Balearic Islands or mainland Portugal but not in peninsular Spain and an other four with mainly terrestrial habits within the genus *Helophorus* (for taxonomic coherence). The complete dataset is provided in Darwin Core Archive format.

Keywords

Aquatic, Coleoptera, freshwater, Iberian peninsula, occurrence, Portugal, Spain

General description

Purpose: The purpose of this paper is to provide all the available distributional information on water beetles from the Iberian Peninsula and Balearic Islands. These data were compiled in the ESACIB (‘EScarabajos ACuáticos IBéricos’) database and published in the “Atlas de

los Coleópteros Acuáticos de España Peninsular” (Millán et al. 2014). However, there are some mismatches between the species presented in Millán et al. (2014) and those presented in this dataset (see the section “additional information” for details). Water beetles have high species richness in the Mediterranean region, inhabiting virtually every kind of fresh and brackish water habitat, from the smallest ponds to lagoons and wetlands, and from streams to irrigation ditches, large rivers, and reservoirs (e.g. Ribera et al. 1998, Ribera 2000, Millán et al. 2002). In comparison to other groups of freshwater invertebrates in the Iberian peninsula and the Balearic islands, water beetles are well known in their systematics and biogeography (Ribera et al. 1998, Ribera 2000, Millán et al. 2006). In this context, the ESACIB database was developed to provide all the available distributional information on water beetles from this region. This database represents the most complete information available for a major group of freshwater invertebrates in the study area.

Additional information: The species included in this dataset but not considered in Millán et al. (2014) are:

i) species endemic to the Balearic Islands (*Deronectes brannanii* (Schaufuss, 1869); *Hydroporus lluci* Fery, 1999; *Hydraena (Hydraena) balearica* Orchymont, 1930; *Graptoodytes kuchtai* (Breit, 1908); *Limnebius minoricensis* Jäch, Valladares & García-Avilés, 1996; *Ochthebius (Ochthebius) javieri* Jäch, 2000; *O. (Ochthebius) pedroi* Jäch, 2000; *Oulimnius echinatus* Berthélemy, 1979);

ii) species endemic to mainland Portugal (*Rhithrodytes agnus agnus* Foster, 1992; *Rhithrodytes agnus argaensis* Bilton & Fery, 1996; *Hydraena (Hydraena) malagrica* Jäch & Díaz, 2012; *Hydraena (Hydraena) optica* Jäch & Díaz, 2012; *Hydraena (Hydraena) zezerensis* Díaz Pazos & Bilton, 1994);

iii) species present in mainland Portugal (*Porhydrus vicinus* (Aubé, 1838)) or the Balearic Islands (*Ochthebius lobicollis* Rey, 1885) but not in the Iberian mainland;

iv) for taxonomic coherence four species of *Helophorus* (subgenus *Empleurus*) are included in this dataset that were not treated in Millán et al. 2014 due to their mostly terrestrial habits (*Helophorus (Empleurus) hispanicus* (Sharp, 1915); *Helophorus (Empleurus) porculus* (Bedel, 1881); *Helophorus (Empleurus) rufipes* (Bosc, 1791); *Helophorus (Empleurus) schmidti* A. Villa & G.B. Villa, 1838).

It should be noted that some Iberian species such as *Macronychus quadrituberculatus* P.W.J. Müller, 1806, *Halipplus (Halipplus) sibiricus* Motschulsky, 1860, *Berosus bispina* Reyche & Saulcy 1856, *Helophorus cincticollis* Guillebeau, 1893 and *Hydraena assimilis* Rey, 1803 are not included in this dataset due to the lack of geographical precision of their records in the study area. In addition, part of the data presented here are also included in the “Inventario Español de Especies Terrestres (MAGRAMA)”.

Project details

Project title: Atlas de los coleópteros acuáticos de España peninsular

Personnel: Andrés Millán (IP), David Sánchez-Fernández (co-IP), Pedro Abellán, Félix Picazo, José A. Carbonell, Jorge M. Lobo, Ignacio Ribera.

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Study area descriptions/descriptors: The Iberian peninsula and Balearic islands are two closely bio-geographically related areas which extend more than 585,644 km². The territory includes a variety of biomes, relief, climates, and soil types, where altitude ranges from sea level to 3483 m.a.s.l. in the Sierra Nevada (SE Iberia). These areas are of great biogeographic interest, being regarded as one of the richest European regions in terms of species diversity (Domínguez-Lozano et al. 1996, Médail and Quézel 1999, Reyjol et al. 2007). Insects in general, and beetles in particular, make up the highest percentage of the biodiversity of this area. Close to 98% of the total Iberian fauna are invertebrates, and roughly 81% are insects (Ramos et al. 2001). The Iberian peninsula has a wide range of habitat types, including some aquatic environments very rare in Europe (Millán et al. 2011). Some of these freshwater ecosystems are subjected to strong human influence, and are in consequence under risk of suffering high rates of biodiversity loss (Allan and Flecker 1993, Saunders et al. 2002).

Design description: The database compiles all available taxonomic and distributional data of the families of strictly aquatic Coleoptera from the literature as well as from museum and private collections, PhD theses, and other unpublished sources. The bibliographic references providing more records are mainly papers compiling distributional data for several families (Fery and Fresneda 2007), PhD thesis (Valladares 1988, Garrido 1990, Millán 1991), and some regional catalogues (Rico 1996, Ribera et al. 1996, Millán et al. 2002, Sánchez-Fernández et al. 2003). It is note worthy that the single most important source of records of this dataset was the IBE collection (Institut de Biologia Evolutiva, CSIC-UPF, Barcelona), with more than 7000 records.

Data published through

GBIF: <http://www.gbif.es/ipt/resource?r=esacib>

Taxonomic coverage

General taxonomic coverage: We focus here exclusively on the “strictly aquatic beetles”, i.e., those that spend most of their life submerged, at least in its adult stage, in any type of aquatic ecosystem (Jäch and Balke 2008). More concretely, we focus on 13 families of water beetles belonging to three suborders (Fig. 1).

Taxonomic ranks

Kingdom: Animalia

Phylum: Arthropoda

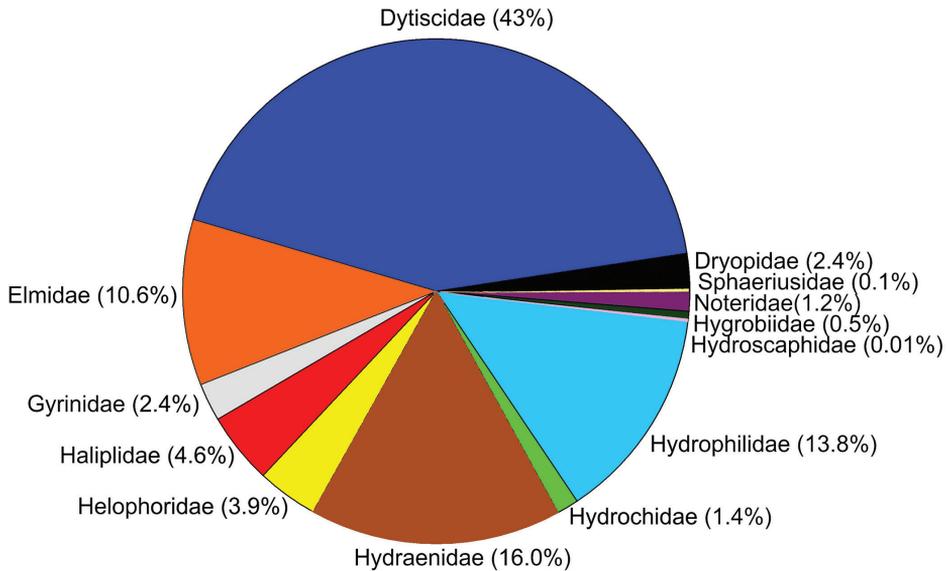


Figure 1. Taxonomic distribution of the dataset (percentage of species per family).

Class: Insecta

Order: Coleoptera

Suborder: Myxophaga, Adepaga, Polyphaga

Family: Hydroscaphidae, Sphaeriusidae, Dytiscidae, Gyrinidae, Haliphilidae, Hygrobiidae, Noteridae, Dryopidae, Elmidae, Helophoridae, Hydraenidae, Hydrochidae, Hydrophilidae

Spatial coverage

General spatial coverage: The study area is the Iberian peninsula (Spain and Portugal mainland) and Balearic islands, located in the southwest of Europe (Fig. 2).

Coordinates: 35°23'60"N and 43°58'48"N Latitude; 10°2'24"W and 4°48'36"E Longitude.

Living time period: 1840–2013 (Fig. 3).

Methods

Method step description:

1. Primary sources of the data
 - 1a. Sampling
 - 1b. Bibliographic compilation
 - 1c. Public and private collections
2. Georeferenciation at 10×10 km grid cells

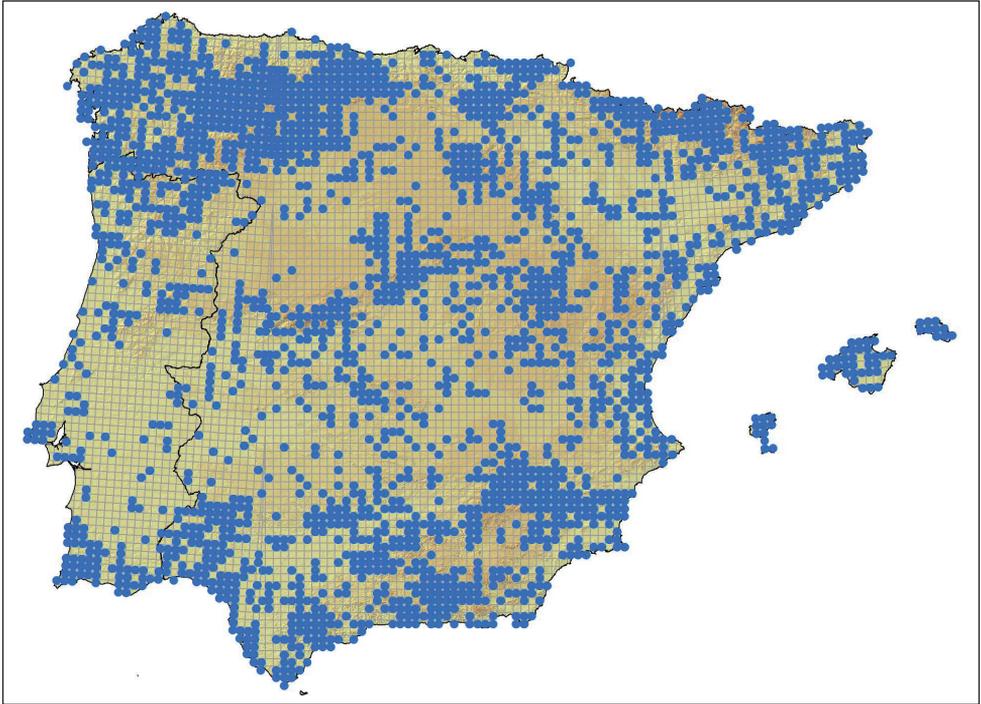


Figure 2. Geographic distribution of the records.

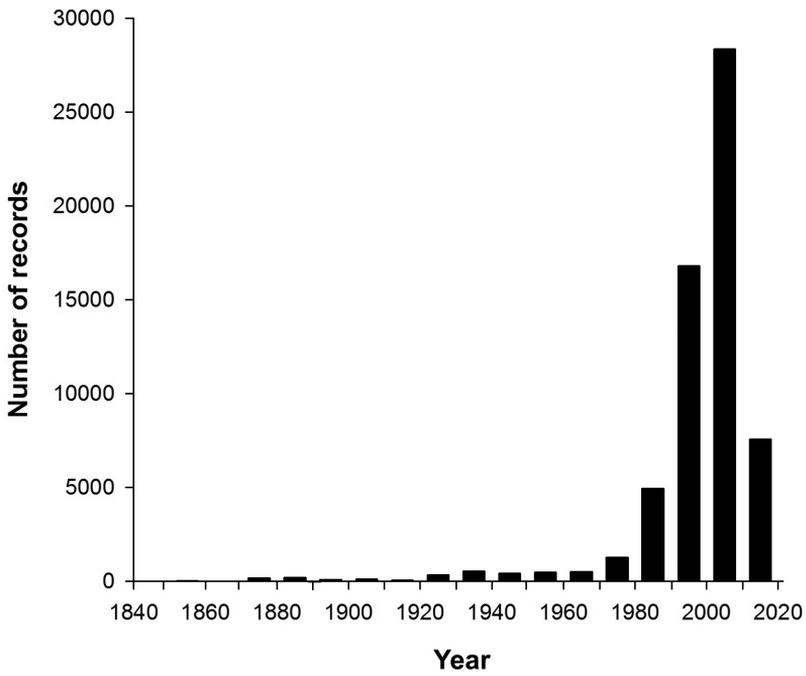


Figure 3. Temporal distribution of the records.

Records were assigned to 10×10 km grid cells based on the Universal Transverse Mercator (UTM) projection and the Military Grid Reference System (MGRS) from the spatial coordinates provided in the original sources. In those cases in which records did not attach spatial coordinates, the locations of the records were identified in Google Earth, translated to UTM coordinates and assigned to 10×10 km grid cells. Those records that could not be unambiguously georeferenced were discarded.

3. Introduction in the database.
4. Elaboration of distributional maps.
5. Checking for doubtful records.
6. Modifications of records (taxonomy or coordinates).
7. Elaboration of new distributional maps.

Study extent description: The Iberian peninsula and Balearic islands. The frequency of sampling has been irregular, as data were obtained from bibliographic sources, field sampling, and the revision of private collections.

Sampling description: For the unpublished data, in most cases at each sampling site beetles were collected from a representation of all mesohabitat types with a kick-net of 500 µm mesh, following in most cases a multihabitat protocol (Jaimez-Cuellar et al. 2002). Each kick-sample was examined in the field and successive samples were taken until no new morpho-types were found. The kick-sample contents were pooled into a unique site-sample, preserved in 70% or 96% ethanol and identified to species level in the laboratory.

Quality control description: Distributional maps for each species were generated that were checked by all members of the project and some external reviewers. Doubtful records were double-checked (identifications, geographical coordinates, etc.). In the case of doubtful data from published sources, the original papers were reviewed again, and in some cases (whenever possible) additional information was requested from the authors on the doubtful records.

Datasets

Dataset description

Object name: Darwin Core Archive Atlas of Iberian water beetles (ESACIB database)

Character encoding: UTF-8

Format name: Darwin Core Archive format

Format version: 1.0

Distribution: <http://www.gbif.es/ipt/archive.do?r=esacib>

Publication date of data: 2015-04-29

Language: English

Licences of use: To the extent possible under law, the publisher has waived all rights to these data and has dedicated them to the <http://creativecommons.org/public-domain/zero/1.0/legalcode>>Public Domain (CC0 1.0). Users may copy, modify, distribute and use the work, including for commercial purposes, without restriction.

Metadata language: English

Date of metadata creation: 2015-01-26

Hierarchy level: Dataset

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