

A new species of Fauveliopsidae (Annelida) from the North Sea

Anna Zhadan^{1,†}, Margarita Atroshchenko^{2,‡}

1 Moscow State University, Faculty of Biology, Leninskie Gory, 1–12, Moscow, 119234 Russia **2** State Biological Museum, Moscow, Malaya Gruzinskaya str., 15, 123242 Russia

† [urn:lsid:zoobank.org:author:BA1BE0BE-6925-4A19-9560-6CD643D93A79](https://zoobank.org/urn:lsid:zoobank.org:author:BA1BE0BE-6925-4A19-9560-6CD643D93A79)

‡ [urn:lsid:zoobank.org:author:C0A6EE85-6A3C-4889-9D44-87709846A552](https://zoobank.org/urn:lsid:zoobank.org:author:C0A6EE85-6A3C-4889-9D44-87709846A552)

Corresponding author: Anna Zhadan (azhadan@wsbs-msu.ru)

Academic editor: C. Glasby | Received 18 January 2012 | Accepted 27 March 2012 | Published 6 April 2012

[urn:lsid:zoobank.org:pub:446BB681-3BF9-4F49-8FEA-029CC2C624A8](https://zoobank.org/pub:446BB681-3BF9-4F49-8FEA-029CC2C624A8)

Citation: Zhadan A, Atroshchenko M (2012) A new species of Fauveliopsidae (Annelida) from the North Sea. ZooKeys 181: 1–10. doi: 10.3897/zookeys.181.2712

Abstract

A new species of the genus *Laubieriopsis* Petersen, 2000 is described based on 28 specimens collected in the north-east part of the North Sea. It is characterized by fixed number of chaetigers (22), paired genital papillae, bidentate neurochaeta of chaetigers 1–4, the absence of acicular chaetae on chaetigers 5–21 and, on the last chaetiger, one acicular and three capillary chaetae enlarged and directed backward. The present study brings the number of known species of *Laubieriopsis* to five and the number of Northeast Atlantic species of this genus to two.

Keywords

Annelida, Polychaeta, Fauveliopsidae, *Laubieriopsis norvegica*, new species, taxonomy, Norway, Northeast Atlantic

Introduction

The Fauveliopsidae are a small (about 20 species) family of detritus-feeding polychaetes. They are small benthic animals, mainly found on deep bottoms down to 6000 m (Levenstein 1970), although a few species can be found above 100 m deep (Katz-

mann and Laubier 1974, Riser 1987, Núñez et al. 1997). Members of the family are either free-living or inhabit dead shells of scaphopods, gastropods or foraminiferans (Blake and Petersen 2000, Petersen 2000).

The family Fauveliopsidae was erected by Hartman (1971) and initially consisted of one genus, *Fauveliopsis* McIntosh, 1922; later Petersen (2000) divided this genus into two – *Fauveliopsis* and *Laubieriopsis* Petersen, 2000. Fauveliopsidae are usually grouped together with Flabelligeridae, based on the presence of a retractable anterior region of the body, but differ from Flabelligeridae by the lack of a cephalic cage, retractile oral branchiae and palps. The presence of an interrampal papilla is the characteristic feature for the family, though Fauchald and Rouse (1997) considered it to be the same as the interrampal papillae of Flabelligeridae and thus found no evidence for monophyly of the family. Genital papilla (single or paired) could be another characteristic feature for the family Fauveliopsidae (Petersen 2000).

The internal morphology and ultrastructure of fauveliopsids have been poorly studied. Riser (1987) described some features for *Laubieriopsis arenicola* Riser, 1987, *L. brevis* (Hartman, 1965) and *Fauveliopsis glabra* (Hartman, 1960). Purschke (2011) gave a detailed description of the anterior end of *Fauveliopsis* cf. *adriatica*, its brain and sensory structures. Thiel et al. (2011) described some anatomical features of *Fauveliopsis confusa* Thiel, Purschke and Böggemann, 2011. Common characters of all the studied species are a thick cuticle, a brain with four posterior lobes, longitudinal muscles forming four muscle bands, strong prostomium protractors, muscular dissepiments, two ventral mesenteries, and epidermal glands associated with parapodia.

Zhadan and Atroshchenko (2010) described the inner organization of *Laubieriopsis* sp. They examined the morphology of the body wall, introvert, and body cavities as well as the digestive, reproductive, and nervous systems. In the present work we formally describe this species.

Material and methods

Specimens of *Laubieriopsis* sp. were collected in the North Sea (60°34'N, 03°41'E; 03°26'E, 60°54'N) (Fig. 1) by the company Akvaplan-niva in May 1998 (collector Andrey Sikorski). All specimens were fixed in a 4% formaldehyde seawater solution and later transferred to 70% ethanol. The material is stored in the Zoological Museum of M.V. Lomonosov Moscow State University (ZMMU).

Twenty-one specimens were examined under a stereomicroscope after dehydration in a glycerin series. Additional data were obtained using scanning electron microscopy: seven specimens were critical point-dried after dehydration in an ethanol series and acetone, and then coated with gold prior to examination with a Scanning Electron Microscope HITACHI S-405 A and Camscan-S2.

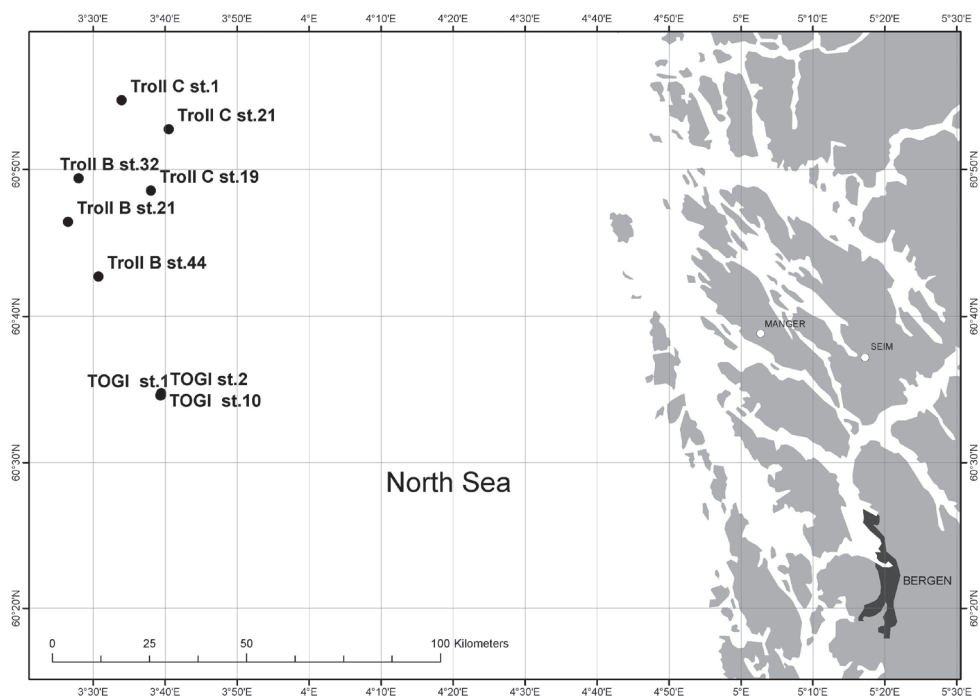


Figure 1. Map of stations of Akvaplan-niva, 1998, showing findings of *Laubieriopsis norvegica* sp.n.

Results

Genus *Laubieriopsis* Petersen, 2000

<http://species-id.net/wiki/Laubieriopsis>

Diagnosis. Body cylindrical, weakly divided into two regions by segment shape and type of chaetae. Fixed number of segments. Cuticle smooth, without papillae or papillae minute and inconspicuous. Chaetae include capillaries and acicular types, some might be bidentate. Interramal papillae small, mostly sessile. Last segment similar in size to preceding ones, often bilobed, pygidium retracted inside it.

Laubieriopsis norvegica sp. n.

urn:lsid:zoobank.org:act:5D46C0C3-6974-4683-81BF-C09964602392

http://species-id.net/wiki/Laubieriopsis_norvegica

Figs 2, 3, 4

Laubieriopsis sp. Zhadan and Atroshchenko 2010: 876–885, Figs 1–4

Type locality. Norway, North Sea, 60°52.69'N, 03°40.48'E, 340 m, mud. Akvaplan-niva, Troll C stn. 21–4; 14.05.1998. coll. A. Sikorski.

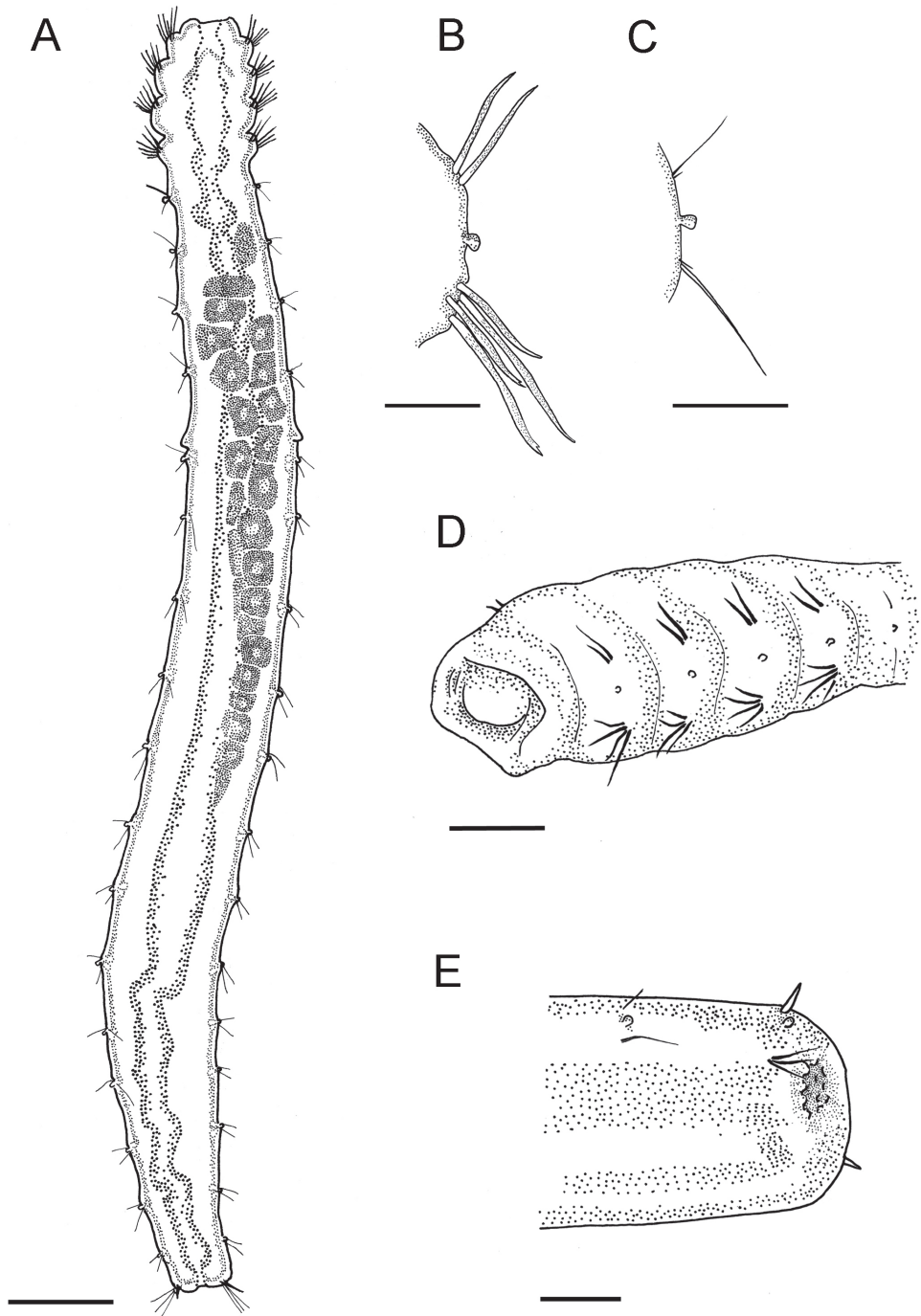


Figure 2. *Laubiertopsis norvegica* sp.n. **A** dorsal view of female with oocytes **B** parapodia of chaetiger 3 **C** parapodia of chaetiger 14 **D** anterolateral view of anterior end (prostomium visible) **E** dorsolateral view of posterior end. Scale (μm): **A** – 400, **B, D, E** – 100, **C** – 30.

Type material. Holotype female with 32 oocytes, 6.8 mm long; width in dorsal view on chaetiger 4–400 μm , stored in 70% alcohol. Original label: “Spec.: Polychaeta indet., stn.: 21–4, Date: 14.05. Troll C 1998. Akvaplan-niva A.Sikorski”. ZMMU № W141 HOLOTYPE, *Laubieriopsis norvegica* (printed label).

Paratypes: Akvaplan-niva Troll C stn. 21–4, 60°52.69'N, 03°40.48'E, 14.05.1998, 340 m, mud, one specimen without visible gametes, ZMMU № W142. Akvaplan-niva Troll C stn. 19–3, 60°48.54'N, 03°38.03'E, 14.05.1998, 334 m, three specimens, two without visible gametes, one female with 39 oocytes, ZMMU № W143. Akvaplan-niva Troll C stn 1–5, 60°54.65'N, 03°33.95'E, 15.05.1998, 341 m, two specimens without visible gametes, ZMMU № W144. Akvaplan-niva TOGI stn.10–4, 60°34.58'N, 03°39.34'E, 10.05.1998, 304 m, four specimens, three without visible gametes, one male with sperm, ZMMU № W144. Akvaplan-niva TOGI stn.1–4, 60°34.59'N, 03°39.45'E, 9.05.1998, 305 m, three specimens with small oocytes, ZMMU № W146. Akvaplan-niva TOGI stn. 2–4, 60°34.72'N, 03°39.46' E, 9.05.1998, 305 m, one specimen, male with sperm, ZMMU № W147. Akvaplan-niva Troll B, stn.32–3, 60°49.37'N, 03°27.98'E, 14.05.1998, 335 m, three specimens, two females with oocytes, one without visible gametes, ZMMU № W148. Akvaplan-niva Troll B, stn. 21–3, 60°46.40'N, 03°26.51'E, 14.05.1998, 322 m, two specimens without visible gametes, ZMMU № W149. Akvaplan-niva Troll B, stn.44–4, 60°42.68'N, 03°30.74'E, 14.05.1998, 319 m, one specimen without visible gametes, ZMMU № W150.

Diagnosis. Adult specimens with 22 chaetigers. First four chaetigers with sigmoidal acicular chaetae, neurochaetae often bidentate. Chaetigers 5–21 without acicular chaetae, with one thin capillary chaeta and one very small and thin additional chaeta in each ramus. Chaetiger 22 with one thick acicular notochaetae and three capillary neurochaetae. Two weltlike genital papillae present at posterior edge of segment 8.

Description. Adult specimens 6–8 mm long and 0.35–0.4 mm wide with 22 chaetigers. Only one specimen has 23 chaetigers. Body slender, often c-shaped or s-shaped by fixation, colorless in preserved material. Four anteriormost chaetigers swollen, following segments cylindrical, without clear borders (Figs 2A, 3A, B).

Cuticle thick (about 8 μm), smooth, with very thin ring wrinkles, bearing scattered minute inconspicuous micropapillae (Figs 3D, 4C), visible under higher magnification and in SEM. Relatively larger micropapillae surrounding inverted parts – prostomium and pygidium (Fig. 4B).

Ventral nerve cord visible by transparency. Ganglia longitudinally elongated and indistinctly separated from each other.

Prostomium small, from round to triangular, lacking appendages, with ciliary nuchal organs, without eyes (Figs 2D, 3B). In most specimens prostomium completely retracted into peristomium (Fig. 4A). Peristomium forms complete ring; it bears micropapillae.

Parapodia biramous, best developed on chaetigers 1–4 and hardly distinct on posteriormost segments except last one (22). Interramal papillae short-stalked, pyriform, situated midway between noto- and neuropodia (Figs 3C, 4 A, B, C, E, F). Two epidermal glands visible in each parapodium.

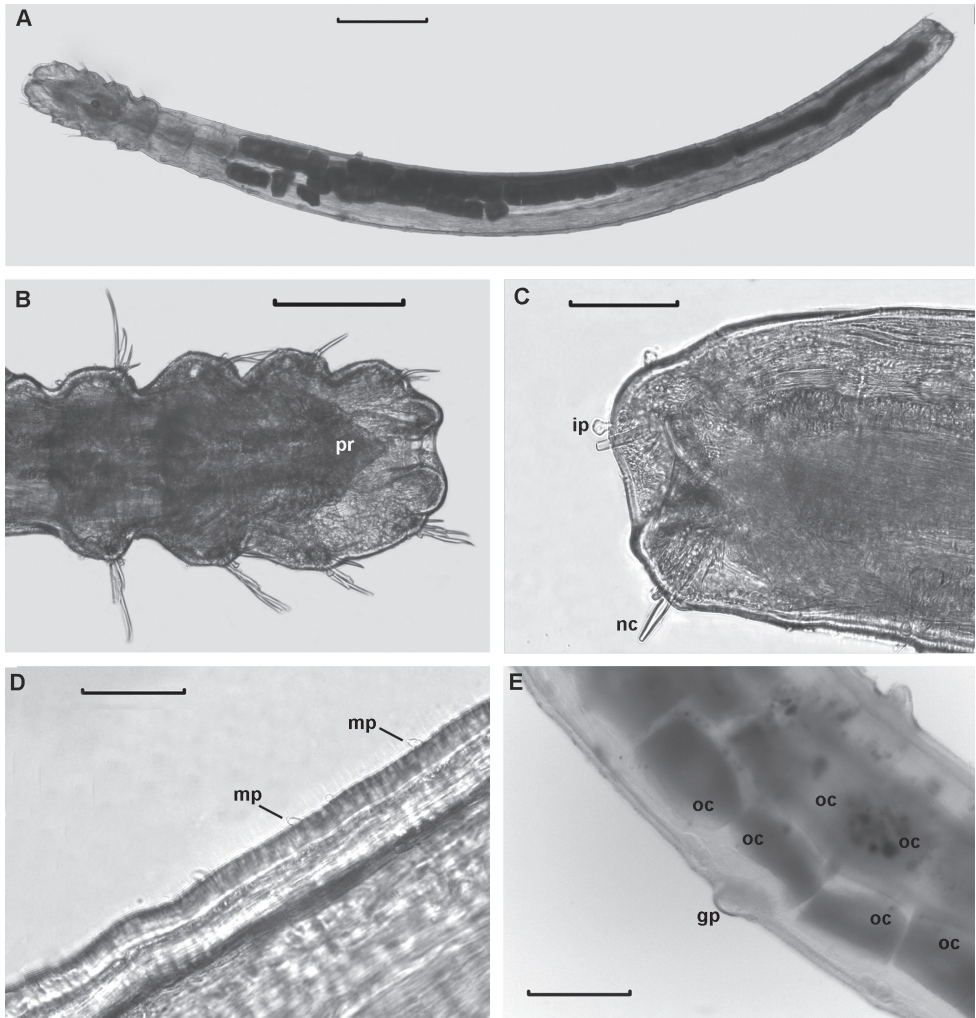


Figure 3. *Laubieriopsis norvegica* sp.n., **A** entire view from the dorsal side **B** anterior end, ventral view **C** posterior end, ventral view **D** micropapillae **E** area of chaetigers 7–9, genital papillae. Scale (μm): **A** – 500; **B**– 200; **C, E** – 100; **D**– 50.

Chaetigers 1–4 bear thick sigmoidal hirsute chaetae. Two chaetae in notopodia and four (2 long and 2 shorter ones) in neuropodia (Figs 2B, 4C). Some specimens possess five instead of four neurochaetae in anterior segments. Neurochaetae mostly bidentate (Fig. 4D), notochaetae usually unidentate, sometimes slightly bidentate. Some specimens possess 3–4 bidentate neurochaetae whereas others have only 1–2 bidentate neurochaetae. Both short and long neurochaetae can be bidentate or unidentate. Two specimens have transitional fifth segment with chaetae more similar to acicular chaetae of anterior four segments than to thin capillary chaetae of rest of the body.

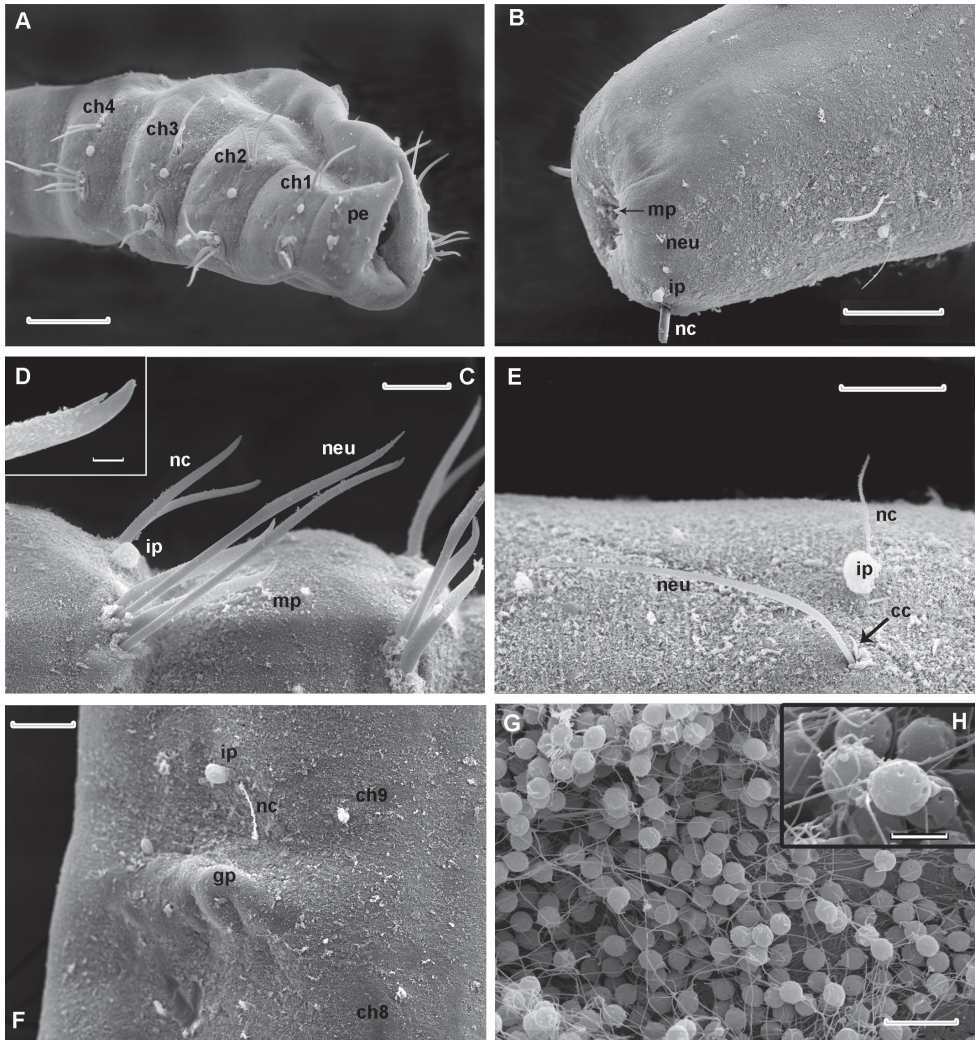


Figure 4. *Laubieriopsis norvegica* sp.n. **A** anterior end, lateral view **B** posterior end, ventro-lateral view **C** parapodia of chaetigers 2–3 **D** tip of neurochaeta enlarged **E** parapodia of chaetiger 12 **F** genital papilla and parapodia of chaetiger 9; neurochaetae are broken **G, H** sperm cell. Scale (μm): **A, B** – 100, **C** – 30, **D** – 3, **E, F** – 30, **G, H** – 10, **H** – 2. Abbreviation: **cc** accessory chaeta, **ch1–ch4** chaetigers 1–4, **ch8** chaetiger 8, **ch9** chaetiger 9, **gp** genital papilla, **ip** interramal papilla, **mp** micropapilla, **neu** neurochaeta, **nc** notochaeta, **oc** oocyte, **pe** peristomium, **pr** prostomium

Chaetigers 5–21 bear one thin capillary chaeta and one accessory very short, thin and barely distinct capillary chaeta per ramus (Figs 2C, 4E, F). Notopodial chaetae thinner and shorter than neuropodial ones; accessory chaetae in notopodia often absent.

Chaetiger 22 bears one thick acicular chaeta in notopodia and three capillary chaetae in neuropodia (Figs 2E, 3C, 4B).

Paired genital papillae (Figs 3 E, 4 F) situated at posterior edge of chaetiger 8 in all studied individuals with gametes as well as in specimens without recognizable gametes, except for one specimen with unpaired genital papilla.

Pygidium retracted within last chaetiger, anus terminal. Boundary between distal part of last segment and pygidium indistinct. Inverted part (distal to chaetae) with a number of conical papillae, which are larger than in rest of body (Figs 2E, 3C, 4B).

Oocytes up to 90 µm in diameter, arranged in two ovisacs, extend through chaetigers 6–15, each contains 20–40 oocytes (Figs 2A, 3A, E).

Sperm cells hardly visible through body wall, but observed in SEM in body cavity of dissected specimen. They have rounded heads about 3 µm in diameter, an acrosome with elongated distal part and free flagellum at least 10 µm long (Fig. 4G, H).

Etymology. The species name refers to its type locality.

Distribution. Northeast part of the North Sea.

Ecology. Inhabits muddy sediments with small admixture (0.8 – 7.7 %) of fine and medium sand, in depth of 300–350 m.

Discussion. *L. norvegica* sp. n. differs from all previously described species of the family Fauveliopsidae by the highly reduced chaetal bunds in median and posterior parapodia. Whereas all fauveliopsid species have one, rarely two acicular spines in each parapodial ramus, noto- and neuropodia of chaetigers 5–21 in *L. norvegica* sp. n bear one fine, slender capillary and one even smaller chaeta which may be absent.

Species of the genus *Laubieriopsis* are characterized by a fixed number of chaetigers; previously described species have 16, 21 and 25 chaetigers (Petersen 2000). *L. norvegica* sp.n. is most similar to *L. cabiochi* (Amoureux, 1982) in the number of chaetigers (22 and 21, respectively) but it has significant differences. *Laubieriopsis norvegica* have paired genital papillae while in *L. cabiochi* the genital papilla is unpaired. Paired genital papillae were observed in some other species of the genus, for example *L. arenicola*. Parapodia of the last chaetiger in *L. norvegica* differ from the other parapodia, bearing one thick acicular chaeta and three capillary chaetae. Enlarged and backward-directed chaetae of the terminal chaetiger – “pygidial cage” – were described also for other *Laubieriopsis* species (*L. brevis*, *L. arenicola*) (Riser 1987, Petersen 2000). *Laubieriopsis arenicola* differs from *L. norvegica* by having 25 thoracic chaetigers and in the location of genital papillae on chaetiger 7.

Our study confirms Petersen’s (2000) statement on characters useful for distinguishing species in the genus *Laubieriopsis*: number of chaetigers, number of anterior chaetigers with acicular chaetae, presence of bidentate anterior neurochaetae, enlarged and directed backward chaetae of the last chaetiger, and paired/unpaired genital papilla. Interestingly, one of 28 specimens studied here has 23 instead 22 thoracic chaetigers and another one has an unpaired genital papilla. As intraspecific variability was noted for all characters (present work, Petersen 2000), description of new as well as redescription of existing taxa should be based on examination of several specimens and the whole complex of characters.

There was only one species of the genus *Laubieriopsis* previously known from the Northeast Atlantic, *L. cabiochi*. The present study increases the number of known species of *Laubieriopsis* to five and the number of Northeast Atlantic species of this genus to two.

Acknowledgements

We are grateful to Andrey Sikorski (Akvaplan-niva, Norway) who kindly provided the material. We thank Anton Makarov (Moscow State University, Russia) for making the map. We are very thankful to Alexander Tzetlin (Moscow State University, Russia) for his permanent patience and great support. The present study was supported by the Russian Foundation for Basic Research, projects № 11-04-01695, 10-04-01547, and federal target program of the Ministry of Education and Science of Russian Federation.

References:

- Amoureux L (1982) Annélides polychètes recueillies sur la pente continentale de la Bretagne à l'Irlande, Campagne 1973 de la "Thalassa" (suite et fin) avec la description de quatre nouvelles espèces pour la Science. II. Inventaire taxonomique annoté de toutes les polychètes sédentaires. *Cahiers de Biologie Marine* 23: 179–214.
- Blake JA, Petersen ME (2000) Family Fauveliopsidae Hartman, 1971. In: Blake JA, Hilbig B, Scott PV (Eds) *Taxonomic Atlas of the Benthic Fauna of the Santa Maria Basin and the Western Santa Barbara Channel. The Annelida part 4. Polychaeta: Flabelligeridae to Sternaspidae*. Santa Barbara Museum of Natural History, Santa Barbara, California, 31–45.
- Fauchald K, Rouse G (1997) Polychaete systematics: Past and present. *Zoologica Scripta* 26(2): 71–138. doi: 10.1111/j.1463-6409.1997.tb00411.x
- Hartman O (1960) Systematic account of some marine invertebrate animals from the deep basins of Southern California. In: Hartman O, Barnard JL (Eds). *The Benthic Fauna of the Deep Basins off Southern California, Part 2*. Allan Hancock Pacific Expeditions 22(2): 65–297.
- Hartman O (1965) Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic areas. Allan Hancock Foundation Publications, Occasional Paper 28:1–378.
- Hartman O (1971) Abyssal polychaetous annelids from the Mozambique Basin off Southeast Africa, with a compendium of abyssal polychaetous annelids from world-wide areas. *Journal of the Fisheries Research Board of Canada* 28: 1407–1428. doi: 10.1139/f71-219
- Katzmann W, Laubier L (1974) Le genre *Fauveliopsis* (polychète sédentaire) en Méditerranée. *Mikrofauna des Meeresbodens* 50: 357–371. <http://archimer.ifremer.fr/doc/1974/publication-5449.pdf>
- Levenstein RJ (1970) New and rare species of the abyssal genus *Fauveliopsis* McIntosh (Polychaeta, Annelida) and the peculiarities of its distribution. *Trudy instituta oceanologii* 88: 227–235.
- McIntosh WC (1922) Notes from the Gatty Marine Laboratory, St. Andrews, 44: 1. On new and rare Polychaeta, Gephyrea, etc., from various regions; 2. Recent additions to the Brit-

- ish marine Polychaeta (continued). Annals and Magazine of Natural History, series 9, 9: 1–30.
- Núñez J, Ocaña O, Brito MC (1997) Two new species (Polychaeta: Fauveliopsidae and Nerillidae) and other polychaetes from the marine lagoon cave of Jameos del Agua, Lanzarote (Canary Islands). Bulletin of Marine Science 60: 252–260. <http://www.ingentaconnect.com/content/umrsmas/bullmar/1997/00000060/00000002/art00007?crawler=true#expand/collapse>
- Petersen M (2000) A new genus of Fauveliopsidae (Annelida: Polychaeta), with a review of its species and redescription of some described taxa. Bulletin of marine science 67(1): 491–515. <http://www.ingentaconnect.com/content/umrsmas/bullmar/2000/00000067/00000001/art00041>
- Purschke G (2011) Sipunculid-like ocellar tubes in a polychaete, *Fauveliopsis* cf. *adriatica* (Annelida, Fauveliopsidae): implications for eye evolution. Invertebrate Biology 130 (2): 115–128. doi: 10.1111/j.1744-7410.2011.00226.x
- Riser NW (1987) A new interstitial polychaete (family Fauveliopsidae) from the shallow subtidal of New Zealand with observations on related species. Bulletin of the Biological Society of Washington (7): 211–216.
- Thiel D, Purschke G, Böggemann M (2011) Abyssal Fauveliopsidae (Annelida) from the South East Atlantic. Journal of Natural History 45 (15–16): 923–937. doi: 10.1080/00222933.2010.540046
- Zhadan AE, Atroshchenko MM (2010) The morphology of *Laubieriopsis* sp. (Polychaeta, Fauveliopsidae) and the position of fauveliopsids in the polychaete system. Biology Bulletin 37 (9): 876–885. doi: 10.1134/S1062359010090025

Description of a new species of *Sternocoelis* from Morocco with proposal of the *Sternocoelis marseulii* species group (Coleoptera, Histeridae)

Tomáš Lackner^{1,†}, Peter Hlaváč^{2,‡}

¹ Czech University of Life Sciences, Faculty of Forestry and Wood Sciences, Department of Forest Protection and Game Management, Kamýcká 1176, CZ-165 21 Praha 6 – Suchbátka, Czech Republic ² Na Doline 14, 044 14, Košice, Slovakia

† [urn:lsid:zoobank.org:author:E1DA422B-F56F-4253-A55D-481479D933B8](https://zoobank.org/E1DA422B-F56F-4253-A55D-481479D933B8)

‡ [urn:lsid:zoobank.org:author:3FA8D0BB-B332-4173-8239-9AB9F6DC9150](https://zoobank.org/3FA8D0BB-B332-4173-8239-9AB9F6DC9150)

Corresponding author: Tomáš Lackner (tomaslackner@me.com)

Academic editor: M. Caterino | Received 15 February 2012 | Accepted 23 March 2012 | Published 6 April 2012

[urn:lsid:zoobank.org:pub:3AF9D612-817C-43B2-ABDA-2DD0DB0DF03F](https://zoobank.org/pub:3AF9D612-817C-43B2-ABDA-2DD0DB0DF03F)

Citation: Lackner T, Hlaváč P (2012) Description of a new species of *Sternocoelis* from Morocco with proposal of the *Sternocoelis marseulii* species group (Coleoptera, Histeridae). ZooKeys 181: 11–21. doi: 10.3897/zookeys.181.2953

Abstract

The *Sternocoelis marseulii* species group is proposed based on antennal and prosternal characters. Five species are included in the group: *S. marseulii* (Brisout de Barneville, 1866) (Spain), *S. viaticus* Lewis, 1892 (Algeria), *S. vaucheri* Lewis, 1896 (Morocco), *S. berberus* Lackner & Yélamos, 2001 (Morocco) and *S. yelamosi* sp. n. (Morocco). The external morphology of *Sternocoelis yelamosi* sp. n. is described and illustrated, the illustrations of genitalia of all species of the group (except for *S. vaucheri*) are provided and a key to the species of the group is given.

Keywords

Coleoptera, Histeridae, Haeteriinae, *Sternocoelis*, new species, Morocco, Spain, Algeria

Introduction

The genus *Sternocoelis* Lewis, 1888 is a small genus of myrmecophilous histerids with 27 described species distributed in the Mediterranean area with most species described from Morocco and Algeria (Yélamos 1995, Mazur 1997). The genus has been recently revised by Yélamos (1995). Since then only one further species has been described,

from the Moroccan High Atlas (Lackner and Yélamos 2001). Recently, mainly Slovak and Czech coleopterists have carried out numerous expeditions with focus on the myrmecophilous beetles of Morocco. The newly described species from the Moroccan Middle Atlas results from one such expedition and shows that our knowledge of the Moroccan fauna of *Sternocoelis* is still incomplete.

Material and methods

Beetles, after being removed from original cards, were side-mounted on triangular points and examined under Nikon 102 binocular microscope with diffuse light. Male genitalia were first macerated in 10% KOH solution for about 15 minutes, cleared in 80% alcohol and macerated in lactic acid with fuchsine heated up to 60°C for another two hours. After that, they were treated with aceto-salicylate heated up to 60°C for 15 minutes and cleared in xylene. They were subsequently examined in α -terpineol in a small dish. Digital photographs were taken by a Nikon 4500 Coolpix camera and edited in Adobe Photoshop CS3. Based on the photographs, observing the actual genitalia, pencil art was drawn; pen art followed, re-tracing the pencil art and making minor corrections. SEM photographs were taken by Hitachi S-2250N camera.

The following acronyms of museums and private collections are used throughout the text:

- BMNH** The Natural History Museum, London, United Kingdom (R. Booth);
CTLA Tomáš Lackner collection, Leiden, The Netherlands;
CTYB Tomás Yélamos collection, Barcelona, Spain;
MNHN Muséum National d'Histoire Naturelle, Paris, France (A. Taghavian).

Abbreviations

Abbreviations of morphological measurements follow Ôhara (1994) and are used throughout the text as follows:

- APW** width between anterior angles of pronotum
EL length of elytron along elytral suture
EW maximum width between outer margins of elytra
PEL length between anterior angles of pronotum and apices of elytra
PPW width between posterior angles of pronotum.

Separate lines of the same label are marked by slash (/); separate labels are marked by double slash (/). Morphological conventions and terminology and methods of illustration preparation follow Lackner (2010).

Taxonomy

Sternocoelis marseulii species group

All members of the group can easily be distinguished from all other *Sternocoelis* by the combination of the following character states:

1. prosternal lobe at the same level or slightly below the level of prosternal keel;
2. prosternal lobe not divided medially and without deep emargination;
3. prosternal keel with carinal prosternal striae distinct and (almost) joined anteriorly;
4. elytra with first dorsal elytral stria complete;
5. antennal scape with a 'hook' (except for *S. viaticus*).

Yélamos (1995), in his revision of the genus placed species *S. marseulii* (Brisout de Barneville, 1866), *S. vaucheri* Lewis, 1896 and *S. viaticus* Lewis, 1892 in a small clade closest to the out-group (*Haeterius ferrugineus* (Olivier, 1789)). *Sternocoelis berberus* Lackner & Yélamos, 2001 and *S. yelamosi* (described here) share with the three afore-mentioned species identical character states, so we believe that these two species also belong in this plesiotypic clade, which we define as '*Sternocoelis marseulii* species group'. Although the monophyly of this group is highly likely, it requires testing by a phylogenetic analysis in the future. A phylogenetic analysis of *Sternocoelis* would be, however, outside of the scope of this paper. We are aware of the fact that proposing new taxonomic structure without a real phylogenetic analysis to support it should not be a standard measure. In the present work we therefore designate this species-group mostly on pragmatic grounds as an informal taxonomic unit that should serve as a pointer for the future studies of this difficult genus. We believe that male terminalia should be examined in all extant species with the special focus on the male terminalia and spiculum gastrale in particular (see Discussion). All five species included in the group are externally rather similar and, for secure identification, the examination of the male terminalia, especially spiculum gastrale, is necessary.

Key to the species of the *S. marseulii* group:

- 1 (8) Antennal scape with a 'hook' (Fig. 20)
- 2 (3) Prosternum, especially apically, weakly punctate (Fig. 4)
..... ***S. yelamosi* sp. n. (Morocco: Jebel Tazzeka)**
- 3 (2) Prosternum apically very coarsely and densely punctate, rugose (Fig. 17)
- 4 (5) Pronotal sides strongly explanate (see Lackner and Yélamos 2001: fig. 1),
well-separated from disc, body size 1.40–1.60 mm
..... ***S. berberus* Lackner & Yélamos, 2001 (Morocco: High Atlas)**
- 5 (4) Pronotal sides weakly explanate, not well separated from the disc (Fig. 30),
body size 1.30–1.40 mm.

- 6 (7) Anterior pronotal angles strongly produced, elytra along widest point rather narrow, ratio width: length 1.14 (Fig. 18), meso-metaventral excavation deep (Fig. 19)..... ***S. marseulii* (Brisout de Barneville, 1866) (Spain)**
- 7 (6) Anterior pronotal angles weakly produced, elytra along widest point rather broad, ratio width : length 1.20 (Fig. 30), meso-metaventral excavation shallow (Fig. 31)..... ***S. vaucheri* Lewis, 1896 (Morocco: Tangier)**
- 8 (1) Antennal scape without a 'hook' ***S. viaticus* Lewis, 1892 (Algeria)**

***Sternocoelis yelamosi* sp. n.**

urn:lsid:zoobank.org:act:6E3C4ACC-F2D0-44F6-8BA0-303324D266A7

http://species-id.net/wiki/Sternocoelis_yelamosi

Figs 1–16

Type locality. Morocco, Jebel Tazzeke.

Type material examined. Holotype, ♂: MOROCCO: Moyen Atlas / Taza: Jebel Tazzeke / N34°12.226', W04°03.908' / 11.V.2009 / Hlaváč lgt., under stone with ants, 1360 m (printed); // HOST ANT: *Aphaenogaster mauritanicus* Emery / H. Cagniant, det. 2009 (printed); // HOLOTYPE *Sternocoelis yelamosi* Lackner & Hlaváč, det. 2010 (red label, printed); CTLA. **Note.** One specimen of minor worker ant is pinned together with the holotype.

Description. PEL:1.425; APW:0.625; PPW:1.075; EL:0.875; EW:1.20. Body (Fig. 1) colour reddish-brown, shiny, weakly convex, oval, dorsal surface with sparse setation, ventral surface lacking setae.

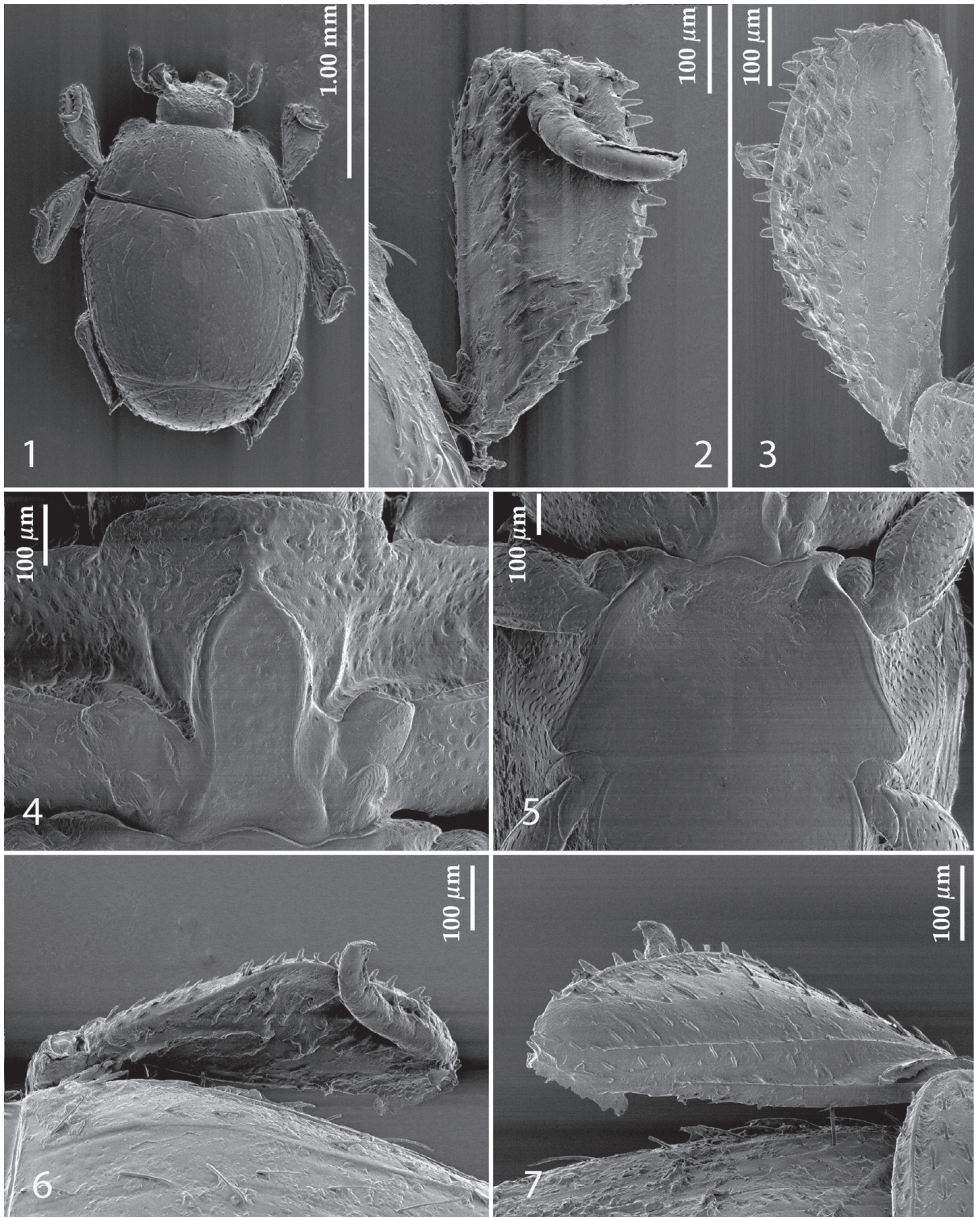
Frons wide, almost rugulose-lacunose, coarsely punctate, punctures with sparse setae; eyes well-visible from above; frontal stria carinate, prolonged onto clypeus; antennal scape thickened, apically with a 'hook', antennal club cylindrical, truncate at apex.

Pronotum with dense fine punctulation anteriorly and evanescent punctulation laterally and basally, with sparse setae (many setae probably worn off); pronotal sides weakly separated from disc by shallow marginal depression; marginal pronotal stria present on basal three-quarters; anterior pronotal angles produced, truncate; posterior angles of pronotum acute, not produced.

Elytra weakly convex, with long but sparse setae; disc densely punctate, punctures separated by several times their diameter; marginal elytral stria complete, carinate, briefly continued as apical elytral stria; first dorsal elytral stria complete, second dorsal elytral stria reaching approximately two-thirds of elytral length apically, third elytral stria the shortest, fine, reaching approximately mid elytral length apically, other elytral striae absent.

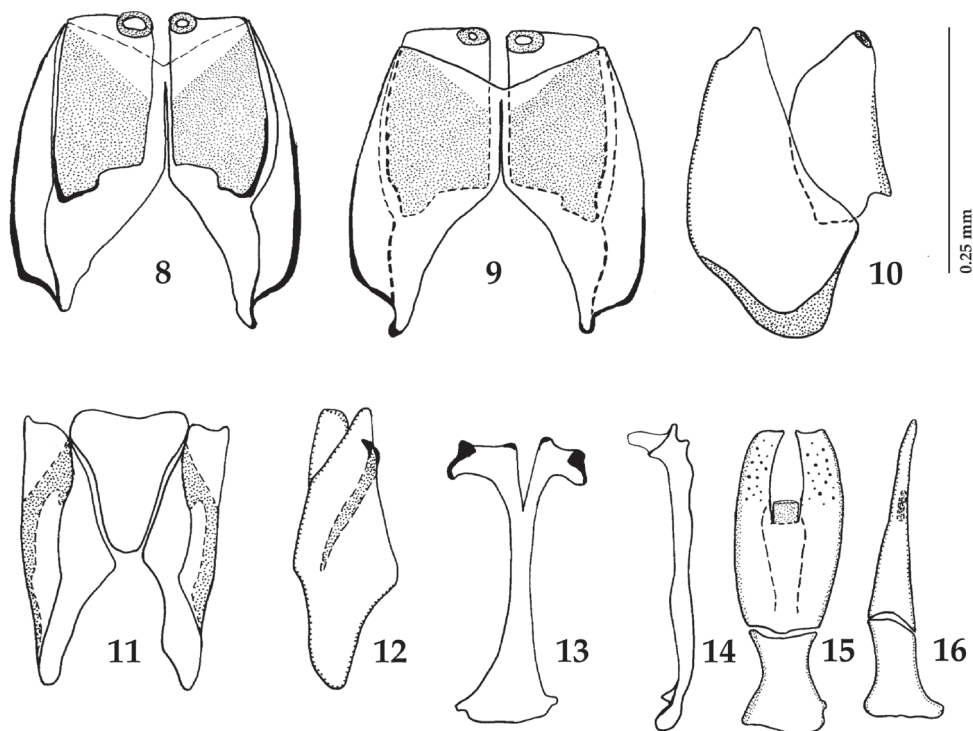
Propygidium about 2.5 times as long as pygidium, both with sparse punctulation and sparse long setae.

Prosternal process (Fig. 4) at slightly higher level than prosternal lobe; carinal prosternal striae bisinuate, joined anteriorly, interspace between them rugose; prosternal lobe roughly punctulate.



Figures 1–7. *Sternocoelis yelamosi* sp. n. **1** habitus dorsal view **2** protibia dorsal view **3** ditto, ventral view **4** prosternum **5** meso-metaventricle **6** mesotibia, dorsal view **7** ditto, ventral view.

Mesoventrite smooth, shiny, asetose; meso-metaventral depression shallow; mesoventral foveae incipient (Fig. 5); three lateral mesoventral striae present; inner one middle of mesoventrite posteriorly. Metaventricle with sparse and fine punctulation present mainly in anterior part, shiny.



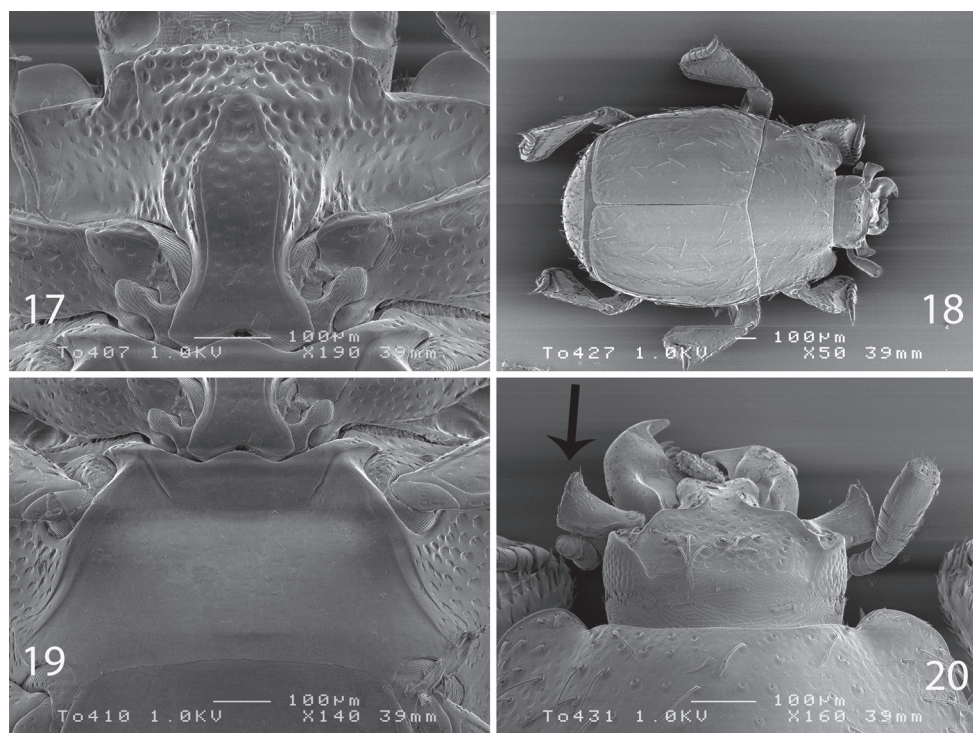
Figures 8–16. *Sternocoelis yelamosi* sp. n. male terminalia. **8** Eighth sternite and tergite, ventral view **9** ditto, dorsal view **10** ditto, lateral view **11** ninth and tenth tergites, ventral view **12** ditto, lateral view **13** spiculum gastrale, ventral view **14** ditto, lateral view **15** aedeagus, dorsal view **16** ditto, lateral view.

All visible abdominal sternites smooth, asetose, second visible abdominal sternite medially about twice as long as sternite I and III.

Legs (Figs 2–3, 6–7) relatively short, with sparse but strong setae, all tibiae expanded from base to apex, with strong setae, mainly on outer margins.

Male genitalia. Eighth sternite (Figs 8–9) longitudinally divided medially, apically without velae, each half of divided eighth sternite apically with round sclerotized ‘ring’; eighth tergite much larger than sternite, longitudinally almost divided medially, its halves widely separated; eighth sternite and tergite completely separated laterally (Fig. 10). Ninth tergite (Figs 11–12) widely separated by tooth-shaped tenth tergite. Spiculum gastrale (Figs 13–14) dilated on both ends, apical end dilated, with medio-apical notch. Aedeagus (Figs 15–16) typical for the genus (see Yélamos 1995: 147, Figs 69–83 for comparison), truncated apically; apical third with pseudopores, laterally barely curved; ratio of basal piece of aedeagus to the parameres approximately 1:2.

Differential diagnosis. *S. yelamosi* sp. n. closely resembles other species included in the *S. marseulii* species group, but it can be differentiated from all of them as follows: from *S. viaticus* by presence of ‘hook’ on antennal scape [lacking only in *S. viaticus*]; from *S. marseulii* by weaker punctuation of the prosternum and coarser punctuation



Figures 17–20. *Sternocoelis marseulii* (Brisout de Barneville, 1866). **17** prosternum **18** habitus, dorsal view **19** meso-metaventrite **20** head, dorsal view.

of dorsum [prosternum of *S. marseulii* is rugose and densely punctate; dorsum with weaker punctation]; from *S. vaucheri* by weaker punctation of the prosternum and coarser punctation of elytra [prosternum of *S. vaucheri* is rugose, coarsely punctate and elytra are almost impunctate]: from *S. berberus* by smaller body size [1.425 *vs.* 1.60 mm in *S. berberus*] as well as by weakly explanate pronotal sides [pronotal disc of *S. berberus* is clearly separated from strongly explanate pronotal sides].

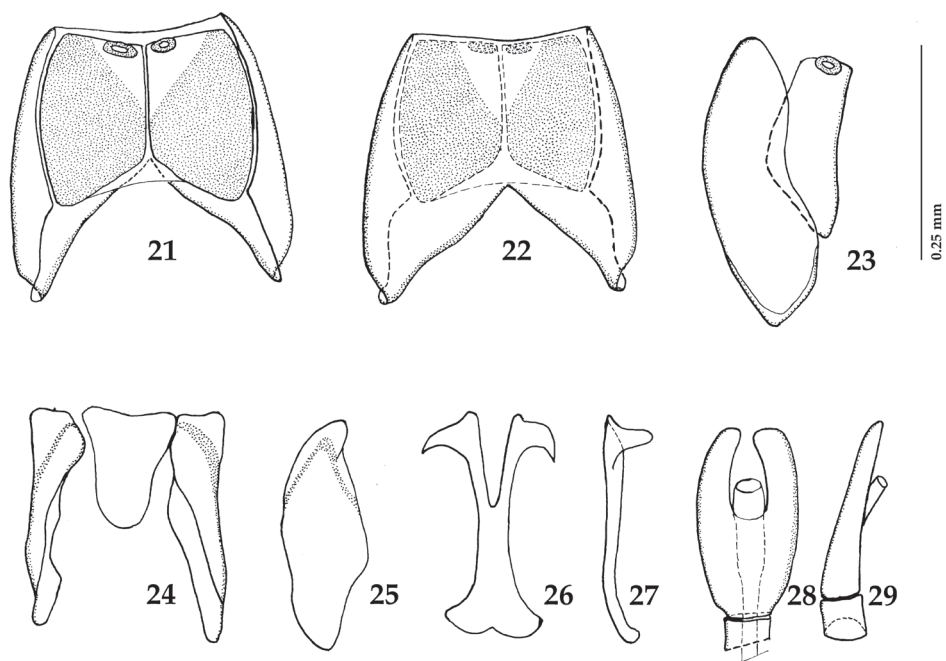
Etymology. Patronymic, named after and dedicated to our friend Tomás Yélamos (Barcelona, Spain), who revised the genus *Sternocoelis* and confirmed this to be a new species.

Distribution. So far known only from the type locality, Jebel Tazzeke in Middle Atlas, northeast Morocco.

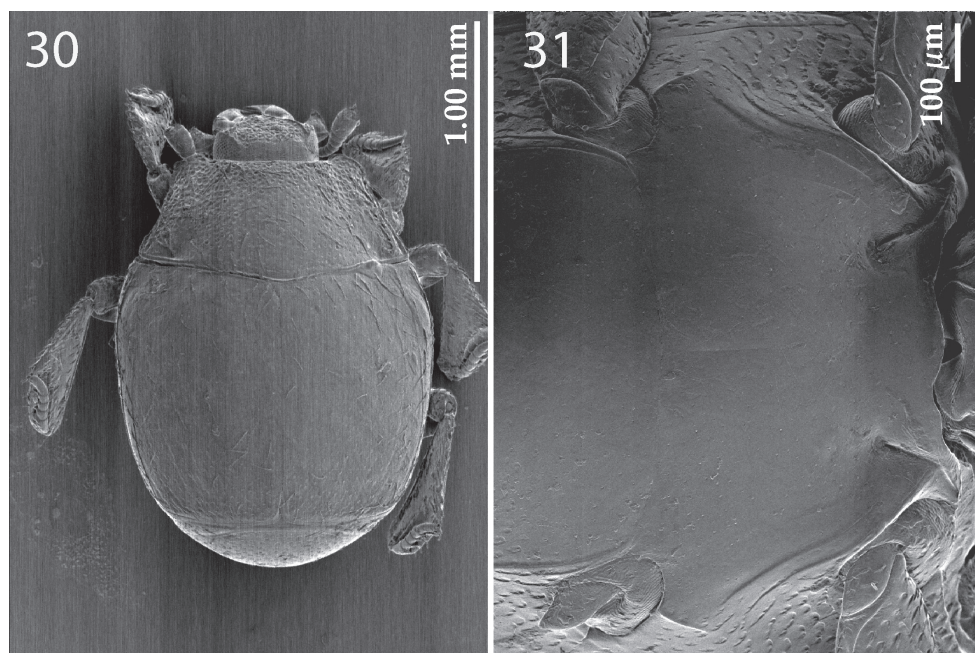
Host ant. *Aphaenogaster mauritanicus* Dalla Torre, 1893.

Other material studied. *Sternocoelis berberus* Lackner & Yélamos, 2001: 26 exs (sex undetermined): Morocco, Oikameden, 2646m - 2656m (N31°11.605', W07°51.172' – N31°11.609', W07°51.168'), 20.V. 2009, P. Koniar & M. Švarc lgt., under rocks on open slope.

Sternocoelis viaticus Lewis, 1892: Paralectotype, ♂, side-mounted on a triangular point with dismembered genitalia glued to the same triangular point as specimen, with another mounting card bearing an ant, with written label: „Meskoutin / G. Lewis



Figures 21–29. *Sternocoelis marseulii* (Brisout de Barneville, 1866) male terminalia. **21** Eighth sternite and tergite, ventral view **22** ditto, dorsal view **23** ditto, lateral view **24** ninth and tenth tergites, ventral view **25** ditto, lateral view **26** spiculum gastrale, ventral view **27** ditto, lateral view **28** aedeagus, dorsal view **29** ditto, lateral view.



Figures 30–31. *Sternocoelis vaucheri* Lewis, 1896. **30** habitus, dorsal view **31** meso-metaventrite.

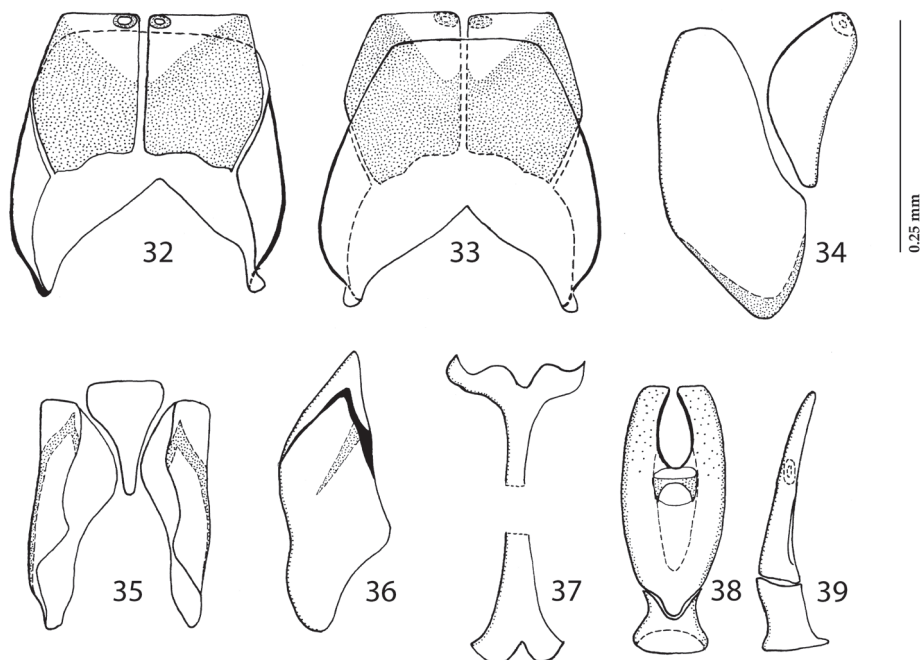
/ 22.4.[18]92“, followed by another written label „*Sternocoelis* / *viaticus* / Co-Type Lewis“, followed by round, yellow-margined label „Co- / type“ and by another printed label „G. Lewis Coll. / B.M. 1926-369“, followed by another red label, printed „Paralectotypus / T. Yélamos / Des. 1993“.

Sternocoelis marseulii (Brisout de Barneville, 1866): 2 ♀♀ & 2 ♂♂ Spain, Escorial (MNHN).

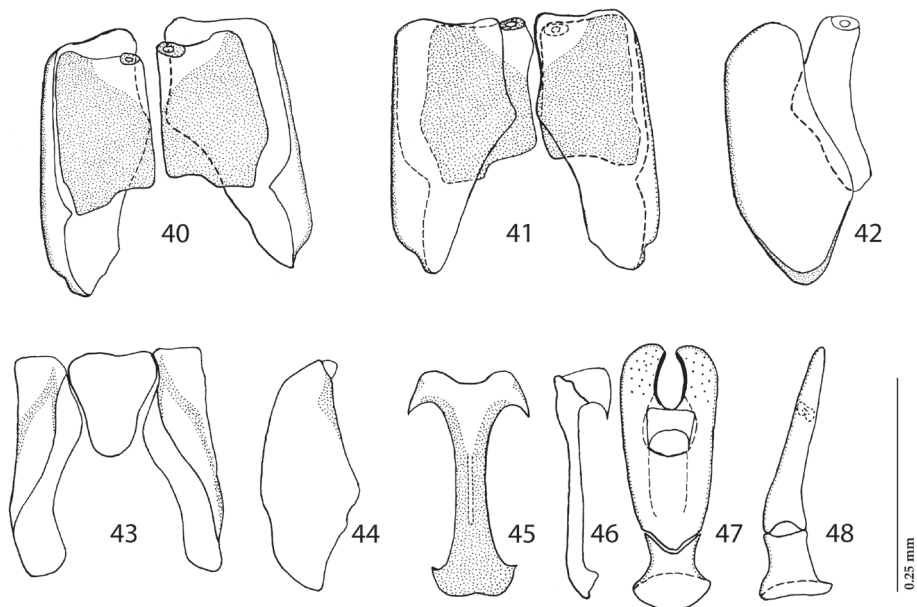
Sternocoelis vaucheri Lewis, 1896: Paralectotype, ♂, side-mounted on a triangular point with dismembered genitalia glued to the same triangular point as specimen (spiculum gastrale missing), with another mounting card bearing an ant, with written label: „Tanger / 1896 / Vaucher“, followed by another written label: „Much more / convex than / *Marseulii* Bris.“, followed by printed label: „G. Lewis Coll. / B.M. 1926-369“, followed by another printed label: „*Sternocoelis* / *vaucheri* / Lewis, 1896 / T. Yélamos Det.“, followed by another red label, printed „Paralectotypus / T. Yélamos / Des. 1993“ (BHMN).

Discussion

S. yelamosi shares numerous character states with another four species (*S. vaucheri*, *S. berberus*, *S. viaticus* and *S. marseulii*) that are found in Morocco, Algeria and Spain, respectively. Most of these character states, according to the phylogenetic analysis performed by Yélamos (1995), are presumed to be symplesiomorphies (Yélamos 1995:168). Yélamos (1995) determined polarities of character states using the Palaearctic genus *Haeterius* Dejean, 1833 for the out-group. Yélamos (1995) did not study male terminalia in detail; he only mentioned that „...male genitalia are very constant, with an almost imperceptible intraspecific variability“ (Yélamos 1995: 168). According to our study of the male terminalia of members of the ‘*Sternocoelis marseulii* species group’ the most reliable character for distinguishing species of this group is the shape of spiculum gastrale, especially its apical and basal ends (compare Figs 13, 26, 37 and 45). *S. marseulii* is the only species of *S. marseulii* species group that already had its spiculum gastrale illustrated (Yélamos 1995: 147, Fig. 84). However, our study of the male terminalia of *S. marseulii* indicates that the spiculum gastrale of this species (Fig. 26) is rather different from that illustrated by Yélamos (1995). The rest of the male terminalia (Figs 8–12, 14–16; 21–25, 27–29; 32–36, 38–39; 40–44, 46–48) show more uniformity and are less valuable tools for the intra-specific recognition. The spiculum gastrale of *S. vaucheri* could not have been examined; therefore the validity of this taxon remains dubious. *S. vaucheri* is known only from two males and two specimens of unidentified sex, all collected more than hundred years ago in Tangier (northern Morocco). Two males of this species belonging to the type series housed in BMNH have been examined, unfortunately their male terminalia (except for the aedeagus already drawn by Yélamos 1995) are damaged and unsuitable for drawing. According to Yélamos (1995), there should be another two specimens of unidentified sex housed in MNHN; these two specimens, however, have not been found in the col-



Figures 32–39. *Sternocoelis berberus* Lackner & Yélamos, 2001, male terminalia. **32** Eighth sternite and tergite, ventral view **33** ditto, dorsal view **34** ditto, lateral view **35** ninth and tenth tergites, ventral view **36** ditto, lateral view **37** spiculum gastrale, ventral view **38** aedeagus, dorsal view **39** ditto, lateral view.



Figures 40–48. *Sternocoelis viaticus* Lewis, 1892, male terminalia. **40** Eighth sternite and tergite, ventral view **41** ditto, dorsal view **42** ditto, lateral view **43** ninth and tenth tergites, ventral view **44** ditto, lateral view **45** spiculum gastrale, ventral view **46** ditto, lateral view **47** aedeagus, dorsal view **48** ditto, lateral view.

lections (Taghavian pers. comm., 2011). A lot of effort was devoted to find this rare species in the surroundings of Tangier, Morocco, but without success. According to Yélamos (pers. comm. 2011), *S. vaucheri* might be a junior synonymy of *S. marseulii*. *S. marseulii* occurs predominantly in the mountains of central and eastern Spain, with few findings also in southern Spain (Yélamos 1995). One locality, Sierra de Córdoba is approximately 300 km from the type locality of *S. vaucheri* (Tangier, Morocco). However, without the examination of the male terminalia of *S. vaucheri* it would be premature to synonymize the two species and therefore both species are kept in their current taxonomic status. We believe that a newly performed phylogenetic analysis including the male terminalia, with desired molecular characters would help to elucidate the relationships among the *Sternocoelis* species.

Acknowledgements

We are grateful to our colleague and friend Tomás Yélamos (Barcelona, Spain) for his time to verify the identity of this new species as well as for his help with the myrmecophilous histerids of Morocco in general. Thanks are due to the curators of the above-mentioned institutions for their help with the specimens as well as to J. Cooter (Oxford, UK) for the grammatical revision of the manuscript.

Open access to this paper was supported by the Encyclopedia of Life (EOL) Open Access Support Project (EOASP).

References

- Lackner T (2010) Review of the Palaearctic genera of Saprininae. Acta Entomologica Musei Nationalis Pragae, Supplement 50: 1–254.
- Lackner T, Yélamos T (2001) Contribution to the knowledge of the Moroccan fauna of *Sternocoelis* Lewis, 1888 and *Eretmotus* Lacordaire, 1854 (Coleoptera: Histeridae). ZAPATERI, Revista Aragonesa de Entomologia 9: 99–102.
- Mazur S (1997) A world catalogue of the Histeridae (Coleoptera: Histeridae). Genus, Supplement, 373pp.
- Ôhara M (1994) A revision of the superfamily Histeroidea of Japan (Coleoptera). Insecta Matsumurana (N. S.) 51: 1–238.
- Yélamos T (1995) Revision of the genus *Sternocoelis* Lewis, 1888 (Coleoptera: Histeridae), with a proposed phylogeny. Revue Suisse de Zoologie 102 (1): 113–174.

A synopsis of the genus *Cypholoba* Chaudoir (Coleoptera, Carabidae, Anthiini) known to occur in the Republic of South Africa

Jonathan R. Mawdsley¹, Terry L. Erwin¹, Hendrik Sithole², Alice S. Mawdsley³

1 Department of Entomology, MRC 187, National Museum of Natural History, Smithsonian Institution, P. O. Box 37012, Washington, DC 20013-7012 USA **2** Research Manager: Invertebrates, South African National Parks, P. O. Box 110040 Hadison Park, Kimberley South Africa **3** Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44114 USA

Corresponding author: Jonathan R. Mawdsley (mawdsley@heinzctr.org)

Academic editor: A. Casale | Received 21 February 2011 | Accepted 27 March 2012 | Published 6 April 2012

Citation: Mawdsley JR, Erwin TL, Sithole H, Mawdsley AS (2012) A synopsis of the genus *Cypholoba* Chaudoir (Coleoptera, Carabidae, Anthiini) known to occur in the Republic of South Africa. ZooKeys 181: 23–43. doi: 10.3897/zookeys.181.2984

Abstract

Nearly one third of the described species of *Cypholoba* Chaudoir (Coleoptera: Carabidae) are known to inhabit the Republic of South Africa. A key and diagnostic notes are provided for their identification, as well as notes about way of life for some of the species based on observations in the Kruger National Park. Fifteen species and subspecies of the genus are recorded from the Republic of South Africa; adult specimens of each species and subspecies are illustrated and information about the distribution of each species in the Republic of South Africa is summarized and mapped: *C. alstoni* (Péringuey), *C. alveolata* (Brême), *C. amatonga* Péringuey, *C. fritschi* (Chaudoir), *C. gracilis gracilis* (Dejean), *C. gracilis scrobiculata* (Bertoloni), *C. gracilis zuluana* Basilewsky, *C. graphipteroides graphipteroides* (Guérin-Méneville), *C. leucospilota semilaevis* (Chaudoir), *C. macilenta* (Olivier), *C. notata* (Perroud), *C. oberthueri seruana* Strohmeier, *C. opulenta* (Boheman), *C. rutata* (Péringuey), and *C. tenuicollis aenigma* (Dohrn).

Keywords

Identification key, distribution, savanna and woodland ecosystems, conservation

Introduction

The genus *Cypholoba* Chaudoir is one of the most diverse lineages within the tribe Anthiini of the beetle family Carabidae, with 156 described species and subspecies (Lorenz 2005) distributed throughout southern and eastern Africa. These beetles are conspicuous elements of savanna and woodland ecosystems, where they are typically found running in bright sunshine over bare ground, or in short grasses (Fig. 1; Marshall and Poulton 1902). Like most other members of the tribe Anthiini, species of *Cypholoba* have the ability to excrete formic acid from their pygidial glands as a defensive behavior (Péringuey 1896). Most species in this genus are black and many species have white setal patches or setal tufts (Fig. 1) that are thought to have evolved through mimicry of Mutillidae, Formicidae, and other stinging Hymenoptera (Marshall and Poulton 1902). These beetles are of potential interest to entomologists and evolutionary biologists studying phenomena such as mimicry, aposematic coloration, and the evolution of chemical defenses. Species of *Cypholoba*, like many other Anthiini, also show close associations with particular ecosystems or vegetation communities and their activity patterns are closely tied with environmental variables such as temperature and rainfall, and overall climate patterns such as seasonal monsoons (Schmidt 2001; Mawdsley et al. 2011). Given the relatively large adult body size of most *Cypholoba* species (length 15–33 mm), their diagnostic color and setal patterns (Strohmeyer 1928) and their conspicuous activity patterns and behaviors (Schmidt 2001), these beetles could easily be incorporated into environmental monitoring programs which track overall ecosystem condition, status, and trends.

As with other Anthiini, the taxonomic history of *Cypholoba* is rather convoluted. Chaudoir (1850) initially established two genera, *Cypholoba* on p. 43 for the single species *Anthia alveolata* Brême and a second, *Polyhirma*, on p. 44 for the group of species that included *Anthia macilentata* Olivier, *A. gracilis* Dejean, *A. intermedia* Boheman, *A. ferretti* Reiche, *A. tetrastigma* Chaudoir, *A. leucospilota* Bertoloni, *A. caillaudi* Gory, and *A. polioloma* Chaudoir. Péringuey (1896) combined these two genera in his revision of southern African Carabidae, treating *Cypholoba* as a synonym of *Polyhirma* and recognizing 27 species-level taxa, ten of which were recorded from what is now the Republic of South Africa. Further descriptive work by other workers, primarily in the east African fauna, led to the recognition of 102 species in the genus by the time of Csiki's (1929) comprehensive catalogue. In the "Coleopterorum Catalogus," Csiki (1929) followed Péringuey in placing *Cypholoba* in synonymy with *Polyhirma*. Strohmeyer (1928) was the first to recognize the page priority of *Cypholoba* and also the first to attempt a comprehensive revision of this group. His revision radically altered the taxonomy of the genus, recognizing just 16 species and relegating most of the former species to subspecies status. This approach came under criticism from Basilewsky (1948, 1955) who pointed out a number of errors in Strohmeyer's revision and argued that many of the so-called "subspecies" of Strohmeyer were separated by characteristics that suggested they were in fact perfectly good species. Basilewsky published a series of studies in which he argued against Strohmeyer's reductionist approach while at the same time continuing to describe new species and subspecies of *Cypholoba* (Basilewsky 1948; 1955; 1963; 1967; 1980; 1983).



Figure 1. Adult female of *Cypholoba graphipteroides graphipteroides* (Guérin-Ménéville), photographed in a dry sandy streambed adjacent to the Sabie River in the Kruger National Park, RSA.

This paper is intended to provide an overview of the species of *Cypholoba* currently known from the Republic of South Africa (RSA). This is a fauna very much in need of good diagnostic materials, particularly keys and illustrations that can be used by non-specialists. Most of the *Cypholoba* species in RSA have never been illustrated and none of the published keys include all of the taxa now known to occur in RSA. The government of RSA has taken recent positive steps towards protecting certain carabid beetles under the South African Biodiversity Act of 2004 (Harrison and Müller, pers. comm.) and carabid beetles are increasingly being incorporated into ecosystem and agricultural monitoring programs in southern Africa (Kotze 2000; Magagula 2003). Both types of conservation approaches (carabid beetles as the subject of direct conservation efforts, and carabid beetles as environmental monitoring targets and ecological indicators) are clearly contingent on the availability of high-quality identification materials for the carabid fauna of interest. Fortunately, development of these identification materials is relatively straightforward. The species of *Cypholoba* from RSA are well represented in museum collections, as a result of large-scale survey activities that began in the 1950s and have continued more or less until the present day. The species-level taxonomy of the RSA fauna is also reasonably well known; there have been only modest changes in the taxonomy of the RSA species of *Cypholoba* since the first revision by Péringuey (1896), despite the major changes introduced by Strohmeier (1928) in other parts of the genus. In the synopsis that follows, we follow the classification of this genus presented by Lorenz (2005) in the most recent catalogue of world Carabidae. It is hoped that this short communication will help to inspire further interest and field studies of these remarkable beetles.

Materials and methods

We examined collections of adult *Cypholoba* Chaudoir and allied genera in the following institutional collections: Field Museum of Natural History, Chicago, Illinois (FMNH); Kruger National Park Museum (Scientific Services), Skukuza, South Africa (KNPC); South African National Collection of Insects, Pretoria, South Africa (SANC); National Museum of Natural History, Smithsonian Institution, Washington, D.C. (NMNH); Transvaal Museum, Pretoria, South Africa (TMSA). In the case of NMNH and TMSA, the specimens examined were authoritatively identified by the late P. Basilewsky, who studied this genus for many years (Basilewsky 1948; 1955; 1963; 1967; 1980; 1983).

Museum collections were augmented by a series of field visits in 2007, 2008, 2009, 2010, and 2011 to the Kruger National Park in northeastern South Africa, where adults of three species (*C. alveolata*, *C. graphipteroides*, and *C. notata*) were collected. Survey methods for *Cypholoba* species and other Anthiini involved systematic walking along roads, dry river washes, or sandy areas adjacent to major rivers. Surveys in the main park area were conducted from dawn until dusk and under all available weather conditions. Head-lamping surveys were conducted at night for Anthiini and other Carabidae in the N'waswitshaka Research Camp at Skukuza. Pitfall traps were also widely deployed throughout the Skukuza Ranger District of the Kruger National Park, to study the distribution of species of Carabidae across different vegetation communities and to record the responses of carabid assemblages to common landscape-scale disturbances such as mammalian grazing and ground fire. Driving surveys (Mawdsley et al. 2011) were also employed as a survey technique for larger Anthiini, as well as species of *Tefflus* Leach (Coleoptera: Carabidae: Panagaeini); adults of larger species of *Cypholoba* such as *C. graphipteroides* can be detected during driving surveys. When encountered during diurnal surveys, the individual beetles were highly conspicuous and could be easily captured by hand. Further information about our survey methods is available in papers by Mawdsley and Sithole (2008; 2009), Mawdsley (2009; 2011), and Mawdsley et al. (2011).

Methods and species concepts follow those previously described (Erwin and Kavanaugh, 1981; 1991). The species validation and diagnosis format follows as closely as possible that suggested in Erwin and Johnson (2000). Measurements of length (ABL) and width (TW) follow those of Ball (1972) and Kavanaugh (1979): ABL (apparent body length), measured from apex of labrum to apex of longer elytron.

Cypholoba Chaudoir, 1850

<http://species-id.net/wiki/Cypholoba>

Cypholoba Chaudoir, 1850:43, type species *C. alveolata* Brême, 1844:293

Polyhirma Chaudoir, 1850:44; synonymy by Strohmeyer (1928:298)

Diabatus Gistel, 1857:36; synonymy by Csiki (1929:381)

Diagnosis. Medium to large Anthiini (length 15–33 mm) with the following combination of adult attributes: head with distinct basal constriction or “neck;” second labial pal-

pomere distinctly longer than third; pronotum rectangular or cordiform (heart-shaped), not at all markedly elevated or swollen, lacking lateral or basal flanges; elytron with rows of elevated costae which alternate with rows of large, deep punctures (in most species); elytral punctures with brown, yellow, or orange setae at bottom in many species; rows of punctures and setae not extending to apex of elytron in most species; apex of elytron flat or planate in most species; elytron often with patterned setae and/or pubescence; posterior margin of elytron usually obliquely truncate; elytron lacking an apical spine.

Distribution. Southern and eastern Africa.

Included species in the Republic of South Africa. The species list below and the arrangement of descriptions that follow are ordered alphabetically.

- Cypholoba alstoni* (Péringuey), Botswana, Namibia, Republic of South Africa (RSA)
- Cypholoba alveolata* (Brême), Botswana, RSA, Zimbabwe
- Cypholoba amatonga* Péringuey, Mozambique, RSA
- Cypholoba fritschi* (Chaudoir), Botswana, Namibia, RSA, Zambia
- Cypholoba gracilis gracilis* (Dejean), Botswana, Mozambique, Namibia, RSA
- Cypholoba gracilis scrobiculata* (Bertoloni), Mozambique, RSA
- Cypholoba gracilis zuluana* Basilewsky, RSA
- Cypholoba graphipteroides graphipteroides* (Guérin-Méneville), Mozambique, RSA, Zimbabwe
- Cypholoba leucospilota semilaevis* (Chaudoir), Mozambique, RSA
- Cypholoba macilenta* (Olivier), Lesotho, RSA, Zimbabwe
- Cypholoba notata* (Perroud), RSA, Swaziland
- Cypholoba oberthueri seruana* Strohmeier, Botswana, RSA
- Cypholoba opulenta* (Boheman), Namibia, RSA
- Cypholoba rutata* (Péringuey), Mozambique, RSA, Zimbabwe
- Cypholoba tenuicollis aenigma* (Dohrn), Botswana, RSA, Zambia, Zimbabwe

Key to adults of *Cypholoba* Chaudoir species from the Republic of South Africa.

- 1 Elytron without white or yellow patches or linear bands of setae in apical half; basal half of elytron along suture at base with at most a narrow white or yellow patch or band of setae..... **2**
- Elytron with distinct white or yellow patches or linear bands of setae in apical half; basal half of elytron typically also with basal patches of white or yellow setae..... **10**
- 2 Pronotum elongate, slender, spindle-shaped..... **3**
- Pronotum broader, either rounded, rectangular, or heart-shaped..... **5**
- 3 Pronotum with well-defined longitudinal median impression; elytral punctures round ***C. gracilis gracilis* (Dejean)**
- Pronotum with median line feebly impressed at base and otherwise not at all impressed along most of length, sometimes with a narrow raised ridge; elytral punctures oval..... **4**

- 4 Pronotum median line with a round impression at base and becoming obsolete on disc..... ***C. gracilis scrobiculata* (Bertoloni)**
- Pronotum median line with a narrow impression at base and a narrow, slightly raised ridge on disc..... ***C. gracilis zuluana* Basilewsky**
- 5 Elytral punctures large, those on disc with orange-red setae deep within each puncture **6**
- Elytral punctures small, those on disc without orange-red setae deep within each puncture **7**
- 6 Apparent body length (ABL) 24–28 mm; pronotum elongate and somewhat heart-shaped, width of pronotum less than width of eyes; elytra with six costae and large alveolate punctures ***C. amatonga* Péringuey**
- Apparent body length (ABL) 31–33 mm; pronotum broader and distinctly heart-shaped, width of pronotum distinctly greater than width of eyes; elytra with six costae, fifth costa as measured from lateral margin short, only reaching to basal sixth of elytra; elytral punctures large, alveolate ***C. alveolata* (Brême)**
- 7 Elytra and pronotum without patterned pubescence or setae of any kind; elytral costae and rows of punctures continuing to apex... ***C. alstoni* (Péringuey)**
- Elytra and often pronotum with small setal tufts or patches at base; elytral costae and rows of punctures usually becoming obsolete on disc, apex of elytra smooth **8**
- 8 Pronotal base, scutellum and base of elytra each with a small white or yellow setal tuft, barely noticeable; elytral costae broad, smooth, and shining, becoming obsolete at apical third; apical third of elytra shining ***C. fritschi* (Chaudoir)**
- Pronotum and/or elytra with more extensive setal patches; elytral costae narrow; apical third of elytra matte, not shining..... **9**
- 9 Elytral surface markedly rugose, each elytron with a narrow linear band of yellow setae along suture on basal third..... ***C. rutata* (Péringuey)**
- Elytral punctures very small, costae present but not markedly elevated, elytral surface largely smooth; each elytron with an ovate or rectangular patch of yellow setae adjacent to suture on basal fifth..... ***C. opulenta* (Boheman)**
- 10 Elytra with patches of grey or white setae on disc at apical third or apical fourth **11**
- Elytra with patches of pale setae at base and/or apex of elytra only, adjacent to the suture..... **13**
- 11 Apparent body length (ABL) 15–17 mm; pronotum elongate, slender, spindle-shaped; each elytron with a second transverse band of white setae at mid-elytron ***C. tenuicollis aenigma* (Dohrn)**
- Apparent body length (ABL) 24–28 mm; pronotum broader, heart-shaped; each elytron with a patch of setae at apical third and a narrow line of white

- setae along the suture on the basal third of elytra; no second band of white setae at mid-elytron **12**
- 12 Pronotal and elytral setae uniformly greyish-white; a narrow linear band of setae along midline of pronotum, a narrow linear band of setae along elytral suture on basal two-fifths, and a pair of arcuate patches located at apical third ***C. graphipteroides graphipteroides* (Guérin-Ménéville)**
- Pronotal setae and the setal band at the base of the elytra yellowish orange; elytral setal patch placement similar to above, except for coloration, discal patches bright white..... ***C. leucospilota semilaevis* (Chaudoir)**
- 13 Elytra with apical third smooth, shining, except for apical patch of white setae; basal two-thirds of elytra with rows of costae and large punctures, which lack reddish-orange pubescence ***C. notata* (Perroud)**
- Elytra with apical fourth or fifth smooth, shining, except for apical patch of white setae; basal three-quarters of elytra with rows of costae and large punctures, reddish-orange pubescence present deep inside each puncture..... **14**
- 14 Elytra with apical fourth smooth, shining, except for apical patch of white setae; each elytron with seven costae, of which the sixth is very short.....
..... ***C. macilenta* (Olivier)**
- Elytra with apical fifth smooth, shining, except for apical patch of white setae; each elytron with seven costae subequal in size.....
..... ***C. oberthueri servana* Strohmeier**

***Cypholoba alstoni* (Péringuey, 1892a)**

http://species-id.net/wiki/Cypholoba_alstoni

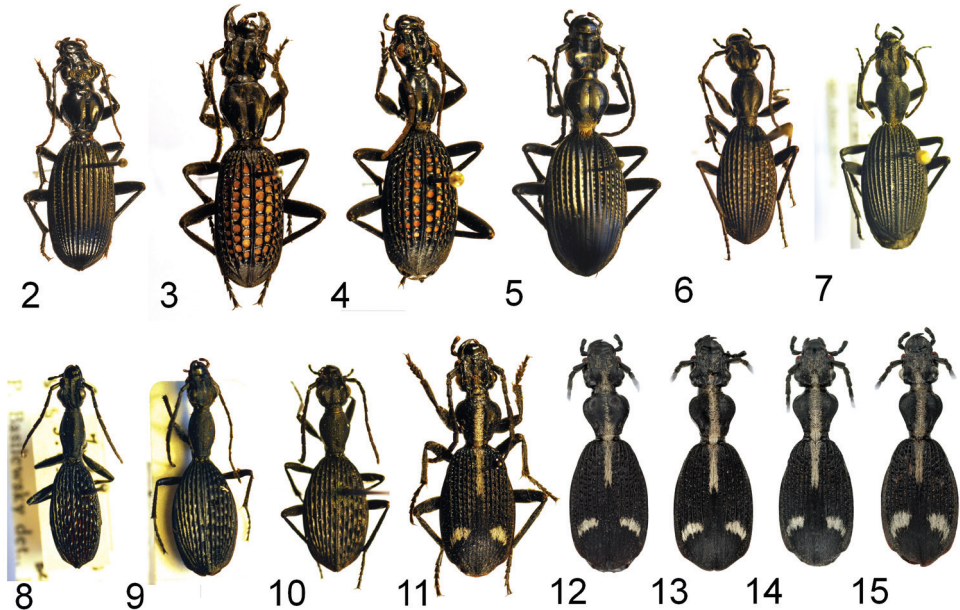
Figs 2, 29

Polyhirma alstoni Péringuey (1892a:14; type locality “British Bechuanaland,” syntype series in South African Museum, Cape Town)

Cypholoba alstoni (Péringuey) Lorenz (2005:513)

Diagnosis. Apparent body length (ABL) 23–24 mm; easily separated from all other species of *Cypholoba* in RSA by the lack of patterned pubescence on the elytra and the continuation of the rows of elytral punctures and costae until the elytral apices. The sympatric species *C. fritschi* is similar but is larger in ABL (25–31 mm in length) and its elytral costae become obsolete on the disc shortly after mid-elytron, leaving the apical third of the elytra almost entirely smooth.

Materials examined. 107 specimens from the following localities: RSA: Northern Cape Province: Farm Brulpan, Marydale, Mata Mata, Niekerk’s Hope in Griqualand West, 30 km E Pofadder, 46 km N Pofadder, Tswalu Nature Reserve, Twee Rivieren.



Figures 2–15. Adult specimens of *Cypholoba* species, from the TMSA collection unless otherwise indicated. **2** *C. alstoni* (Péringuey), male, Niekerk's Hope in Griqualand West, Northern Cape Province, RSA **3** *C. alveolata* (Brême), male, Warmbath, Limpopo Province, RSA **4** *C. amatonga* Péringuey, male, Soutpansberg, Limpopo Province, RSA **5** *C. fritschi* (Chaudoir), male, Twee Rivieren, Northern Cape Province, RSA **6** *C. gracilis gracilis* (Dejean), male, Zoutpan, Gauteng Province, RSA **7** *C. gracilis gracilis* (Dejean), female, Vanwyksfontein Farm, Northern Cape Province, RSA **8** *C. gracilis scrobiculata* (Bertoloni), male, Zoutpansberg, Limpopo Province, RSA **9** *C. gracilis scrobiculata* (Bertoloni), female, Inhambane, Mozambique **10** *C. gracilis zuluana* Basilewsky, male, "E. Zululand," KwaZulu/Natal Province, RSA **11** *C. graphipteroides graphipteroides* (Guérin-Ménéville), male, 20–26 km NE of Pietersburg, Limpopo Province, RSA **12–15** *C. graphipteroides graphipteroides* collected at sites along the Sabie River west of Paul Kruger Gate in the Kruger National Park, showing intrapopulational variation in elytral setal patterns (NMNH collection).

Cypholoba alveolata (Brême, 1844)

http://species-id.net/wiki/Cypholoba_alveolata

Figs 3, 25, 29, 34

Anthia alveolata Brême (1844:293, pl. 7 f. 5, type locality "Port-Natal," holotype in Museum National d'Histoire Naturelle, Paris)

Polyhirma alveolata (Brême) Péringuey (1896:351)

Cypholoba caillaudi alveolata (Brême) Strohmeyer (1928:322)

Cypholoba alveolata (Brême) Strohmeyer (1928:448)

Diagnosis. Apparent body length (ABL) 31–33 mm; one of the largest species of *Cypholoba* in RSA and the largest species that has the distinctive alveolate elytral

punctures with reddish-orange pubescence at the bottom of the punctures (see Fig. 3). It can be separated from *C. macilenta*, *C. notata*, and *C. oberthueri servana* by the lack of a white patch of setae across both elytral apices and from *C. amatonga* by its larger ABL (24–28 mm length in *C. amatonga*) and the differences in elytral surface sculpture mentioned in the key above. Some specimens have a patch of white setae on each elytron immediately adjacent to the scutellum, while others also have a patch of white setae on the scutellum and/or a line of white setae along the midline of the pronotum.

Materials examined. 159 specimens from the following localities: RSA: Gauteng Province: Johannesburg, Moloto, Pienaars River Dam, Pretoria, Rosslyn, Zoutpan. KwaZulu/Natal Province: Duku duku, Durban, Ndumu, Pongola River, St. Lucia Bay, Umfolozi, Waterval, “E Zululand.” Limpopo Province: Bandelierkop, 25 km NE Ellisras, Farm Scrutton, Haenertsburg, Klein Letaba, 18 miles W Letaba Rest Camp, Letaba Rest Camp in Kruger National Park, Marblehall, Messina, Mokeetse, Pietersburg, 22 miles E Pietersburg, 20–26 km N Pietersburg, Potgietersrus, Sedula near Leydsdorp, Shilouvane, Warmbath, Woodbush. Mpumalanga Province: Barberton, 3 km NW Barberton, Grootdraai, Lydenburg, Nelspruit, Nkuhlu Plots in Kruger National Park, Sabie River road west of Paul Kruger Gate, Skukuza. North West Province: Marico River, Rustenburg, Vryberg. Province not specified: “Transvaal.”

Notes on biology. During our surveys (see Materials and Methods above), adults of this species were found in riverine and upland areas of the Kruger National Park. Specific vegetation communities (Gertenbach 1983) where adults were collected included riverine gallery forest and upland *Acacia nigrescens* Olivier - *Combretum apiculatum* Sonder savanna. Adults were collected diurnally, in pitfall traps, and with headlamps at night.

Cypholoba amatonga Péringuey, 1892b

http://species-id.net/wiki/Cypholoba_amatonga

Figs 4, 29

Cypholoba amatonga Péringuey (1892:102b, type locality “Amatongaland, Delagoa Bay,” syntype series in South African Museum, Cape Town)

Polyhirma amatonga (Péringuey) Péringuey (1896:350)

Cypholoba chaudoiri amatonga (Péringuey) Strohmeyer (1928:321)

Cypholoba amatonga (Péringuey) Strohmeyer (1928:448)

Diagnosis. Apparent body length (ABL) 24–28 mm; similar to *C. alveolata* but separated from that species by the smaller ABL (31–33 mm in *C. alveolata*) and by the differences in elytral surface sculpture mentioned in the key above.

Materials examined. 2 specimens from the following locality: RSA: Limpopo Province: Soutpansberg.

***Cypholoba fritschi* (Chaudoir, 1883)**

http://species-id.net/wiki/Cypholoba_fritschi

Figs 5, 29

Polyhirma fritschi Chaudoir (1883:27, type locality “Kuruman,” holotype in Museum National d’Histoire Naturelle, Paris)

Cypholoba opulenta fritschi (Chaudoir) Strohmeyer (1928:368)

Cypholoba fritschi (Chaudoir) Strohmeyer (1928:450)

Diagnosis. Apparent body length (ABL) 25–31 mm; distinctive for its large body size and for the lack of patterned pubescence or setae on the elytra, although some specimens may have a small patch of yellow setae adjacent to the scutellum. As noted by Péringuey (1896), there is also a tuft of pubescence on the scutellum. The elytral surface sculpture is also diagnostic, with the rows of costae becoming obsolete shortly after mid-elytron and the apical third smooth and strongly shining.

Materials examined. 11 specimens from the following localities: RSA: Northern Cape Province: Niekerk’s Hope in Griqualand West, Twee Rivieren.

Notes on taxonomy. Strohmeyer (1928) had placed this species as a subspecies of *C. opulenta* but as pointed out by Basilewsky (1948) this is clearly an error as the two species are quite distinct and have different proportions, vestiture, and surface sculpture.

***Cypholoba gracilis gracilis* (Dejean, 1831)**

http://species-id.net/wiki/Cypholoba_gracilis_gracilis

Figs 6, 7, 30

Anthia gracilis Dejean (1831:468, type locality “cap de Bonne-Espérance,” holotype in Museum National d’Histoire Naturelle, Paris).

Polyhirma gracilis (Dejean) Péringuey (1896:342–343)

Cypholoba gracilis gracilis (Dejean) Strohmeyer (1928:303)

Diagnosis. Apparent body length (ABL) 17–20 mm; easily recognized by its slender body form, spindle-shaped pronotum, and lack of white setae or pubescence on the elytra. The shape and size of the elytral punctures (described in the key) will separate this subspecies from both *C. g. scrobiculata* and *C. g. zuluana*. In both *C. g. gracilis* and *C. g. scrobiculata* the shape of the elytra is sexually dimorphic and thus both forms are illustrated here.

Materials examined. 35 specimens from the following localities: RSA: Eastern Cape Province: Aliwal North, Dorset. Free State Province: Bothaville. Gauteng Province: 11 km SE Bronkhorstspuit, Bronkhorstspuit, Brooklyn, Florida, Pretoria, Rood-eplaas, Rosslyn, Welgedacht, Zoutpan, Zusterstroom. KwaZulu/Natal Province: Wa-

terval. Limpopo Province: Messina, Moordrift, Naboomspruit, Nylsvley, Pietersburg, Rhenosterpoort, Smith Farm. Mpumalanga Province: Argent, 14 miles E Middelburg, Middelburg, Moloto, Waterval Onder. Northern Cape Province: Niekerk's Hope in Griqualand West, Vanwyksfontein Farm. North West Province: Lichtenburg, Rustenburg.

***Cypholoba gracilis scrobiculata* (Bertoloni, 1847)**

http://species-id.net/wiki/Cypholoba_gracilis_scrobiculata

Figs 8, 9, 30

Anthia scrobiculata Bertoloni (1847:90, type locality “in provincia Inhambanensi Monzambici,” holotype in Accademia della Scienze dell'Istituto Bologna, Italy)

Polyhirma scrobiculata (Bertoloni) Péringuey (1896:342)

Cypholoba gracilis scrobiculata (Bertoloni) Strohmeyer (1928:315)

Diagnosis. Apparent body length (ABL) 19–20 mm; similar in general body form and appearance to *C. g. gracilis* but differing from that subspecies in having the median line of the pronotum not markedly impressed and in having the elytral punctures oval rather than round.

Materials examined. 1 specimen from the following locality: RSA: Limpopo Province: Zoutpansberg.

Notes on taxonomy. Based on the rather significant differences in the pronotal and elytral surface sculpture, *C. g. scrobiculata* may ultimately prove to represent a species distinct from *C. gracilis*. A thorough review of the *C. gracilis* species complex with a rigorous evaluation of the 41 subspecific taxa proposed by Strohmeyer (1928) is needed.

***Cypholoba gracilis zuluana* Basilewsky, 1948**

http://species-id.net/wiki/Cypholoba_gracilis_zuluana

Figs 10, 30

Cypholoba gracilis zuluana Basilewsky (1948:111, type locality “Zululand,” syntype series in Naturhistoriska Riksmuseet, Stockholm)

Diagnosis. Apparent body length (ABL) 19–20 mm; similar in general body form and appearance to *C. g. gracilis* and *C. g. scrobiculata* but differing from those two subspecies in the pronotal surface sculpturing, as noted in the key. There are also subtle differences in the arrangement and size of the elytral punctures and elytral costae between all three subspecies (see Figs 6–10).

Materials examined. 6 specimens from the following localities: RSA: KwaZulu/Natal Province: “E. Zululand,” “Zululand.”

***Cypholoba graphipteroides graphipteroides* (Guérin-Ménéville, 1845)**

http://species-id.net/wiki/Cypholoba_graphipteroides_graphipteroides

Figs 1, 11, 12, 13, 14, 15, 26, 31, 33

Anthia graphipteroides Guérin-Ménéville (1845:285, type locality “in regione Massili-catzi,” holotype in Museum National d’Histoire Naturelle, Paris)

Polyhirma graphipteroides (Guérin-Ménéville) Péringuey (1896:346)

Cypholoba graphipteroides graphipteroides (Guérin-Ménéville) Strohmeier (1928:343)

Diagnosis. Apparent body length (ABL) 24–27 mm; this species is easily separated from most of the other sympatric species of *Cypholoba* by the distinctive pattern of pubescence and setae on the elytra (Fig. 11). Adults could potentially be confused with *C. leucospilota*, but in that species the pubescence on the pronotum and elytral suture is yellowish and the patches of setae at apical third are brilliant white (Fig. 16).

Materials examined. 77 specimens from the following localities: RSA: Gauteng Province: Pretoria, Rosslyn. KwaZulu/Natal Province: Hluhluwe, Mkuze Game Reserve, Ndumu, St. Lucia Bay. Limpopo Province: Alma, Koedoesrivier, Letaba Rest Camp in the Kruger National Park, 9–14 miles E Louis Trichardt, 75 km W Messina, Nylstroom, Nylsvley, Pietersburg, 9 miles N Pietersburg, 20–26 miles NE Pietersburg, Potgietersrus, Punda Maria Rest Camp in the Kruger National Park, Rust de Winter, Shilouvane, Thabina, Warmbath. Mpumalanga Province: Kaapmuiden, 20 km SW Kaapmuiden, Louws Creek, Lydenburg, N’waswitshaka Research Camp, Pretoriuskop, Sabie river banks west of Paul Kruger Gate, Skukuza. Province uncertain: Lebombo Mountains.

Notes on biology. This is easily the most abundant and frequently encountered species of Anthiini in the Kruger National Park, RSA. Adults (Fig. 1) emerge in the early rainy season (November–December) and can be locally abundant. Adults are active both nocturnally and diurnally and are typically found in riverine or riparian areas near flowing water. The characteristic behavior observed in this species, as with most Anthiini, is a rapid walking behavior which appears to be associated primarily with foraging but also is likely involved in searching for conspecifics and prospective larval habitats. We have observed adults of this species in several distinct vegetation communities (riverine gallery forest, open *Combretum apiculatum* – *Acacia nigrescens* savanna, *Phragmites* reed beds) and microhabitats (sand or dirt roads adjacent to riverine communities, dry sand wash, and riverine sand bars). This species also occasionally enters human-inhabited areas; we have found individuals in the N’waswitshaka Research Camp at Skukuza and also in the main tourist areas at the Skukuza tourist camp. Marshall and Poulton (1902) suggest that this species may be a mimic of Mutillidae and other stinging Hymenoptera. Our observations suggest that, while there are certainly similarities in color pattern and behavior between adults of *C. graphipteroides* and sympatric Mutillidae, adults of *C. graphipteroides* are actually significantly larger in size than most of the sympatric black-and-white mutillid wasps, rendering the resemblance less than exact. We infer from these observations that selection pressures may not be operating as intensely

on this species as on other Carabidae (for examples of carabid beetles which are much more convincing mimics of Mutillidae, see Marshall and Poulton 1902).

Notes on taxonomy. Strohmeyer (1928) recognized 20 subspecies in *C. graphipteroides*, many of which were separated on the basis of differences in the setal patterns of the elytra. Many of these taxa are doubtfully distinct from the nominate form and the whole group is in need of a careful revision. Such a revision should include examination of extensive series in order to determine the extent of intrapopulational variation in the elytral setal patterns. Figures 12–15 illustrate the variation in elytral setal patterns within a single population of this species, located west of Paul Kruger Gate along the Sabie River in the Kruger National Park.

***Cypholoba leucospilota semilaevis* (Chaudoir, 1861)**

http://species-id.net/wiki/Cypholoba_leucospilota_semilaevis

Figs 16, 31

Polyhirma semilaevis Chaudoir (1861:571–572, type locality “de la baie Delagoa,” syntype series in Museum National d’Histoire Naturelle, Paris)

Polyhirma semilevis Péringuey (1896:344–345), unjustified emendation

Cypholoba leucospilota semilaevis (Chaudoir) Strohmeyer (1928:351)

Diagnosis. Apparent body length (ABL) 22–28 mm; similar in general appearance to *C. graphipteroides* but distinguished from that species by the color of the pronotal and elytral pubescence and setae. The bands of pubescence on the pronotum and elytral base are yellow in *C. leucospilota* (white or grey in *C. graphipteroides*), and the bands of pubescence on the apical third of the elytra are a much brighter white in *C. leucospilota* than in *C. graphipteroides*.

Materials examined. 5 specimens from the following localities: RSA: KwaZulu/Natal Province: “E. Zululand,” “Zululand.”

***Cypholoba macilenta* (Olivier, 1795)**

http://species-id.net/wiki/Cypholoba_macilenta

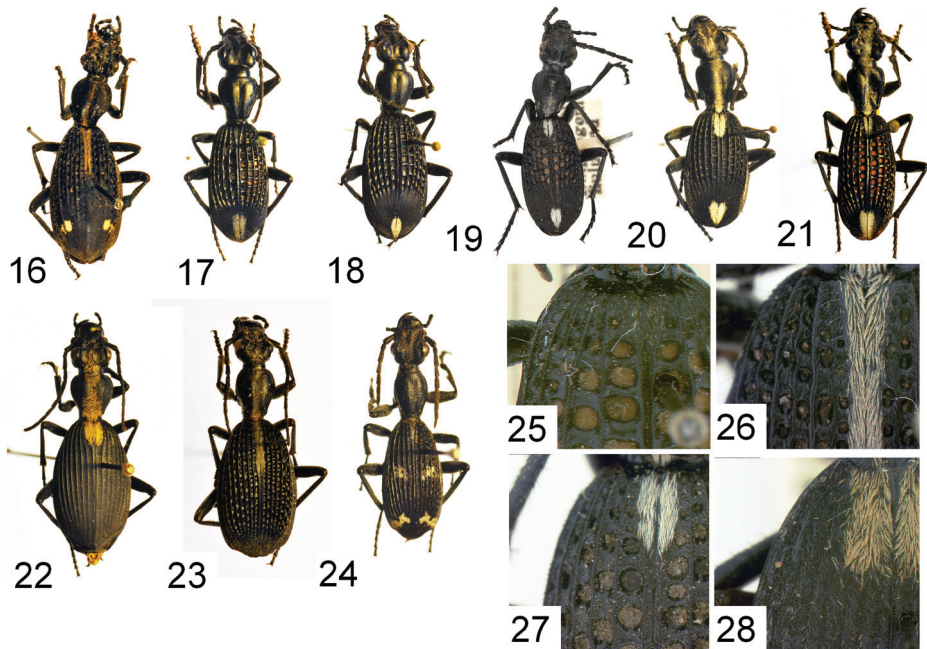
Figs 17, 32

Carabus macilentus Olivier (1795:26, pl. 11 f. 130, type locality “Cap de Bonne-Espérance,” holotype in Museum National d’Histoire Naturelle, Paris)

Polyhirma macilenta (Olivier) Péringuey (1896:348)

Cypholoba macilenta (Olivier) Strohmeyer (1928:335)

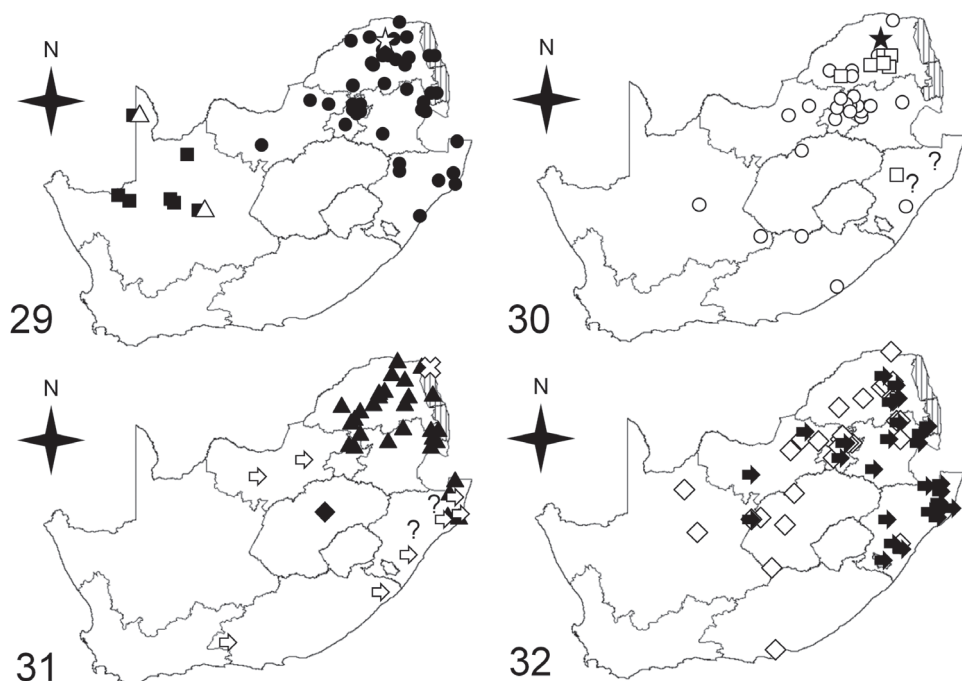
Diagnosis. Apparent body length (ABL) 20–23 mm; similar to *C. notata* and *C. oberthueri* but differing from both species in the length of the elytral costae. The elytral costae are roughly equal in length in *C. macilenta* (see Fig. 17) while the lateral costae



Figures 16–28. Adult specimens and diagnostic features of *Cypholoba* species, from the TMSA collection unless otherwise indicated **16** *C. leucospilota semilaevis* (Chaudoir), male, “E. Zululand,” KwaZulu/Natal Province, RSA **17** *C. macilenta* (Olivier), male, Groenkloof, Gauteng Province, RSA **18** *C. notata* (Perroud), male, Shilouvane, Limpopo Province, RSA **19** *C. notata* (Perroud), male, Empangeni, KwaZulu/Natal Province, RSA (NMNH collection) **20** *C. notata* (Perroud), male, Pongola River, KwaZulu/Natal Province, RSA **21** *C. oberthueri servana* Strohmeier, male, Allemanskraal, Free State Province, RSA **22** *C. opulenta* (Boheman), male, Us Pass, Khomas Hochland, Namibia **23** *C. rutata* (Péringuey), male, Pafuri, Kruger National Park, Limpopo Province, RSA **24** *C. tenuicollis aenigma* (Dohrn), Zimbabwe **25** base of elytra showing punctures, *C. alveolata* **26** base of elytra showing punctures, *C. graphipteroides graphipteroides* **27** base of elytra showing punctures, *C. notata* **28** base of elytra showing punctures, *C. opulenta*.

are distinctly longer than those on the disc in *C. notata* and *C. oberthueri* (see Figs 18–21). Adults could potentially also be confused with *C. amatonga* but that species lacks the patch of white pubescence at the apex of the elytra and the apical portion of the elytra is more rugosely punctate, not flat and strongly shining as in *C. macilenta*.

Materials examined. 127 specimens from the following localities: RSA: Eastern Cape Province: De la Rey. Free State Province: Boshof, Bothaville, H. F. Verwoerd Dam, Krugersdrift Dam. Gauteng Province: Bronkhorstspuit, Groenkloof, Johannesburg, Moloto, Muldersdrift, Pretoria, Randfontein, Rosslyn, Valhalla, Zoutpan. KwaZulu/Natal Province: Waterval. Limpopo Province: Messina, Moordrift, Pienaars River, Pietersburg, 20–26 km NE Pietersburg, Shilouvane, Woodbush. Mpumalanga Province: Lydenburg, Middelburg, 14 miles E Middelburg, Waterval Onder. Northern Cape Province: Kimberley, Niekerk’s Hope in Griqualand West, Vanwyksfontein Farm. North West Province: Lichtenburg, Marico, Rustenburg.



Figures 29–32. Maps showing distributions of *Cypholoba* species in RSA **29** black squares for *C. alstoni*, black circles for *C. alveolata*, white star for *C. amatonga*, white triangles for *C. fritschi* **30** white circles for *C. gracilis gracilis*, black star for *C. gracilis scrobiculata*, question marks (indeterminate localities) for *C. gracilis zuluana*, white squares for *C. tenuicollis aenigma* **31** black triangles for *C. graphipteroides graphipteroides*, question marks (indeterminate localities) for *C. leucospilota semilaevis*, black diamond for *C. oberthueri seruana*, white arrow for *C. opulenta*, white X for *C. rutata* **32** white diamonds for *C. macilenta*, black arrows for *C. notata*.

Cypholoba notata (Perroud, 1846)

http://species-id.net/wiki/Cypholoba_notata

Figs 18, 19, 20, 27, 32

Anthia notata Perroud (1846:50–56, type locality “de l’ intérieur de Natal,” syntypes in Museum National d’Histoire Naturelle, Paris)

Polyhirma notata (Perroud) Péringuey (1896:348)

Cypholoba semisuturata notata (Perroud) Strohmeyer (1928:337)

Cypholoba notata (Perroud) Strohmeyer (1928:452)

Diagnosis. Apparent body length (ABL) 21–23 mm; elytral surface sculpture distinctive with the entire apical third lacking the rows of punctures and costae found on the more basal portions of the elytra. The lateral elytral costae are longer than the costae on mid-disc or the suture. The patterns of pubescence vary in this species; some specimens possess white linear bands of setae on the head, pronotum, and base of the elytra while



Figure 33. Riverine/riparian area along the south bank of the Sabie River west of Paul Kruger Gate in the Kruger National Park, RSA. A collecting site for *Cypholoba graphipteroides graphipteroides*.

other specimens lack these bands (Figs 14, 15). We have also examined highly abraded specimens that lack all of the elytral and pronotal setae but in these specimens the elytral surface sculpturing is still diagnostic.

Materials examined. 96 specimens from the following localities: RSA: Gauteng Province: Malvern, Pretoria. KwaZulu/Natal Province: Empangeni, 30 miles N of Empangeni, Hluhluwe, Maritzburg, Mkuze, “Natal,” Ntambanana, Pinetown, Pongola, Pongola River, St. Lucia Bay, Thorny Bush, Ubombo Mountains, Weenen, “Zululand,” “E. Zululand.” Limpopo Province: Koedoes Rivier, Sedula near Leydsdorp, Shilouvane, Thabina, Zoutpansberg. Mpumalanga Province: Barberton, Grootdraai, Lydenburg, Nelspruit, Sabie River bank west of Paul Kruger Gate, Waterval River Pass. Northern Cape Province: Kimberley. North West Province: Marico River, Vryburg.

Notes on biology. We observed adults of this species running along dirt roads in bright sunshine through open *Combretum apiculatum* – *Acacia nigrescens* savanna in close proximity to the Sabie River in the Kruger National Park, RSA.

***Cypholoba oberthueri seruana* Strohmeier, 1928**

http://species-id.net/wiki/Cypholoba_oberthueri_seruana

Figs 21, 31

Cypholoba semisuturata seruana Strohmeier (1928:337, type locality “Ost-Betschuana-land,” one syntype from “Sogosse” the other syntype from “Serue” in Museum für Naturkunde, Berlin)



Figure 34. Upland *Acacia nigrescens* – *Combretum apiculatum* savanna near Skukuza in the Kruger National Park, RSA. A collecting site for *Cypholoba alveolata*.

Diagnosis. Apparent body length (ABL) 21–24 mm; similar in appearance to *C. macilentata* and *C. notata* but differing from both species in the length of the elytral costae (as noted in the key) and the elytral proportions which are somewhat longer and narrower in *C. o. seruana* than in the other two species. This is apparently a rare form, at least in collections; it is known at present from two localities in Botswana and the nominate subspecies is known only from Zimbabwe (Strohmeyer 1928).

Materials examined. 1 specimen from the following locality: RSA: Free State, Allemanskraal.

***Cypholoba opulenta* (Boheman, 1860)**

http://species-id.net/wiki/Cypholoba_opulenta

Figs 22, 28, 31

Polyhirma opulenta Boheman (1860:9, type locality “juxta fluvium Svakop,” holotype in Naturhistoriska Riksmuseet, Stockholm)

Cypholoba opulenta (Boheman) Strohmeyer (1928:367)

Diagnosis. Apparent body length (ABL) 18–24 mm; easily recognized by the golden-yellow pubescence on the head, pronotum, and base of elytra adjacent to the suture (Fig. 22). The elytral surface sculpturing is also diagnostic, with small narrow costae separated by flat intervals with a single row of minute punctures in each interval (Fig. 28).

Materials examined. 45 specimens from the following localities: Eastern Cape Province: Nduma, Willowmore. KwaZulu/Natal Province: Maritzburg, Melmoth, Mkuze, St. Lucia, “E. Zululand.” North West Province: Lichtenburg, 30 km W Vryberg.

***Cypholoba rutata* (Péringuey, 1892b)**

http://species-id.net/wiki/Cypholoba_rutata

Figs 23, 31

Polyhirma rutata Péringuey (1892b:101, type locality “Zambeze,” syntype series in South African Museum, Cape Town)

Cypholoba divisa rutata (Péringuey) Strohmeyer (1928:359–360)

Cypholoba rutata (Péringuey) Strohmeyer (1928:453)

Diagnosis. Apparent body length (ABL) 22–27 mm; similar in body proportions and surface sculpturing to *C. graphipteroides* and *C. leucospilota* but lacking the oblique bands of white setae and pubescence that are found at the apical third of the elytra in those species (see Figs 11–16).

Materials examined. 1 specimen from the following locality: RSA: Limpopo Province: Pafuri in Kruger National Park.

***Cypholoba tenuicollis aenigma* (Dohrn, 1881)**

http://species-id.net/wiki/Cypholoba_tenuicollis_aenigma

Figs 24, 30

Anthia aenigma Dohrn (1881:326, type locality not indicated, type material originally in Natural History Museum, Stettin, and apparently destroyed in World War II)

Polyhirma aenigma (Dohrn) Péringuey (1896:343–344, pl. 4 f. 6)

Cypholoba gracilis aenigma (Dohrn) Strohmeyer (1928:303)

Cypholoba aenigma (Dohrn) Strohmeyer (1928:447)

Diagnosis. Apparent body length (ABL) 15–17 mm; similar in size, body proportions, and surface sculpturing to smaller individuals of *C. gracilis* but easily separated from that species by its patterned elytral pubescence (Fig. 19) and smaller body size (adults of all subspecies of *C. gracilis* are 17–22 mm in ABL). Adults are also superficially similar to those of species in the genus *Eccoptyptera* Chaudoir but in species of that genus the pronotum is broadly rounded and markedly convex (Péringuey 1896; Strohmeyer 1928).

Materials examined. 84 specimens from the following localities: RSA: KwaZulu/Natal Province: Entabeni. Limpopo Province: Haenertsburg, Moddernek, Pietersburg, Shilouvane, Woodbush, Wylie’s Poort.

Acknowledgements

For assistance with the fieldwork associated with this project, we thank F. Venter, V. Ndlovu, J. Baloyi, O. Sithole, P. Khoza, A. Manganyi, and T. Khoza of South African National Parks. For additional field assistance we thank R. D. and J. L. Mawdsley, as well as J. du G. Harrison and his family. For assistance with visits to museum collections, we thank A. Newton and M. Thayer (FMNH), G. Zambatis (KNPC), R. Staals and B. Grobbelaar (SANC), and J. du G. Harrison and Ruth Müller (TMSA). For assistance with additional specimen imaging and image editing, we thank K. Darrow (NMNH). For comments on the manuscript during the review process, we thank A. Casale as well as two anonymous reviewers.

References

- Ball GE (1972) Classification of the species of *Harpalus* subgenus *Glanodes* Casey (Carabidae: Coleoptera). *Coleopterists Bulletin* 26: 179–204.
- Basilewsky P (1948) Descriptions de Coleoptères Carabiques nouveaux d'Afrique et notes diverses sur des espèces déjà connues, III. *Bulletin de la Société Entomologique de France* 53: 107–111.
- Basilewsky P (1955) Notes sur les Cypholobini (Col. Carabidae Anthiinae), I. *Bulletin de l'Institut Royal des Sciences naturelles de Belgique* 31(25): 1–8.
- Basilewsky P (1963) Notes sur les Cypholobini (Col. Carabidae Anthiinae), II. *Bulletin et Annales de la Société Royale d'Entomologie de Belgique* 99(10): 150–158.
- Basilewsky P (1964) Notes sur les Cypholobini (Col. Carabidae Anthiinae). III. Sur les *Cypholoba* du groupe *divisa*. *Bulletin et Annales de la Société Royale d'Entomologie de Belgique* 100(11): 145–153.
- Basilewsky P (1967) Coleoptères Carabidae africains nouveaux 16. *Revue de Zoologie et de Botanique Africaines* 75: 319–330.
- Basilewsky P (1980) Note sur les Cypholobini 4 (Coleoptera Carabidae Anthiinae). *Revue de Zoologie Africaines* 94(2): 489–503.
- Basilewsky P (1983) Note sur les Cypholobini 5 (Coleoptera Carabidae Anthiinae). *Revue de Zoologie Africaines* 97(3): 563–566.
- Bertoloni G (1847) Sezione di zoologia anatomia comparata e fisiologia. *Diario dell'ottavo Congresso degli scienziati Italiani convocati in Genova 1847*: 90–91.
- Boheman CH (1860) Coleoptera samlade af J. A. Wahlberg i Syd-Vestra Afrika. *Öfversigt af Kongliga Vetenskaps-Akademien Föreläsningar* 17(1): 3–22.
- Brême F le Marquis de (1844) Insectes Coleoptères nouveaux ou peu connus, première et deuxième décades. *Annales de la Société Entomologique de France, Ser. 2* 2: 287–313 + pls. 7–9.
- Chaudoir M Baron de (1850) Mémoire sur la famille des Carabiques, 2^e partie. *Bulletin de la Société Impériale des Naturalistes de Moscou* 23(2): 3–196.

- Chaudoir M Baron de (1861) Matériaux pour servir à l'étude des Cicindélètes et des Carabiques. Bulletin de la Société Impériale des Naturalistes de Moscou 34(2): 491–576.
- Chaudoir M Baron de (1883) Descriptions de Carabiques nouveaux. Coleopterorum Novitates 1: 17–39.
- Csiki E (1929) Carabidae: Harpalinae III. In: Schenkling S (Ed), Coleopterorum Catalogus, Pars 104. W. Junk, Berlin, 347–528.
- Dejean PFMA (1831) Species général des Coléoptères de la Collection de M. le Baron Dejean, Tome V. Mequignon-Marvis Père et Fils, Paris, viii + 883 pp.
- Dohrn CA (1881) Exotisches. Entomologische Zeitung [Stettin] 42: 309–327.
- Erwin TL, Johnson PJ (2000) Naming species, a new paradigm for crisis management in taxonomy: Rapid journal validation of scientific names enhanced with more complete descriptions on the internet. The Coleopterists Bulletin, 54(3): 269–278. doi: 10.1649/0010-065X(2000)054[0269:NSANPF]2.0.CO;2
- Erwin TL, Kavanaugh DH (1981) Systematics and zoogeography of *Bembidion* Latreille: I. The *carlhi* and *erasum* groups of western North America (Coleoptera: Carabidae, Bembidiini). Entomologica Scandinavica, Supplement 15: 33–72.
- Erwin TL, Kavanaugh DH (1991) The tribe Cicindini Banninger (Coleoptera: Carabidae): comparative morphology, classification, natural history, and evolution. Proceedings of the Entomological Society of Washington 93: 356–389.
- Gertenbach WPD (1983) Landscapes of the Kruger National Park. Koedoe 26: 9–121.
- Gistel J (1857) Achthundert und zwanzig neue oder unbeschriebene wirbellose thiere. Schöner'sche Buchhandlung, Straubing, 94 pp.
- Guérin-Méneville FE (1845) Description de quelques-uns des Insectes les plus remarquable découverts par M. A. Delegorgue dans les pays des Boschimans, des Ama Zoulous, des Massilicatz et au Port Natal, pendant les années 1838, 39, 40, 41, 42, 43 et 44. Revue Zoologique, par la Société Cuvierienne 18: 283–286.
- Kavanaugh DH (1979) Studies on the Nebriini (Coleoptera: Carabidae), III. New Nearctic *Nebria* species and subspecies, nomenclatural notes, and lectotype designations. Proceedings of the California Academy of Sciences, Ser. 4 42: 87–133.
- Kotze DJ (2000) Ground beetle diversity patterns across Afromontane forest/grassland ecotones in KwaZulu-Natal, South Africa. In: Brandmayr, P, Lövei GL, Zetto Brandmayr T, Casale A, and Vigna – Taglianti A (Eds) Natural history and applied ecology of carabid beetles. Pensoft, Sofia, 231–240.
- Lorenz W (2005) Nomina Carabidarum, a directory of the scientific names of ground beetles (Insecta, Coleoptera “Geadephaga”: Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae), second edition. Lorenz, Tutzing, 993 pp.
- Magagula CN (2003) Changes in carabid beetle diversity within a fragmented agricultural landscape. African Journal of Ecology 41: 23–30. doi: 10.1046/j.1365-2028.2003.00403.x
- Marshall GAK, Poulton EB (1902) Five years' observations and experiments (1896–1901) on the bionomics of South African insects, chiefly directed to the investigation of mimicry and warning colors. Transactions of the Entomological Society of London 1902: 287–584 + pl. 9–23.

- Mawdsley JR (2009) Taxonomy, ecology, and phylogeny of species of *Lophyra* Motschulsky 1859, subgenus *Eriolophyra* Rivalier 1948 (Coleoptera: Cicindelidae). *Tropical Zoology* 22: 57–70.
- Mawdsley JR (2011) Taxonomy, identification, and phylogeny of the African and Madagascan species of the tiger beetle genus *Chaetodera* Jeannel 1946 (Coleoptera: Cicindelidae). *Insecta Mundi* 0191: 1–13.
- Mawdsley JR, Sithole H (2008) Dry season ecology of riverine tiger beetles in Kruger National Park, South Africa. *African Journal of Ecology* 46(2): 126–131. doi: 10.1111/j.1365-2028.2007.00820.x
- Mawdsley JR, Sithole H (2009) Natural history of the African riverine tiger beetle *Chaetodera regalis* (Dejean) (Coleoptera: Cicindelidae). *Journal of Natural History* 43: 1891–1908. doi: 10.1080/00222930903015816
- Mawdsley JR, Sithole H, Mawdsley AS (2011) Peaceful giant ground beetles: The genus *Tefflus* Latreille (Coleoptera: Carabidae) in the Republic of South Africa. *Insecta Mundi* 0181: 1–7.
- Olivier GA (1795) *Entomologie, ou histoire naturelle des insectes, avec leurs caractères génériques et spécifiques, leur description, leur synonymie, et leur figure enluminée, Coléoptères, tome troisième*. De l’Imprimerie de Lanneau, Paris, 557 pp.
- Péringuey L (1892a) Third contribution to the South-African coleopterous fauna; On beetles collected in tropical south-west Africa by Mr. A. W. Eriksson. *Transactions of the South African Philosophical Society* 6: 1–94.
- Péringuey L (1892b) Fourth contribution to the South African coleopterous fauna: Description of new Coleoptera in the South African Museum. *Transactions of the South African Philosophical Society* 6: 95–136. doi: 10.1080/21560382.1889.9526257
- Péringuey L (1896) Descriptive catalogue of the Coleoptera of South Africa, part II. *Transactions of the South African Philosophical Society* 7: 99–623.
- Perroud BP 1846 Description de quelques Coléoptères nouveaux ou peu connus (Tribu des Carabiques, famille des Truncatipennes, section des Anthiaires). *Annales de la Société Linéenne de Lyon* 2(1): 25–64.
- Schmidt AD (2001) Experimentelle und freilandökologische Untersuchungen zu Aktivitätsrhythmik und mikroklimatischem Präferenzverhalten ausgewählter afrikanischer Laufkäferarten der Gattung *Anthia* und *Thermophilum*. *Mitteilungen des Internationalen Entomologischen Vereins Frankfurt* 26(1/2): 53–84.
- Strohmeyer G (1928) Systematisches und Zoogeographisches über die Cypholobini (Carab. Anthiinae), ein Beitrag zur Kenntnis der Fauna des afrikanischen Trockenwaldes. *Mitteilungen aus dem Zoologischen Museum in Berlin* 14(2): 287–462 + 17 pls.

First report of *Melittobia australica* Girault in Europe and new record of *M. acasta* (Walker) for Italy

Antonino Cusumano¹, Jorge M. González², Stefano Colazza¹, S. Bradleigh Vinson²

¹ Dipartimento DEMETRA, Università degli Studi di Palermo, Viale delle scienze, 90128 Palermo, Italy

² Texas A & M University, Department of Entomology, College Station, Texas 77843-2475, USA

Corresponding author: Jorge M. González (gonzalez.jorge.m@gmail.com)

Academic editor: N. Johnson | Received 26 January 2012 | Accepted 26 March 2012 | Published 6 April 2012

Citation: Cusumano A, González JM, Colazza S, Vinson SB (2012) First report of *Melittobia australica* Girault in Europe and new record of *M. acasta* (Walker) for Italy. ZooKeys 181: 45–51. doi: 10.3897/zookeys.181.2752

Abstract

Melittobia acasta and *M. australica* are newly recorded from Sicily, Italy, and the second species is reported in Europe for the first time. A short historical background about *Melittobia* parasitoid wasps, their hosts, and distribution, with emphasis in those two species is presented together with illustrations to facilitate their identification. Brief discussion about the presence and possible distribution of the species in Sicily is also included.

Keywords

Parasitoid wasp, *Sceliphron spirifex*, *Osmia* sp., Sicily, Europe

Introduction

Melittobia Westwood is a cosmopolitan genus of gregarious ectoparasitoids that primarily attack prepupae of aculeate Hymenoptera, but are also able to parasitize a wide range of hosts from the orders Coleoptera, Diptera and Lepidoptera (Dahms 1984b; González et al. 2004; Matthews et al. 2009). They are frequently associated with mud-dauber wasps (*Sceliphron* spp. and *Trypoxylon* spp.) but some species are frequently found parasitizing several bee species (i.e. *Anthidium*, *Anthophora*, *Apis*, *Bombus*, *Ceratina*, *Chalicodoma*, *Heriades*, *Megachile*, *Osmia*, *Pythirus*, *Stelis*) (González and Terán 1996; González et al. 2004; Maeta 1978; Matthews et al. 2009).

Twelve species are known in the genus (Matthews and González 2008; Matthews et al. 2009). All *Melittobia* species exhibit extreme intrasexual and intersexual polymorphism, where males are blind, and brachypterous, and have highly modified antennae. Females are either brachypterous, and emerge from the pupae containing a large batch of eggs and are ready to mate, or macropterous, and capable of greater dispersal (Cusumano et al. 2010; González and Matthews 2008; Matthews and González 2008; Matthews et al. 2009; Schmiedeknecht 1933). Of these, *Melittobia acasta* (Walker), has been known as the only Eurasian species of *Melittobia* but it occurs widely in other regions of the world (Summarized by González et al. 2004). It has been reported as a dangerous threat to honeybees and/or solitary bees used as crop pollinators (See González and Matthews 2005).

Melittobia australica Girault, was described on the basis of three males and ten females that emerged from *Pison spinolae* Shuckard (Hymenoptera: Sphecidae) (Girault 1912). This parasitoid is a nearly cosmopolitan species that has been reported from Australia and New Zealand, several countries of Africa, Asia, North, Central and South America, including a few Caribbean islands/countries (Assem et al. 1982; Dahms 1984a; González and Matthews 2005; González and Terán 1996; González et al. 2008; Maeta 1978).

Here we confirm the presence of two *Melittobia* species (*M. australica* and *M. acasta*) in Italy and provide the first record of *M. australica* from Europe.

Materials and methods

Collections of trap nests and mud dauber nests were carried out during 2010 in western Sicily. Trap nests were built by pooling together about 10 pieces ($\varnothing=2-3$ cm; length=20 cm) of reed (*Arundo donax* L.) that were hung on trees located both in cultivated and uncultivated fields. Nests of mud dauber wasps were collected mainly from external walls of buildings especially in areas close to water sources.

A number of *Melittobia* wasps emerged from trap nests colonized by *Osmia* bees originally placed in the Palermo University campus (38°06'26"N, 13°21'07"E). The collected parasitoids were mounted and later identified.

One of several mud dauber nests constructed by *Sceliphron spirifex* L. (Hymenoptera: Sphecidae) collected in the town of Contessa Entellina (province of Palermo) (37°44'23"N, 13°08'27"E), had a cell containing a prepupae of its host parasitized with *Melittobia* wasps. Some were collected and placed on calliphorid pupae (*Calliphora* sp., Diptera: Calliphoridae) to be reared, while the rest were studied and identified. The parasitoids that emerged from these cultures were studied, identified and counted (Table 1).

A thorough review of literature was done in order to corroborate that the identified species indeed constituted a new record for the country. We were unable to review other insect collections besides the one at Università degli Studi di Palermo, and that of an amateur entomologist in Palermo. Voucher specimens of both parasitoid species

Table 1. *Melittobia australica* Girault emerged from six pupae of *Calliphora* sp., under laboratory conditions (25°C; 75% RH) at Dipartimento DEMETRA, Università degli Studi di Palermo, Sicily, Italy

Host number	Males	Brachypterous females	Macropterous females	Larvae/Pupae*
1	3	1	60	20
2	2	3	45	28
3	2	1	11	82
4	1	0	5	89
5	4	0	12	68
6	3	1	68	18
Total	15	6	201	305
Mean	2.5	1	33.5	50.83

* These larvae/pupae died during development.

and the hosts are deposited at the TAMU insect collection, College Station, Texas, USA and at Palermo University, Palermo, Sicily, Italy.

Results and discussion

Four males and 63 macropterous females of *Melittobia acasta* were collected (and mounted) from a cell of *Osmia* sp. inside a trap nest.

A total of one male and 376 females of *M. australica* emerged from a *Sceliphron spirifex* prepupa. It is worth noting that the factitious host *Calliphora* pupae appear to be highly nutritious and large allowing the development of brachypterous females (Table 1). From six cultures established using *Calliphora* pupae (four females/fly pupae) as hosts, fifteen males of *M. australica*, as well as six brachypterous females, 201 macropterous females and 305 pupae/larvae emerged (see Table 1).

The easiest way to separate *Melittobia acasta* and *M. australica* is by examining male specimens under a magnifying glass (10×–20×) or a microscope. Males of *M. acasta* are 1.3–1.5 mm long, light brown or amber in color (Figure 1a); antennal scape has a cup-shaped depression, the distal region of the scape is strongly oblique with a broad excavation (Figure 2a) (Dahms 1984a; González and Terán 1996). Males of *M. australica* are 1.1–1.3 mm long, light amber in color and have an antennal scape with a deep ventral longitudinal groove (Figures 1b, 2b) (Dahms 1984a; González and Terán 1996). In order to identify species using females we recommend following Dahms (1984a).

Even though the North American species *Melittobia chalybii* Ashmead was once mentioned in an European country (Denmark) by Holm (1960), it was later clarified that the species was actually *M. acasta* (Holm and Skou 1972; González and Matthews, 2005). Otherwise, all reports we had been able to find of the presence of *Melittobia* in European countries, indicate that the species found was always *M. acasta* (González and Matthews 2005; González et al. 2004). Thus it was not surprising that the first

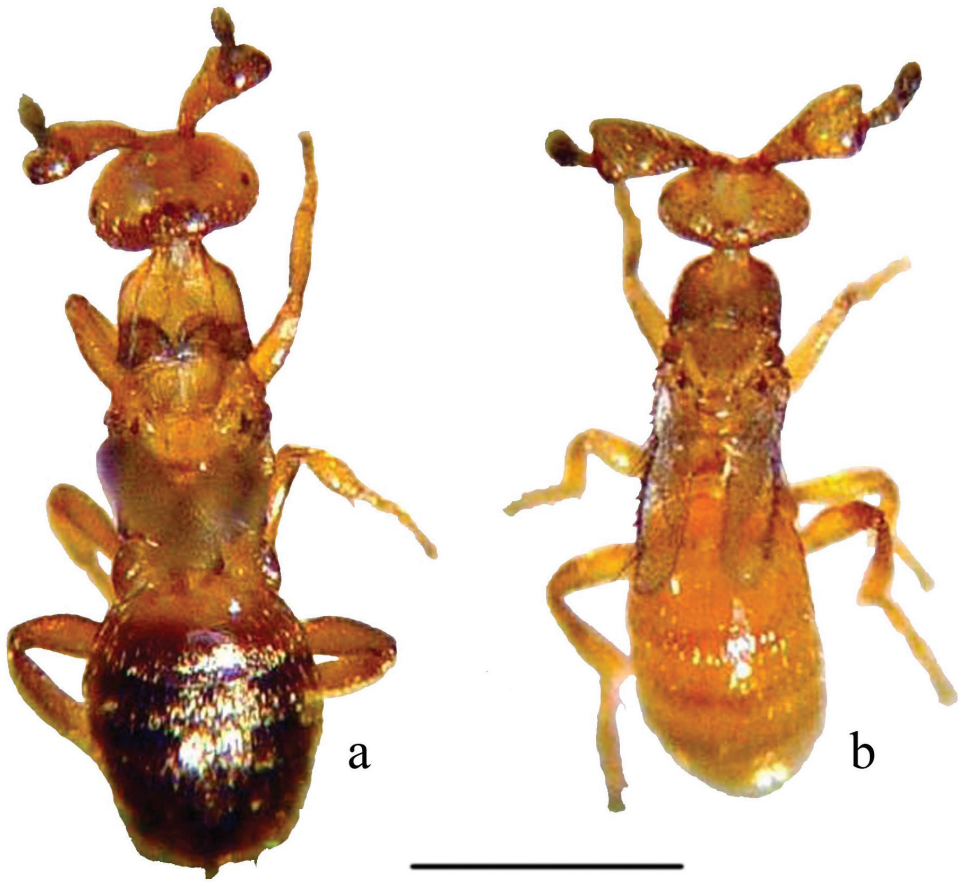


Figure 1. Males of *Melittobia* parasitoid wasps: **a** *M. acasta* **b** *M. australica*. Scale: 0.5 mm

Melittobia we encountered in Sicily was *M. acasta*. Since the species was encountered in Palermo, a major port-city in the island, we might suspect that the species found its way to Sicily through the many ships that come from many lands to this place. Since *M. acasta* is widely distributed in Europe and Asia, we might even speculate that its invasion of Sicily could have occurred centuries ago.

Even though *Melittobia australica* is a nearly cosmopolitan species, it has never been reported from Europe until now. The finding of this species in the town of Contessa Entellina, located about 80-90 km from the port of Palermo raises an interesting question: how long has it been in Sicily? Since the place where we found *M. australica* in Sicily is in the interior of the island, we might suspect that the species is widely distributed on the island. It is important to note that several species of bees and wasps known from Sicily (Incalterra et al. 2003; Rasmont et al. 2008; Schmid-Egger 2003) are suitable hosts for both *Melittobia* wasps found, helping them to easily establish on the island.

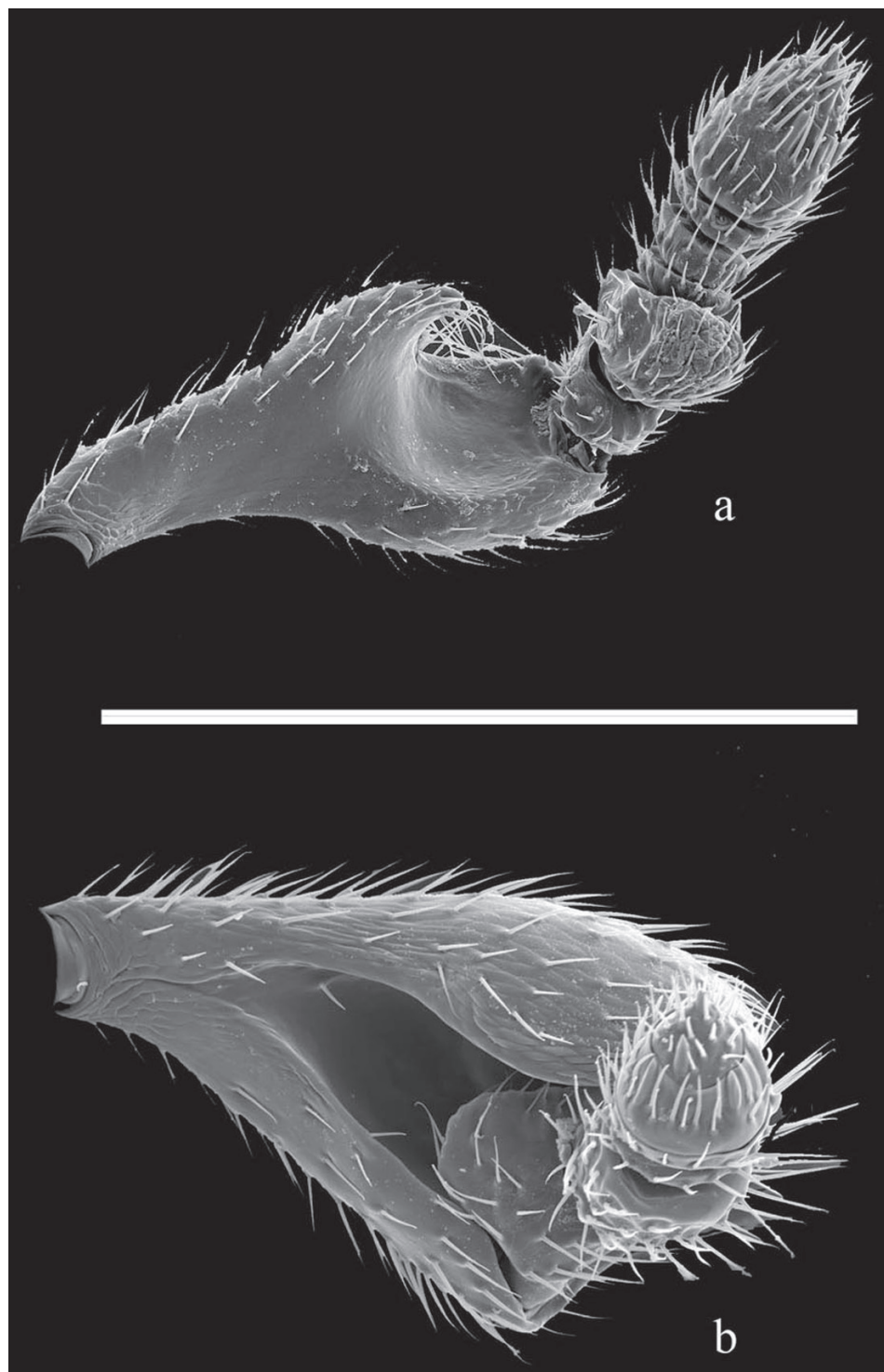


Figure 2. Male antennae of *Melittobia acasta* **a** and *M. australica* **b**. Scale: 0.25 mm.

Acknowledgements

We would like to thank Angela Sinacori (Università degli Studi di Palermo, Sicily, Italy) who kindly gave us useful suggestions about collecting *Osmia* bees and their parasitoids by using trap nests. Thanks also to Carlo Polidori (Museo Nacional de Ciencias Naturales, Madrid, Spain) for identification of *S. spirifex*. We are also indebted to Freder Medina for the SEM photographs of the antennae of both *Melittobia* species that appear in Figure 2. We greatly appreciate the thoughtful comments and suggestions given by Bob Matthews, and an anonymous reviewer, which greatly improved the submitted manuscript.

References

- Assem J van den, Bosch HAJ in den, Prooy E (1982) *Melittobia* courtship behaviour: A comparative study of the evolution of a display. Netherlands Journal of Zoology 32(4): 427–471. doi: 10.1163/002829682X00184
- Cusumano A, González JM, Colazza S, Vinson SB (2010) Behavioral responses of the parasitoid *Melittobia digitata* to volatiles emitted by its natural and laboratory hosts. Entomologia Experimentalis et Applicata 136: 301–307.
- Dahms EC (1984a) Revision of the genus *Melittobia* (Chalcidoidea: Eulophidae) with the description of seven new species. Memoirs of the Queensland Museum 21(2): 271–336.
- Dahms EC (1984b) A review of the biology of species in the genus *Melittobia* (Hymenoptera: Eulophidae) with interpretations and additions using observations on *Melittobia australica*. Memoirs of the Queensland Museum 21(2): 337–360.
- González JM, Matthews RW (2005) An annotated bibliography of *Melittobia* (Hymenoptera: Eulophidae). Caribbean Journal of Science, Special Publication 8: 1–41.
- González JM, Matthews RW (2008) Female and male polymorphism in two species of *Melittobia* parasitoid wasps (Hymenoptera: Eulophidae). Florida Entomologist 91(2): 162–169. doi: 10.1653/0015-4040(2008)91[162:FAMPIT]2.0.CO;2
- González JM, Terán JB (1996) Parasitoides del género *Melittobia* Westwood (Hymenoptera: Eulophidae) en Venezuela. Distribución y hospederos. Boletín de Entomología Venezolana (N.S.) 11(2): 139–147.
- González JM, Matthews RW, Vinson SB (2008) Distribution and host records of *Melittobia* (Hymenoptera: Eulophidae) from Mexico. Revista Mexicana de Biodiversidad 79: 529–531.
- González JM, Terán JB, Matthews RW (2004) Review of the biology of *Melittobia acasta* (Walker) (Hymenoptera: Eulophidae). Caribbean Journal of Science 40(1): 52–61.
- Holm SN (1960) Experiments on the domestication of humble bees (*Bombus* Latr.), in particular *B. lapidarius* and *B. terrestris* L. Royal Veterinary and Agricultural College of Copenhagen, Yearbook 1960: 1–19.
- Holm SN, Skou JP (1972) Studies on trapping, nesting, and rearing of some *Megachile* species (Hymenoptera, Megachilidae) and on their parasites in Denmark. Entomologica Scandinavica 3(3): 169–180. doi: 10.1163/187631272X00274

- Incalterra G, Iapichino G, D'Anna F, Sinacori A (2003) Influences of different pollinators on winter melon Brown under polyethylene tunnel. *Acta Horticulturae* 614: 297–299.
- Maeta Y (1978) A preliminary study on the physical control of *Melittobia acasta* (Walker) by cold treatment (Hymenoptera: Eulophidae). *Bulletin of the Tohoku National Agriculture Experiment Station* 58: 211–229.
- Matthews RW, González JM (2008) The *acasta* conundrum: polymorphism and taxonomic confusion within the parasitoid genus *Melittobia* (Hymenoptera: Eulophidae). *Zootaxa* 1854: 45–54.
- Matthews RW, González JM, Matthews JR, Deyrup LD (2009) Biology of the parasitoid *Melittobia* (Hymenoptera: Eulophidae). *Annual Review of Entomology* 54: 251–266. doi: 10.1146/annurev.ento.54.110807.090440
- Rasmont P, Coppée A, Michez D, Meulemeester T de (2008) An overview of the *Bombus terrestris* (L. 1758) subspecies (Hymenoptera: Apidae). *Annales de la Société Entomologique de France (nouvelle série)* 44(1): 243–250.
- Schmid-Egger C (2003) New records of “Sphecidae” (Hymenoptera: Sphecidae & Crabronidae) from Sicily (Italy) and Malta. *Linzer Biologische Beiträge* 35(2): 747–762.
- Schmieder RG (1933) The polymorphic forms of *Melittobia chalybii* Ashmead and the determining factors involved in their production (Hymenoptera: Chalcidoidea, Eulophidae). *Biological Bulletin of the Marine Biological Laboratory, Woods Hole* 65: 338–352. doi: 10.2307/1537183

Sinocorophium hangangense* sp. n. (Crustacea, Amphipoda, Corophiidae), a new species from Korea, with a key to the genus *Sinocorophium

Young-Hyo Kim^{1,2,†}

1 Canadian Museum of Nature, Research Services, P.O. Box 3443, Station D, Ottawa, Canada K1P 6P4

2 Department of Life Sciences, Dankook University, Cheonan, Korea 330-714

† urn:lsid:zoobank.org:author:1194DE92-AFFD-408D-8E09-FDC8EDA9BAC6

Corresponding author: Young-Hyo Kim (amphipod74@gmail.com)

Academic editor: C.O. Coleman | Received 2 March 2012 | Accepted 27 March 2012 | Published 6 April 2012

urn:lsid:zoobank.org:pub:FA92499B-AC11-4666-A530-5AF77C1CEC61

Citation: Kim Y-H (2012) *Sinocorophium hangangense* sp. n. (Crustacea, Amphipoda, Corophiidae), a new species from Korea, with a key to the genus *Sinocorophium*. ZooKeys 181: 53–65. doi: 10.3897/zookeys.181.3043

Abstract

A new species of the corophiid gammaridean amphipod belonging to the genus *Sinocorophium* Bousfield & Hoover was collected from the lower reaches of the Han River in Gyeonggi-do, Korea. A relatively large body size and morphology of the uropods 1 and 3 are the major characteristics which serve to distinguish the new species from its congeners. The new species is fully illustrated and extensively compared with related species. A key to the species of *Sinocorophium* is also provided.

Keywords

Amphipoda, Corophiidae, *Sinocorophium*, new species, Korea, key

Introduction

The genus *Sinocorophium* is usually found free-burrowing, in intertidal muddy substrata, from marine to brackish waters. To date, sinocorophiid amphipods are comprised of 10 species (Shen 1955, Bousfield and Hoover 1997) and almost all species reported are endemic to warm temperate and subtropical shallows of the Far East region such as China, Japan, Vietnam and Korea. Only one species, *S. alienense* Chapman, 1988 occurs in the northeast

Pacific (delta of San Francisco Bay). According to Chapman (1988), *S. alienense* was introduced from Vietnam through widespread ballast water traffic during the Vietnam War. The genus *Sinocorophium* is morphologically characterized by having uncoalesced urosomites, uropod 1 laterally inserted, peduncular article 2 of antenna 2 with a large gland cone, palp article 2 of maxilliped elongated and a rounded posteroventral corner of epimeron 3. This genus is divided into two groups, a relatively ancestral and more derived subgroup based on characteristics of antenna 2, gnathopod 1, pereopods 3–4 and uropods (Bousfield and Hoover 1997). The derived subgroup is comprised of 6 species: *S. homoceratum* (Yu, 1938), *S. monospinum* (Shen, 1955), *S. intermedium* (Dang, 1965), *S. triangulopedarum* (Hirayama, 1990) from China Sea, *S. japonicum* (Hirayama, 1984) from Japan, and *S. alienense* (Chapman, 1988) from California. In this paper I add one new Korean species *S. hangangense* sp. n., which is placed into the derived subgroup. Hitherto, only one species of the genus, *S. sinense* (Zhang, 1974) has been previously recorded in Korea, from intertidal oyster beds (Jung and Kim 2007). A key to the world *Sinocorophium* species is also given.

Material and methods

Specimens were collected with a hand-net from the mud substratum of Gongreung stream, Paju-si, Korea, where the brackish water region is influenced by the intertidal zone (Fig. 1). The specimens were fixed with 80% ethyl alcohol and dissected in glycerol on Cobb's aluminum hollow slide. Permanent mounts were made using polyvinyl lactophenol with lignin pink added. Drawings and measurements were performed with the aid of a drawing tube, mounted on an Olympus SZX 12 stereomicroscope and Olympus BX 51 interference contrast compound microscope. The body length was measured from the tip of rostrum to the end of the telson, along the dorsal parabolic line of the body. Type specimens are deposited at the National Institute

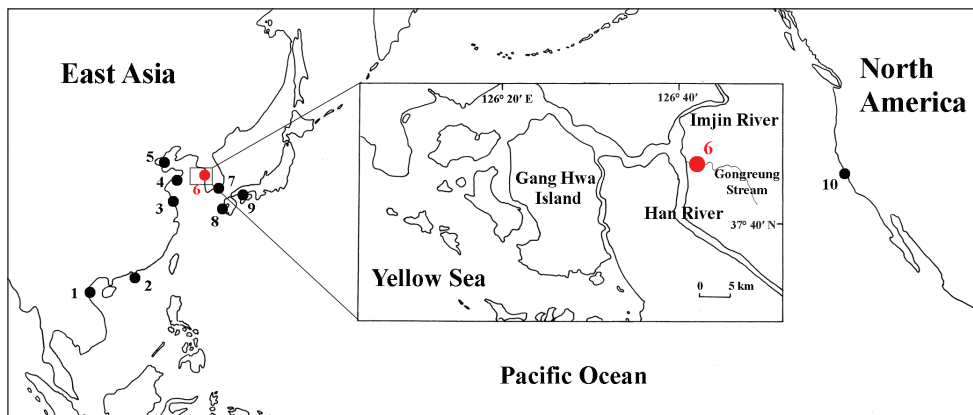


Figure 1. Distribution of world *Sinocorophium* species. 1 *S. minutum*, *S. intermedium* 2 *S. triangulopedarum* 3 *S. monospinum* 4 *S. sinense* 5 *S. heteroceratum*, *S. homoceratum* 6 *S. hangangense* sp. n. 7 *S. sinense* 8 *S. japonicum*, *S. lamellatum* 9 *S. sinense* 10 *S. alienense*.

of Biological Resources (NIBR), Incheon, Korea, Department of Biological Science, Dankook University (DKU), Cheonan, Korea and the Canadian Museum of Nature (CMN), Ottawa, Canada.

Taxonomy

Genus *Sinocorophium* Bousfield & Hoover, 1997

<http://species-id.net/wiki/Sinocorophium>

Type species. *Corophium sinensis* Zhang, 1974

Diagnosis. Body cylindrical. Rostrum vestigial to distinct. Antenna 2 strongly pediform, gland cone of peduncular article 2 large, prominent, peduncular article 4 with ventrodistal tooth. Maxilliped, palp article 2 elongate. Gnathopod 1, palm of propodus distinct and transverse, dactylus rather short. Pereopods 3–4, carpus not shortened, slightly shorter than merus. Pleonal epimeron 3 subquadrate or weakly pointed posteroventrally. Urosomites separate. Uropod 1 laterally inserted. Uropod 3 uniramous, ramus linear to subovate. Telson short and subtriangular.

Species composition. *Sinocorophium alienense* (Chapman, 1988), *S. hangangense* sp. n., *S. heteroceratum* (Yu, 1938), *S. homoceratum* (Yu, 1938), *S. intermedium* (Dang, 1965), *S. japonicum* (Hirayama, 1984), *S. lamellatum* (Hirayama, 1984), *S. minutum* (Dang, 1965), *S. monospinum* (Shen, 1955), *S. sinense* (Zhang, 1974), and *S. triangulopedarum* (Hirayama, 1990).

Sinocorophium hangangense sp. n.

urn:lsid:zoobank.org:act:F79F7494-083C-4E1E-96C7-324B1F712E43

http://species-id.net/wiki/Sinocorophium_hangangense

Korean name: Han-gang-baem-yeop-sae-u, new

Figs 2–5

Material examined. Holotype, adult male, 12.2 mm, NIBRIV0000245089, Gongreung stream, Songchon-ri, Gyoha-eup, Paju-si, Gyeonggi-do, Korea, 37°45'10"N, 126°42'20"E, C.M. Lee and Y.H. Kim, 2 November 2002. Paratype, adult female, 11.0 mm, NIBRIV0000245090, same station data; one male, 11.5 mm and one female, 10.5 mm, CMNC 2012-0002, same station data; three females, 8.7–10.2 mm, DKU 201203, same station data.

Additional material examined. 2 males, same locality as holotype, C.M. Lee, 30 September 2006; 2 males, 3 females, same locality as holotype, C.M. Lee, 4 November 2006; 5 males, 4 females, same locality as holotype, C.M. Lee, 3 May 2008.

Coloration in alcohol. Body yellowish grey; antennae to urosomites with light brownish reticulate pattern dorsally, especially pereonites with 2 longitudinal rows of light brown lines dorsally (Fig. 2).



Figure 2. *Sinocorophium hangangense* sp. n., male, 11.5 mm, Gongreung stream, Songchon-ri, Gyoha-eup, Paju-si, Korea. **A** lateral view **B** dorsal view; female, 8.5 mm, Gongreung stream, Songchon-ri, Gyoha-eup, Paju-si, Korea. **C** lateral view **D** dorsal view.

Description. Holotype, adult male, NIBRIV0000245089.

Body (Fig. 3A) 12.2 mm long, head longer than pereonite 1, rostrum (Fig. 3B) pointed distally, triangular in dorsal view. Eye invisible in alcohol. Cephalic lobe sharply produced. Pereonites 1–2 subequal in length, shorter than pereonite 3. Coxae flat dorsoventrally, except coxa 1, much shallower than pereonites. Urosomites 1–3 separate.

Antenna 1 (Figs. 3B, 3C) weakly setose, subequal in length to head and pereonites 1–4 combined; peduncular article 1 rectangular, distinctly narrowed distally, medial margin irregularly serrated when viewed dorsally, ventrodistal corner with 1 small robust seta and 1 penicillate seta, distal half of ventral margin with 8 setae; length ratio of peduncular articles 1–3 = 1.00 : 0.41 : 0.31; flagellum 13-articulate, shorter than peduncle, several articles bearing club-shaped, small aesthetascs ventrodistally.

Antenna 2 (Figs. 3A, 3D) massive, nearly twice as long as antenna 1; peduncular article 2 with large curved and sharply pointed gland cone; peduncular article 3 longer than wide; peduncular article 4 $1.23 \times$ article 5, with a row of tubercles ventromedially and a ventrodistal large tooth; peduncular article 5 rectangular, with a row of tubercles ventromedially; flagellum biarticulate, proximal one with a row of tubercles, $0.56 \times$ peduncular article 5, distal one short, about $0.2 \times$ proximal one, with 2 unequal setae apically.

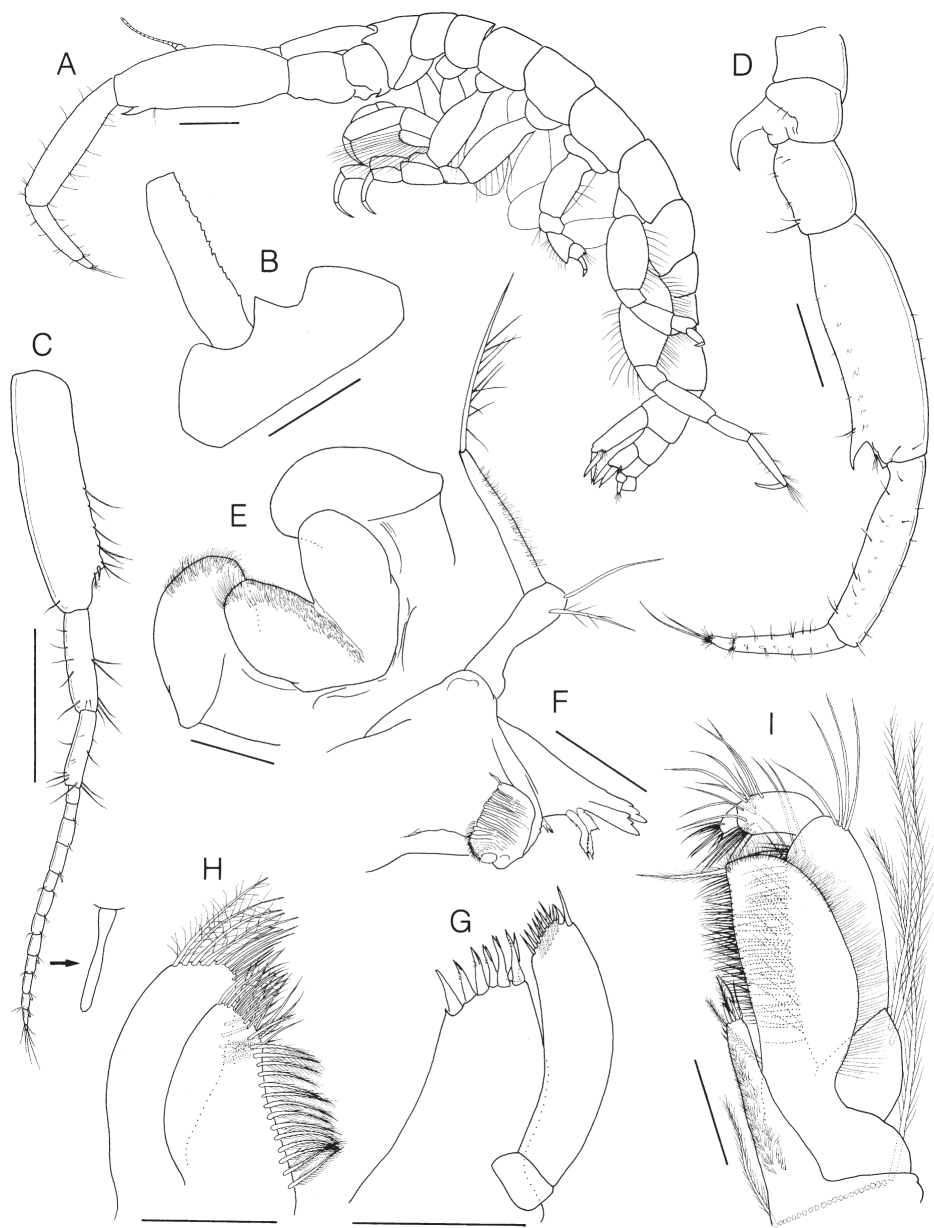


Figure 3. *Sinocorophium hangangense* sp. n., holotype, male, 12.2 mm, Gongreung stream, Songchon-ri, Gyoha-eup, Paju-si, Korea. **A** habitus, lateral **B** head, dorsal **C** antenna 1 **D** antenna 2 **E** lower lip **F** mandible **G** maxilla 1 **H** maxilla 2 **I** maxilliped. Scale bars: 1.0 mm (**A–D**), 0.2 mm (**E–I**).

Lower lip (Fig. 3E) inner lobe subovate, coalescent proximally, rounded apically; mandibular process small and blunt; both lobes covered with patch of pubescence medially.

Left mandible (Fig. 3F) well developed, incisor and lacinia mobilis produced inward, bluntly tridentate; accessory setal row with 2 curved, finely pectinate blades; molar well developed, massive, truncate; palp biarticulate, proximal segment shorter than distal, with 1 simple seta apically and 1 sparse plumose seta subapically, distal segment slender, with pubescence medially and long plumose seta apically.

Maxilla 1 (Fig. 3G) inner plate unknown; outer plate armed with 7 setal-teeth (simple or serrulate) apically; palp biarticulate, proximal segment short, wider than long, distal one extending beyond end of outer lobe, with row of 7 simple setae and 2 unequal robust setae apically, with row of 7 setae subapically.

Maxilla 2 (Fig. 3H) inner plate with longitudinal row of pinnate setae on inner margin, apical margin with 2 rows of simple or pectinate setae; outer plate extending beyond end of inner one, inner distal and apical margins with simple or plumose setae.

Maxilliped (Fig. 3I) inner plate slender and elongate, inner surface covered by pubescence, basal portion with transverse row of about 22 plumose setae, apical margin with 3 unequal robust setae, 1 pinnate and 2 simple setae, respectively; outer plate not reaching distal end of article 2 of palp, inner margin straight, densely setose, with 1 long pinnate seta distally, outer margin pubescent, curved convexly; palp 4-articulate, proximal article with 2 long plumose setae on outer margin, article 2 elongate, more than twice length of proximal one, inner margin densely setose, outer margin with 4 simple setae distally, article 3 subrectangular, with rounded distal corner, surrounded by setae distally, $0.34 \times$ article 2, distal article short, $0.33 \times$ article 3, with apical setae.

Gnathopod 1 (Fig. 4A) subchelate; coxa elongate-ovate, much longer than wide, ventral margin rounded with 3 long plumose setae, anterior margin with 6 setules; basis as long as carpus, anterior margin straight, unarmed, posterodistal corner with unequal setae; ischium quadrate, with long pinnate setae ventrodistally; merus short, subtriangular, with long pinnate setae ventrodistally; carpus slightly narrowing distally, anterior margin with 5 simple setae, distomedial corner with transverse row of 6 simple setae, posterior margin with 2 rows of pinnate setae; propodus subrectangular, posterior margin concave, $0.74 \times$ carpus, anterior and medial portions with pectinate setae, posterior margin with pinnate and simple setae, palm transverse, slightly convex, lined with row of bifid spinules; dactylus falcate, almost fitting palm.

Gnathopod 2 (Fig. 4B) simple; coxa small, wider than long; basis subrectangular, posterodistal margin with cluster of simple setae; ischium flat, depressed; merus convexly curved posteriorly, with 2 rows of long pinnate setae along posterior margin and medial portion; carpus isosceles triangle in shape, strongly widening distally, with several pinnate setae posterodistally; propodus weakly narrowing distally, $1.57 \times$ carpus, both margins with unequal simple setae, proximal half of medial portion with oblique row of pinnate setae; dactylus long and falcate, curved concavely, inner margin with row of setules.

Pereopod 3 (Fig. 4C) coxa small, wider than long; basis weakly expanded medially, anterior margin with 5 setules, distal half of posterior margin with simple setae; merus slightly widening distally, $1.24 \times$ propodus; carpus rather elongated, $0.69 \times$ merus; dactylus simple, $0.52 \times$ propodus.

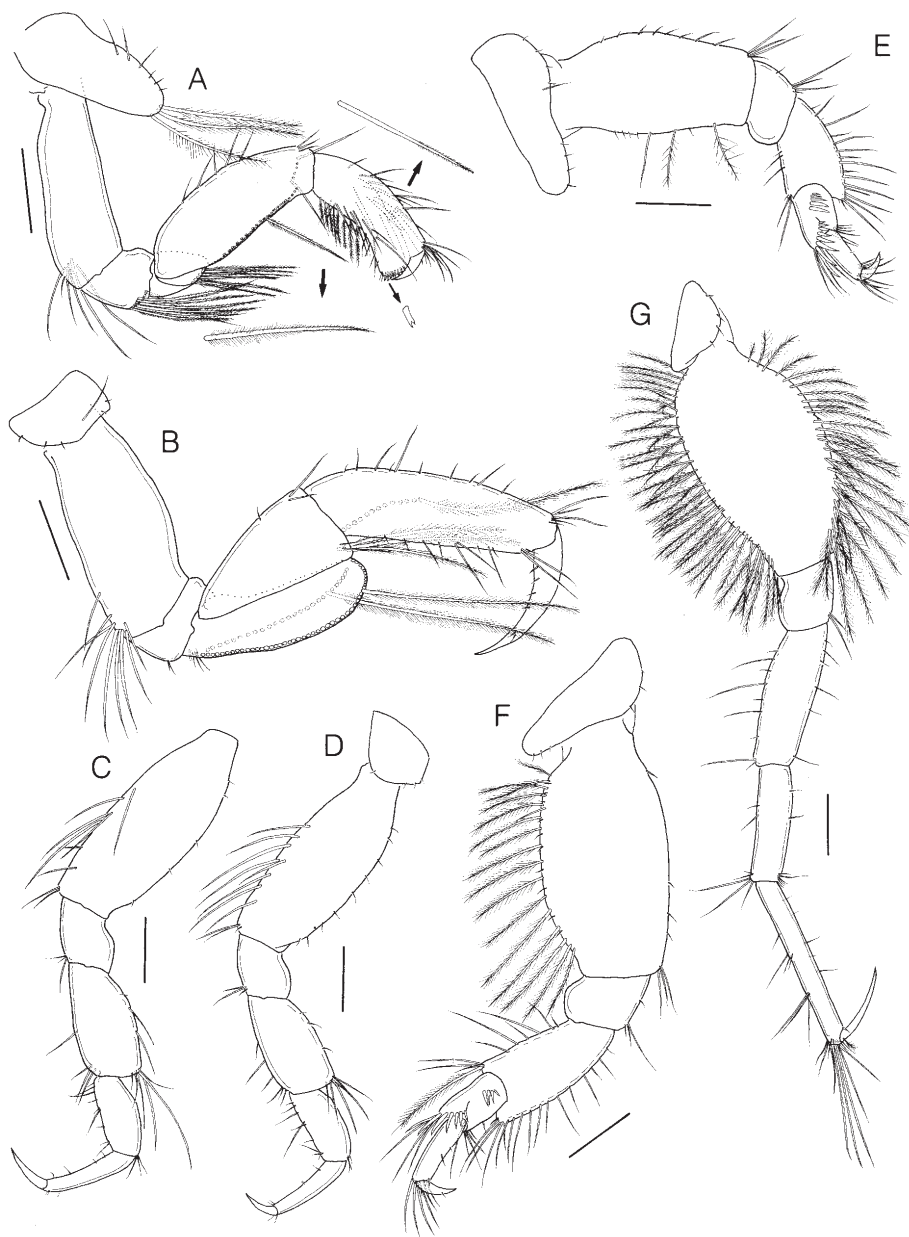


Figure 4. *Sinocorophium hangangense* sp. n., holotype, male, 12.2 mm, Gongreung stream, Songchon-ri, Gyoha-eup, Paju-si, Korea. **A** gnathopod 1 **B** gnathopod 2 **C** pereopod 3 **D** pereopod 4 **E** pereopod 5 **F** pereopod 6 **G** pereopod 7. Scale bars: 0.4 mm (A–G).

Pereopod 4 (Fig. 4D) similar to pereopod 3.

Pereopod 5 (Fig. 4E) coxa depressed, much wider than long, slightly concave mid-ventrally, narrowing distally; basis slightly widened medially, anteromarginally with

row of setules, posteromarginally with sparse setae; merus widening distally, both margins with unequal simple setae; carpus with 2 oblique rows of 5 proximal and 8 distal robust setae respectively, subequal in length to propodus; dactylus short.

Pereopod 6 (Fig. 4F) similar to pereopod 5, but about $1.3 \times$ longer; basis more greatly expanded posteriorly, with a row of setules and plumose setae; merus slightly widening distally, both margins with simple setae, posterodistally with 1 plumose seta; propodus slender, rectangular, $1.13 \times$ carpus; dactylus about $0.5 \times$ propodus.

Pereopod 7 (Fig. 4G) elongate, much longer than either pereopod 5 or 6; coxa small, ventral margin convexly rounded, with 4 setules; basis elongate-ovate, moderately expanded anteroposteriorly, densely setose along both margins with long plumose setae; ischium to propodus linear and rectangular; length ratio of articles 2–7 = $1.00 : 0.31 : 0.67 : 0.48 : 0.75 : 0.36$.

Urosomites 1–3 (Fig. 5A) separate; urosomite 1 longest, widest in middle when viewed dorsally, urosomite 2 longer than 3, nearly rectangular, posterodistal margins rounded in dorsal view; uropods 1–3 arising laterally.

Uropod 1 (Fig. 5A) slightly extending beyond end of uropod 2; peduncle rectangular, $1.84 \times$ outer ramus, ventrodistal process present, triangular, blunt, lateral margin with row of simple setae, medial one with 3 robust setae; outer ramus slightly longer than inner, lateral margin with 6 robust setae, medial one with 5 robust setae, including 2 subdistal robust setae; inner ramus slightly curved medially.

Uropod 2 (Fig. 5A) peduncle slightly longer than rami, with triangular ventrodistal process, apicodistal robust setae and cluster of setae; rami subequal in length, with robust setae marginally.

Uropod 3 (Fig. 5A) uniramous, peduncle short, broader than long, $0.78 \times$ ramus; ramus subelliptical, narrowing distally, margins with unequal simple setae, with apical setae.

Telson (Fig. 5A) fleshy, thickened, grooved centrally, subtriangular, with truncate corners, broadest in middle, dorsolaterally with 2 penicillate setae and 1 setule.

Paratype, female (sexually dimorphic characters), 11.0 mm, NIBRIV0000245090.

Body (Fig. 5B) similar to male including antenna 2, but rostrum (Fig. 5C) weaker; antenna 1 (Fig. 5D) peduncular article 1 without medial serrations, article 3 rather short; antenna 2 (Fig. 5E) subsimilar to that of male, but less robust and shorter, peduncular article 4 with 1 ventromedial robust seta.

Remarks. Bousfield and Hoover (1997) divided *Sinocorophium* into two groups, a relatively ancestral and a more derived subgroup through a numerical taxonomic analysis. According to this classification, the new species belongs to the derived subgroup. Some characteristics which relate to this group include non sexual dimorphic antenna 2, convex palm of the gnathopod 1, short carpi of pereopods 3–4 and short ramus of the uropod 3 (Table 1). However, the new species shows more similarity with the ancestral subgroup in having an elongate-ovate ramus of uropod 3 which is longer than the peduncle. The new species is distinguished from its congeners by having a row of slender setae and not robust setae on the lateral margin of the peduncle of uropod 1. These are unique morphological systematic statuses among

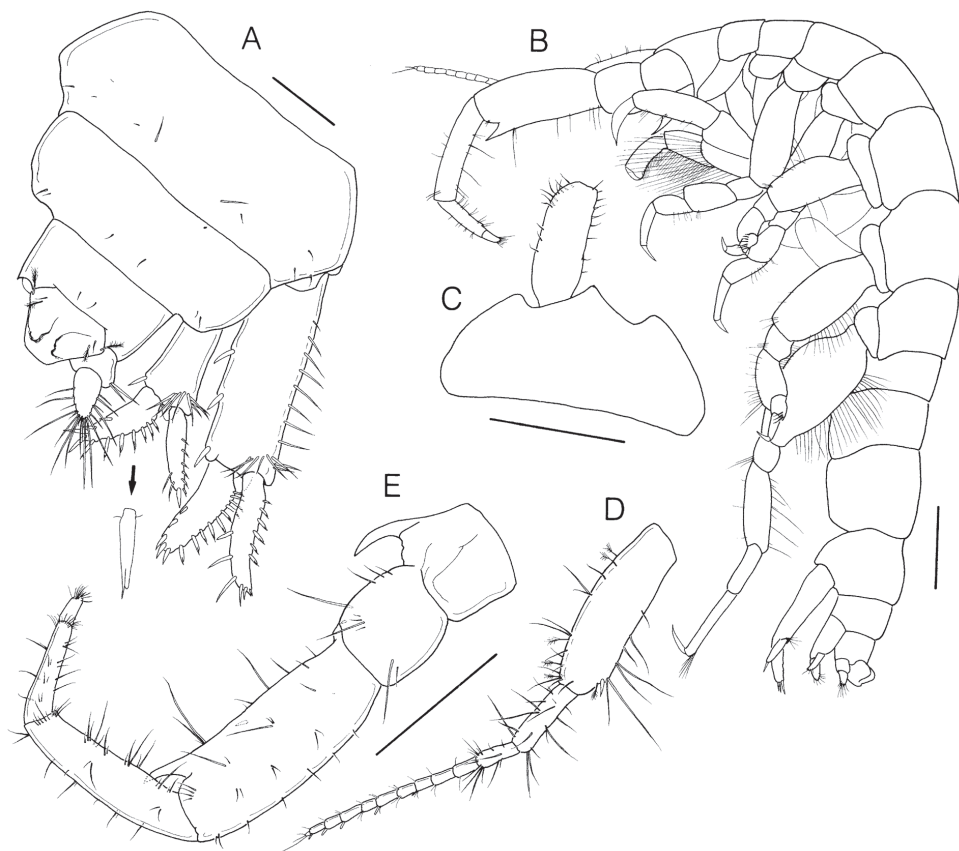


Figure 5. *Sinocorophium hangangense* sp. n., holotype, male, 12.2 mm, Gongreung stream, Songchon-ri, Gyoha-eup, Paju-si, Korea. **A** urosomites, uropods and telson, dorsal; paratype, female, 11.0 mm, Gongreung stream, Songchon-ri, Gyoha-eup, Paju-si, Korea. **B** habitus, lateral **C** head, dorsal **D** antenna 1 **E** antenna 2. Scale bars: 1.0 mm (**B–E**), 0.4 mm (**A**).

the more derived subgroup which is sexually subsimilar in antenna 2. Ecologically the new species inhabits lower stream regions with lowered salinity, while related congeners except *S. intermedium* and *S. minutum* in North Vietnam are mainly abundant in shallow subtidal and intertidal mudflats. Among the derived subgroup species, the new species is more similar to *S. alienense* of the northeast Pacific than to the geographically closer sinocorophiid amphipods in the Far East. These two species have several characteristics in common: 1) prominent ventromedial teeth row on peduncular articles 4–5 and first flagellum of male antenna 2; 2) inner ramus of uropod 1 curved medially and shorter than outer one; 3) antenna 2, posterodistal tooth of peduncular article 4 unidentate; 4) basis of pereopod 5 bearing posteromarginal setae. However, the new species is distinguished from *S. alienense* (different characters of *S. alienense* in brackets) by the combination of the following features:

Table 1. Morphological characters of *Sinocorophium hangangense* sp. n. and related species with sexually subsimilar antenna 2.

Character and distribution	Species						
	<i>S. alienense</i> (♂)	<i>S. homoceratum</i> (♂)	<i>S. intermedium</i> (♂)	<i>S. japonicum</i> (♀)	<i>S. monospinum</i> (♀)	<i>S. triangulopedarum</i> (♂)	<i>S. hangangense</i> sp. n. (♂)
Body length	6.5 mm	10–12 mm	8.0 mm	6.0 mm	4.5 mm	5.5 mm	12.2 mm
Rostrum	subequal to cephalic lobe	subequal to cephalic lobe	subequal to cephalic lobe	vestigial	longer than cephalic lobe	subequal to cephalic lobe	subequal to cephalic lobe
Cephalic lobe	subacute	rounded	rounded	weak	rounded	subacute	subacute
Antenna 1, peduncular article 1 ventral margin	pinnate setae	8 teeth	?	setae	setae	simple setae	simple setae
Antenna 1, length of peduncular article 1 to article 2	=	>>	>	>	>	>	>>
Antenna 1, flagellum, # of articles	13–15	16–18	17	12	14	17?	13
Antenna 2, ventrodistal process	1	2	1	1	1	1	1
Mandible, length of palp articles 1 & 2	proximal < distal	subequal	proximal < distal	subequal	subequal	proximal > distal	proximal < distal
Maxilliped, length of outer plate	less than end of palp article 2	exceeding end of palp article 2	?	less than end of palp article 2	reaching end of palp article 2	less than end of palp article 2	less than end of palp article 2
Pereopod 5, posterior margin of basis	setose	setose	?	not setose	?	not setose	not setose
Uropod 1, lateral margin of peduncle	robust setae	robust setae	?	robust setae	robust setae	robust setae	slender setae
Uropod 1, length of rami	inner < outer	inner << outer	inner << outer	subequal	inner << outer	inner < outer	inner < outer
Uropod 3, shape of ramus	ovate	clavate	ovate	ovate	ovate	semi-circular	elongate-ovate
Uropod 3, length of ramus	< peduncle	= peduncle	= peduncle	= peduncle	= peduncle	< peduncle	> peduncle
Habitat	intertidal	tide-pool	brackish	intertidal	coastal	mangrove marsh	brackish
Distribution	San Francisco Bay, California (Chapman 1988)	Tangku, Hopei, Yellow Sea (Yu 1938)	Thanh hoa, Vietnam, Gulf of Tongking (Dang 1965)	Tomioka Bay, Kyushu, Japan (Hirayama 1984)	Fenghsien, Kiangsu, East China Sea (Shen 1955)	Mai po, Hong Kong, South China Sea (Hirayama 1990)	Paju-si, Korea (present study)

1) peduncular article 1 of male antenna 1 with ventral simple setae (with pinnate setae); 2) peduncular article 1 of antenna 1 about $2.3 \times$ article 2 (peduncular article 1 subequal in length to article 2); 3) peduncular article 1 of antenna 1 with serrated medial margin (with smooth margin); 4) each lobe of telson with mid-lateral 1 simple and 2 penicillate setae, respectively (unornamented).

Etymology. Named for the type locality, Gongreung stream, which is a small tributary on the lower reaches of the Han River in Gyeonggi-do, Korea. The Korean word “Gang” means river.

Habitat. The new species is found in brackish water regions, but it may inhabit intertidal regions, are downstream of the type locality.

Distribution. Songchon-ri, Gyoha-eup, Paju-si, Gyeonggi-do, Korea.

Key to the species of *Sinocorophium*

- 1 Antenna 2 sexually dimorphic; gnathopod 1, palm steeply oblique to transverse; pereopods 3–4, merus subequal to carpus.....2
- Antenna 2 sexually subsimilar; gnathopod 1, palm gently convex; pereopods 3–4, merus longer than carpus5
- 2 Body medium sized, more than 5 mm; mandibular palp, terminal article short, much shorter than proximal article3
- Body small sized, less than 5 mm; mandibular palp, terminal article normal, subequal to proximal article4
- 3 Antenna 2(♀), peduncular article 4 with 2 ventral robust setae; gnathopod 2(♂), basis with dentiform process anterodistally*S. sinense* (Zhang, 1974)
- Antenna 2(♀), peduncular article 4 without ventral robust seta; gnathopod 2(♂), basis straight, without dentiform process anterodistally*S. heteroceratum* (Yu, 1938)
- 4 Rostrum minute, vestigial *S. lamellatum* (Hirayama, 1984)
- Rostrum rather prominent.....*S. minutum* (Dang, 1965)
- 5 Rostrum short, vestigial; uropod 1, rami subequal in length*S. japonicum* (Hirayama, 1984)
- Rostrum elongate; uropod 1, inner ramus shorter than outer.....6
- 6 Antenna 2, peduncular article 4 with 2 ventrodistal large teeth.....*S. homoceratum* (Yu, 1938)
- Antenna 2, peduncular article 4 with 1 ventrodistal large tooth7
- 7 Antenna 2, peduncular article 4 stubby, more than $0.5 \times$ as wide as long.....*S. intermedium* (Dang, 1965)
- Antenna 2, peduncular article 4 elongate, less than $0.5 \times$ as wide as long....8
- 8 Body large sized, more than 10 mm; uropod 1, lateral margin of peduncle with setae; uropod 3, ramus longer than peduncle*S. hangangense* sp. n.
- Body small sized, less than 7 mm; uropod 1, lateral margin of peduncle with robust setae; uropod 3, ramus subequal or shorter than peduncle9

- 9 Antenna 1, peduncular article 1 subequal in length to article 2 ***S. alienense* (Chapman, 1988)**
 – Antenna 1, peduncular article 1 longer than article 2 **10**
 10 Uropod 3, ramus semi-circular, shorter than peduncle ***S. triangulopedarum* (Hirayama, 1990)**
 – Uropod 3, ramus ovate, subequal in length to peduncle ***S. monospinum* (Shen, 1955)**

Acknowledgements

The author cordially thanks Ed A Hendrycks of the Canadian Museum of Nature, Canada for his preliminary critique of the manuscript. Wim Vader of Tromsø Museum, Norway, Ko Tomikawa of Hiroshima University, Japan and Ji-Min Lee of Daegu University, Korea are thanked for providing valuable papers. The author also appreciates Xiao-Chun Wu of the Canadian Museum of Nature, Canada for assistance with translation of the Chinese paper consulted in this study and Chang-Mok Lee of Munsandong Middle School, Korea for specimen collection. This research was supported by the National Research Foundation of Korea Grant funded by the Korean Government [NRF-2009-352-C00121].

References

- Bousfield EL, Hoover PM (1997) The amphipod superfamily Corophioidea on the Pacific coast of North America. Part V. Family Corophiidae. Corophiinae, new subfamily. Systematics and distributional ecology. *Amphipacifica* 2(3): 67–139.
- Chapman JW (1988) Invasions of the Northeast Pacific by Asian and Atlantic Gammaridean amphipod Crustaceans, including a new species of *Corophium*. *Journal of Crustacean Biology* 8(3): 364–382. doi: 10.2307/1548276
- Dang NT (1965) Mot so loai giap xac moi tim thay trong nuroc ngot va nuroc lo mien Bac Viet-nam. *Tap San Sinh Vat-Dia Hoc* 4: 146–152.
- Hirayama A (1984) Taxonomic studies on the shallow water Gammaridean Amphipoda of West Kyushu, Japan. II. Corophiidae. *Publications of the Seto Marine Biological Laboratory* 29(1/3): 1–92.
- Hirayama A (1987) Two peculiar species of corophiid amphipods (Crustacea) from the Seto Inland Sea, Japan. *Zoological Science* 4: 175–181.
- Hirayama A (1990) Marine Gammaridean Amphipoda (Crustacea) from Hong Kong. I. The Family Corophiidae, Genus *Corophium*. In: Morton B (Ed.) *The marine flora and fauna of Hong Kong and southern China II. Proceedings of the Second International Marine Biological Workshop: The Marine Flora and Fauna of Hong Kong and Southern China*, Hong Kong, 2–24 April 1986. Hong Kong: University Press, 449–485.

- Jung JW, Kim W (2007) New record of *Sinocorophium sinensis* (Crustacea: Amphipoda: Corophiidae) in Korea. Korean Journal of Systematic Zoology 23(2): 241–245. doi: 10.5635/KJSZ.2007.23.2.241
- Shen CJ (1955) On some marine crustaceans from the coastal water of Fenghsien, Kiangsu Province. Acta Zoologica Sinica 7: 75–100.
- Yu SC (1938) Descriptions of two new amphipod Crustacea from Tangku. Bulletin of the Fan Memorial Institute of Biology, Zoological Series 8: 83–103.
- Zhang WQ (1974) A new species of the genus *Corophium* (Crustacea, Amphipoda, Gammaridea) from the southern coast of Shantung Peninsula, North China. Studia Marina Sinica 9: 139–146.

On the species status of the root-knot nematode *Meloidogyne mayaguensis* Rammah & Hirschmann, 1988

Gerrit Karssen¹, Jinling Liao², Zhuo Kan²,
Evelyn YJ van Heese¹, Loes JMF den Nijs¹

1 Plant Protection Service, Wageningen Nematode Collection, P.O. Box 9102, 6700 HC Wageningen, The Netherlands **2** Plant Nematode Lab, College of Environment and Natural Resource, South China Agricultural University, Guangzhou, 510642, PR China

Corresponding author: Gerrit Karssen (g.karssen3@chello.nl)

Academic editor: S. Subbotin | Received 30 January 2012 | Accepted 28 March 2012 | Published 6 April 2012

Citation: Karssen G, Liao J, Kan Z, van Heese EYJ, den Nijs LJMF (2012) On the species status of the root-knot nematode *Meloidogyne mayaguensis* Rammah & Hirschmann, 1988. ZooKeys 181: 67–77. doi: 10.3897/zookeys.181.2787

Abstract

Holo- and paratypes of the root-knot nematodes *Meloidogyne mayaguensis* Rammah & Hirschmann, 1988 and *M. enterolobii* Yang & Eisenback, 1983 were morphometrically and morphologically compared. All observed female, male and second-stage juvenile morphometrical and morphological characters are identical for the two studied species. Additionally, contradictions between the original species descriptions were unravelled.

The present study of holo- and paratypes confirms the taxonomical status of *Meloidogyne mayaguensis* as a junior synonym for *M. enterolobii*.

Keywords

Junior synonym, *Meloidogyne*, *M. enterolobii*, *M. mayaguensis*, Nematoda, root-knot nematode, synonymisation

Introduction

In 1983 Yang and Eisenback described the root-knot nematode *Meloidogyne enterolobii* from roots of pacara earpod trees (*Enterolobium contortisiliquum* (Vell.) Morong), on Hainan Island in China. The authors reported severe damage on these pacara earpod trees. In 1988 Rammah and Hirschmann described the root-knot nematode *M. maya-*

guensis from eggplant (*Solanum melongena* L.) roots, from Puerto Rico. *Meloidogyne mayaguensis* was described by the authors as: “superficially resembles *M. enterolobii*”, and reported at the same time “several distinct morphologically features and a unique malate dehydrogenase pattern (N3c)”.

It was Fargette and Braaksma (1990) and Fargette et al. (1996) who reported for the first time on the resistance-breaking behaviour of *M. mayaguensis* in Africa and concluded that it is present in both continents of Africa and America. The authors reported (1996) on *M. enterolobii*: “*M. enterolobii* from China has been described as having the same esterase phenotype as *M. mayaguensis*. However it is not known whether their DNA are closely related”. In 2000 Carneiro et al. published esterase and malate dehydrogenase patterns for a Brazilian population of *M. mayaguensis*, and detected a different (N1a) malate dehydrogenase pattern. Additionally Blok et al. (2002) published mtDNA results from different *M. mayaguensis* populations, including type material from Puerto Rico.

In their comprehensive studies on the characterisation of *Meloidogyne* species from China, with isozymes and mtDNA, Meng et al. (2004) and Xu et al. (2004) included two *M. enterolobii* populations from Hainan Island, isolated from the fruit tree Guava (*Psidium guajava* L.). They proved for the first time that *M. enterolobii* esterase (VS1-S1) and malate dehydrogenase (N1a) patterns and mtDNA results are identical to reported *M. mayaguensis* data, and concluded carefully: “the mtDNA sequence evidence presented here, suggests that *M. mayaguensis* could be conspecific with *M. enterolobii*”.

In 2005–2006 we compared the available holo- and paratypes of *M. enterolobii* and *M. mayaguensis*. Meanwhile our Chinese co-authors collected live *M. enterolobii* material on Hainan Island at the type locality from the type host and we kindly received live *M. mayaguensis* type material from Dr. V. Blok (originating from Dr. M. Fargette). The preliminary isozyme and morphological results were presented by the first author during a Pest Risk Analysis meeting on *M. enterolobii* at EPPO in Paris (Anonymous, 2008). Additionally this type material of both species was compared at DNA level to *Meloidogyne* sp. from Switzerland and we identified the Swiss population as *M. enterolobii* (Kiewnick et al. 2008).

Finally, as again at DNA level no differences were found, the two species were synonymised: “The species *M. enterolobii* (syn. *M. mayaguensis*)” and “...of *M. mayaguensis* (junior synonym of *M. enterolobii*)” (Kiewnick et al. 2009).

Although taxonomical not strictly necessary, we present herein a morphological and morphometrical comparison between the holo- and paratype slides of *M. mayaguensis* and *M. enterolobii*. Additionally we discuss anomalies between the descriptions of *M. mayaguensis* and *M. enterolobii*.

Material and methods

Holo- and paratype slides (Table 1) originating from USDA Nematode Collection (USDANC), Beltsville, USA were kindly provided by Dr. Z. Handoo. The type slides

Table 1. *Meloidogyne mayaguensis* and *M. enterolobii* holo-, allo- and paratype slides studied, including USDANC codes.

	<i>M. mayaguensis</i>		<i>M. enterolobii</i>	
Holotype	1 female	T-428t	1 female	T-360t
Allotype*	1 male	T-429t	1 male	T-361t
Paratype	10 perineal patterns	T-3849p	8 perineal patterns	T-3147p
Paratype	6 males	T-3843p	10 males	T-3149p
Paratypes	25 J2's	T-3846/7p	25 J2's	T-3152p

*According to the ICZN rules (4th edition) the allotype concept is no longer valid, and treated herein as a paratype.

are in good condition and includes female holotypes, male allotypes, perineal patterns and second-stage juvenile paratypes. These slides were observed by compound light microscopy (Olympus BH-2 and Zeiss Axio Imager), including Differential Interference Contrast and photographed by Leica DMC-50 digital camera. For the overall morphological and morphometrical comparison between the types we focussed on the most differential and supplementary *Meloidogyne* characters, as described by Jepson (1987) and as previously applied by Karssen (2002). Live type material of both species was propagated and maintained on tomato at the greenhouse of the PPS the Netherlands. This material was studied morphologically (females, males and second-stage juveniles) and used for isozyme electrophoresis (Mdh; EC 1.1.1.37 and Est; EC 3.1.1.1). For details on the preparation of slides and applied electrophoresis method we respectively refer to Karssen (1996) and Karssen et al. (1995).

Results and discussion

See Figure 1 and 2 for LM photographs of female and second-stage juvenile morphological characteristics.

See Table 2–5 for respectively female, male and second-stage juvenile morphological and morphometrical observations.

Females

The important morphological characters, like female stylet knob and perineal pattern shape do not differ between the species, as can already be observed by comparing the original illustrations between *M. mayaguensis* and *M. enterolobii* (see original descriptions respectively Fig. 2 A–D & Fig. 3 A–D). This perineal pattern type is not species specific within the genus *Meloidogyne* and can best be marked as typical for many species within the *M. incognita*-group, including the observed variation within the dorsal part. Additionally we observed a relatively large tail remnant area, free of any striae, just above the covered anus (Fig. 1 A–D). Also the observed stylet knob position variation,

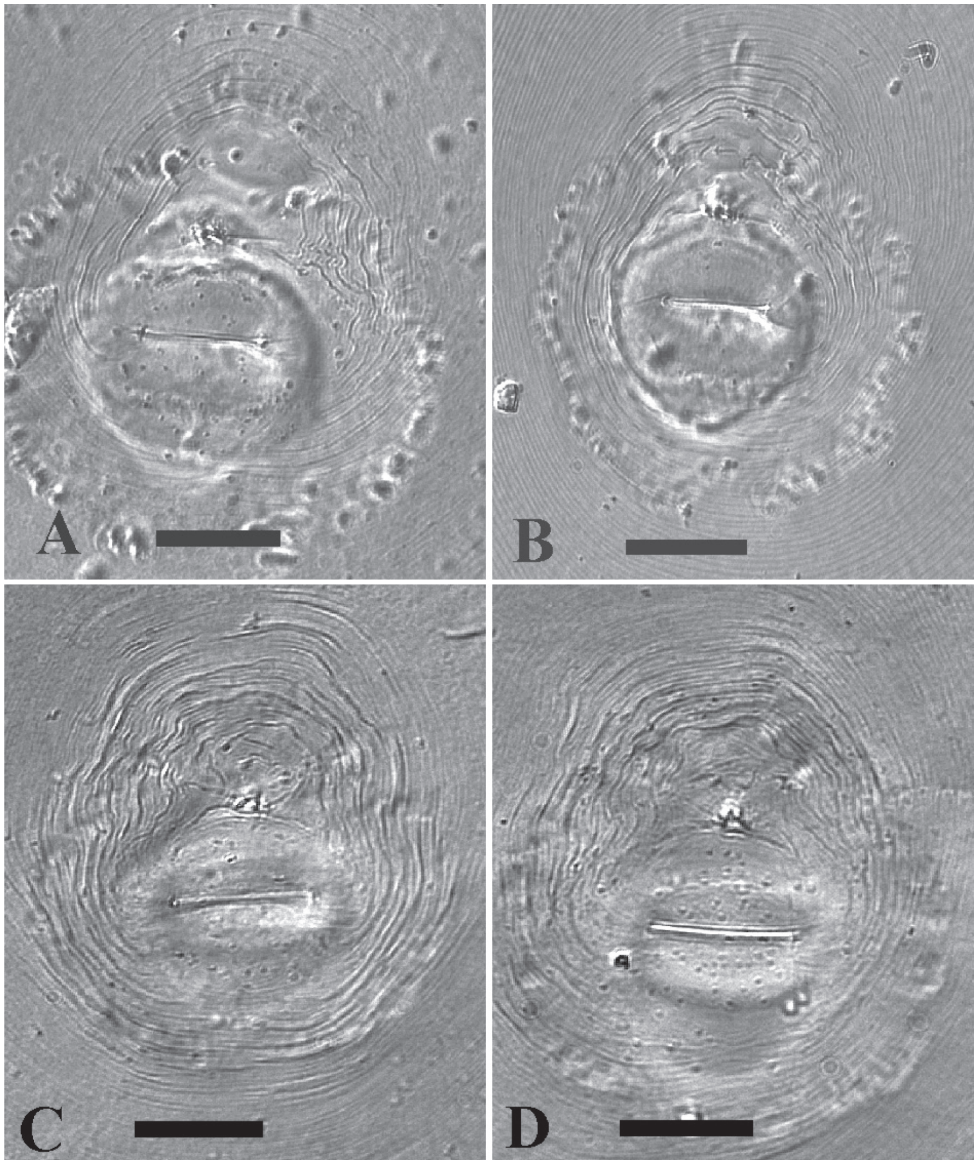


Figure 1. LM photographs of perineal patterns of *M. mayaguensis* (**A, B**) and *M. enterolobii* (**C, D**). Bar = 25 μ m.

slightly sloping backward to set off from the shaft, is a common *Meloidogyne* feature. Strangely this variation is also clearly visible in the SEM photographs of excised female stylets of *M. mayaguensis* (see original description, Fig. 3 A-C), but not described. With the light microscope one can observe a weak longitudinal indentation, for both species, in the female stylet knobs at the anterior side. The reported differences “not divided so conspicuously as those of *M. enterolobii*” as mentioned in the *M. mayaguensis* descrip-

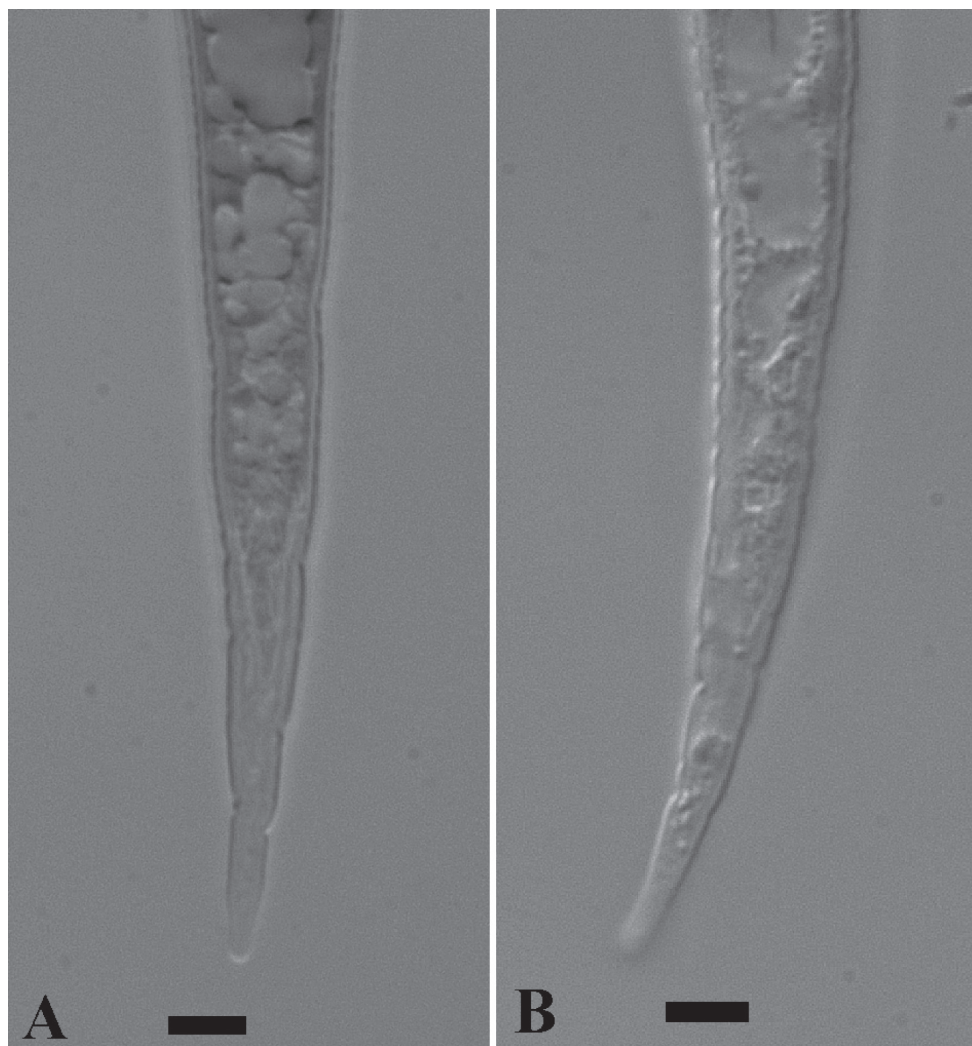


Figure 2. LM photographs of second-stage juvenile tails of *M. mayaguensis* (A) and *M. enterolobii* (B). Bar = 5 μ m.

tion (see diagnosis original description), was not confirmed by our observations. Also the described position of one of the *M. mayaguensis* stylet knobs “the dorsal knob is slightly sloping posteriad in lateral view” was not observed by us.

Males

The male head shape for *M. mayaguensis* is described as “not set off”, while a slightly set off head region was observed as described for *M. enterolobii*. Comparing the original SEM pictures of the head for *M. mayaguensis* and *M. enterolobii* (see original descrip-

Table 2. Morphological observations of primary female, male and second-stage juvenile characters of *Meloidogyne mayaguensis* and *M. enterolobii* holo- and paratypes compared to described data.

Species	<i>M. mayaguensis</i>	<i>M. enterolobii</i>	
Character	described		observed
Female			
Stylet knobs	knobs reniform or transversely elongated, distinctly indented, merging gradually with shaft	knobs set off from shaft, and divided longitudinally by groove so that each knob appears as two	oval, anteriorly often indented, slightly sloping backward to set off
Perineal pattern	round to dorso-ventrally ovoid, dorsal arch rounded, striae fine, single lateral line may occur	oval shaped, with coarse and smooth striae, dorsal arch moderately high to high, often rounded, nearly square in some, lateral lines not distinct	oval shaped, striae mostly fine, dorsal arch rounded to square, weak lateral line(s) sometimes present
Male			
Head shape	head not set off, shallowly rounded to truncate, head region high without annulations	head cap high and rounded, head region only slightly set off from body	head cap high and rounded, head region slightly set off, not annulated
Stylet knobs	knobs large, set off from shaft, rounded, sloping backward, dorsal knob base concave	knobs large, rounded, distinctly set off, in some specimens each knob divided longitudinally	knobs large, ovoid to rounded, slightly sloping backwards
Second-stage juvenile			
Stylet knobs	knobs small, rounded, set off from shaft, distinctly sloping backward	knobs large, rounded, set off from shaft	knobs ovoid to rounded, slightly sloping backwards
Tail shape	slender, gradually tapering to bluntly rounded tip	very thin, tip broad, bluntly rounded	slender, posterior part nearly straight and parallel, tapering to rounded tip
Hyaline tail part	distinctly set off, often containing small fat droplet at tip	clearly defined, a few fat droplets may occur in terminus	anterior part not clearly delimited

tions respectively Fig. 6 A–D & 5 A,B) shows clearly not any differences in head morphology. Also the male stylet knobs have been SEM studied for the original descriptions (Fig. 3 E, F & Fig. 6 B) of both species. Large oval to rounded shaped knobs, slightly sloping backwards are clearly visible. This was also observed by LM for both species, however described as “rounded and set off” for *M. enterolobii* and “set off from the shaft, rounded, sloping backward” for *M. mayaguensis*. The later description of the knobs is rather odd, i.e. set off and sloping backward at the same time! The same results were described and observed for the second-stage juvenile knobs for both species.

Second-stage juveniles

The second-stage juvenile stylet knob size is described as small for *M. mayaguensis* and large for *M. enterolobii*. We indeed observed a larger size variation for *M. enterolobii*

Table 3. Morphometrical (in µm) observations (mean, SD & range) of female *Meloidogyne mayaguensis* and *M. enterolobii* holo- (single female) and paratypes (perineal patterns) compared to described data.

Species	<i>M. mayaguensis</i>		<i>M. enterolobii</i>	
Character	description	observed	description	observed
Holotype (N)	1	1	1	1
Body length	720	674	667	693
Body width	570	576	415	462
Neck length	190	168	265	262
Neck width	160	169	--	--
DGO	6.2	6.4	3.7	4.8
Excretory pore to head end	46.4	45.8	44.8	64.0
Stylet length	15.1	15.7	13.4	14.7
Stylet knob height	2.2	2.0	2.7	2.3
Stylet knob width	4.4	4.5	4.3	4.5
Paratypes (N)	35	10	20	8
Interphasmidial dist.	23.2 ± 2.5 (18.1–29.6)	28.8 ± 3.7 (24.3–33.3)	30.7 ± 4.8 (22.2–42.0)	33.5 ± 7.6 (22.4–41.9)
Vulval slit length	26.1 ± 1.9 (20.9–30.4)	27.0 ± 1.4 (25.0–29.4)	28.7 ± 2.0 (25.3–32.4)	28.0 ± 1.0 (25.9–29.1)
Vulva-anus distance	18.4 ± 1.5 (12.7–21.1)	21.4 ± 3.1 (17.0–27.1)	22.2 ± 1.8 (19.7–26.6)	23.4 ± 1.6 (21.1–26.2)
DGO	4.8 ± 0.8 (3.5–6.7)	–	4.9 ± 0.8 (3.7–6.2)	–
Excretory pore to head end	48.2 ± 13.6 (25.9–86.6)	–	62.9 ± 10.5 (42.3–80.6)	–
Stylet length	15.8 ± 0.8 (13.8–16.8)	–	15.1 ± 1.4 (13.2–18.0)	–

stylet knob width (2.5 – 4.0 µm) compared to *M. mayaguensis* (2.2 – 2.9 µm). However when observing live second-stage juveniles, the same large stylet knob width variation was observed for both species.

As for the males, the published SEM second-stage juvenile head shape is absolute identical for *M. mayaguensis* and *M. enterolobii* (see original descriptions respectively Fig. 7 A–D & Fig. 8 A, B). The tail is distinctly tapering and in the posterior tail (roughly the hyaline tail part) nearly straight and running parallel for both second-stage juvenile paratypes. Also, for both species the hyaline tail part is described as “distinctly set off” or “clearly defined”. We observed for both species however not a clearly anterior delimited hyaline tail part, in fact the body content runs deep into the hyaline tail part (Fig 2 A, B), as comparable to *M. hapla* (Karssen, 2002). The second-stage juvenile drawings for both species descriptions (Fig. 4 E, F & Fig. 7 E–F) show a clearly delimited anterior hyaline tail part, while the original photographs (Fig. 5 F, G & Fig. 9 B) do not show this at all. The fact that both descriptions did not include the hyaline tail measurements (a standard procedure), suggest strongly that the hyaline tail part is not clearly defined. Also in live second-stage juveniles we did not observe a clearly defined hyaline tail part (Table 2).

Table 4. Morphometrical (in μm) observations (mean, SD & range) of male *Meloidogyne mayaguensis* and *M. enterolobii* paratypes compared to described data.

Species	<i>M. mayaguensis</i>		<i>M. enterolobii</i>	
Character	description	observed	description	observed
N	30	7	20	11
Body length	1503 \pm 142 (1175–1742)	1431 \pm 63 (1337–1496)	1600 \pm 160 (1349–1913)	1230 \pm 316 (865–1667)
Greatest body width	37.8 \pm 3.1 (32.2–44.4)	34.5 \pm 1.9 (32.0–37.4)	42.3 \pm 3.6 (37.0–48.3)	32.0 \pm 6.0 (23.7–39.2)
Stylet length	22.9 \pm 0.8 (20.7–24.6)	22.1 \pm 0.7 (20.8–23.0)	23.4 \pm 1.0 (21.2–25.5)	21.5 \pm 1.7 (19.2–23.4)
Stylet knob height	3.0 \pm 0.3 (2.4–3.7)	3.2 \pm 0.3 (2.6–3.4)	3.3 \pm 0.3 (2.6–3.9)	2.5 \pm 0.3 (2.1–3.2)
Stylet knob width	5.0 \pm 0.3 (4.3–5.6)	5.3 \pm 0.5 (4.5–5.8)	5.4 \pm 0.3 (4.5–5.8)	4.5 \pm 0.6 (3.5–5.0)
DGO	4.1 \pm 0.4 (3.3–5.0)	4.1 \pm 0.7 (3.2–5.1)	4.7 \pm 0.4 (3.7–5.3)	4.7 \pm 0.6 (3.7–5.8)
Excretory pore to head end	166.4 \pm 8.8 (147.2–180.8)	158.6 \pm 14.9 (132.5–177.9)	178.2 \pm 11.2 (159.7–206.2)	155.8 \pm 22.3 (129.9–199.7)
Spicule length	28.3 \pm 1.5 (24.4–31.3)	29.0 \pm 2.4 (25.6–32.3)	30.4 \pm 1.2 (27.3–32.1)	28.0 \pm 1.1 (26.2–29.4)
Gubernaculum length	7.1 \pm 0.6 (6.1–9.3)	7.5 \pm 1.0 (6.4–9.0)	6.2 \pm 1.0 (4.8–8.0)	6.5 \pm 0.8 (6.1–8.0)
Tail length	14.3 \pm 1.1 (11.3–16.3)	13.0 \pm 1.1 (10.9–14.7)	12.5 \pm 2.2 (8.6–20.2)	11.9 \pm 1.2 (10.2–13.4)
A	39.9 \pm 3.9 (31.1–49.6)	41.6 \pm 2.9 (37.2–44.7)	37.9 \pm 3.2 (34.1–45.5)	38.1 \pm 4.0 (30.0–43.4)
C	105.7 \pm 10.0 (85.8–124.3)	110.5 \pm 10.8 (98.5–133.7)	131.6 \pm 24.2 (72.0–173.4)	103.2 \pm 23.7 (71.4–135.9)

Morphometrics

The morphometrical characters between the types of *M. mayaguensis* and *M. enterolobii* (Table 3–5), are comparable for the described and observed data, i.e. all mean data are the same or at least within the calculated range. Body length and body width data are generally slightly smaller when comparing observed to described data, this is a well known effect due to a slight shrinking of the nematode body within permanent slides. For *M. enterolobii* males we noticed however an unusual difference in greatest body width between the described 42.3 μm (37–48 μm) and observed 32.0 μm (24–39) μm data. The differences can not only be explained due to a shrinking effect, particularly as the observed greatest body width data agrees with the observed data for *M. mayaguensis*. Also for the *M. enterolobii* female holotype unexplainable differences were noticed between described and observed data for the DGO (3.7 μm versus 4.8 μm) and stylet length (13.4 μm versus 14.7 μm).

The described and discussed *M. mayaguensis* differences (see diagnosis original description) within the female perineal pattern for the interphasmidial distance, vulval

Table 5. Morphometrical (in µm) observations (mean, SD & range) of second-stage juvenile *Meloidogyne mayaguensis* and *M. enterolobii* paratypes compared to described data.

Species	<i>M. mayaguensis</i>		<i>M. enterolobii</i>	
Character	description	observed	description	observed
N	35	25	30	25
Body length	454 ± 28 (390–528)	420 ± 21 (386–456)	437 ± 17 (405–473)	408 ± 18 (380–442)
Greatest body width	14.7 ± 0.5 (13.8–15.8)	13.9 ± 0.7 (13.1–15.4)	15.3 ± 0.9 (13.9–17.8)	14.8 ± 2.1 (11.0–18.0)
Body width at anus	10.9 ± 0.5 (10.2–12.2)	9.8 ± 0.6 (9.0–11.2)	–	9.8 ± 0.9 (8.0–11.0)
Stylet length	11.6 ± 0.3 (11.1–12.2)	11.5 ± 0.4 (10.9–12.1)	11.7 ± 0.5 (10.8–13.0)	11.3 ± 0.7 (10.5–13.0)
Stylet base to head end	15.2 ± 0.3 (14.8–15.8)	15.4 ± 0.3 (14.7–16.0)	–	15.0 ± 0.7 (14.0–16.0)
Stylet knob height	–	1.5 ± 0.1 (1.2–1.7)	1.6 ± 0.1 (1.3–1.8)	1.8 ± 0.3 (1.5–2.0)
Stylet knob width	–	2.5 ± 0.2 (2.2–2.9)	2.9 ± 0.3 (2.4–3.4)	3.0 ± 0.4 (2.5–4.0)
DGO	3.9 ± 0.2 (3.3–4.3)	3.7 ± 0.4 (3.2–4.2)	3.4 ± 0.3 (2.8–4.3)	3.8 ± 0.3 (3.0–4.5)
Excretory pore to head end	87.6 ± 3.3 (79.9–97.9)	88.3 ± 3.0 (83.5–95.3)	91.7 ± 3.3 (84.0–98.6)	80.8 ± 4.4 (70.0–88.0)
Tail length	54.4 ± 3.6 (49.2–62.9)	54.2 ± 2.7 (48.7–58.5)	56.4 ± 4.5 (41.5–63.4)	52.1 ± 3.4 (45.0–57.0)
a	30.9 ± 1.9 (26.4–34.7)	30.1 ± 1.6 (26.9–32.8)	28.6 ± 1.9 (24.0–32.5)	28.0 ± 3.7 (23.3–34.6)
c	8.3 ± 0.4 (7.0–9.2)	7.8 ± 0.3 (7.1–8.4)	7.8 ± 0.7 (6.8–10.1)	7.9 ± 0.6 (7.0–9.0)
Excretory pore (%)	19.4 ± 1.0 (17.8–22.3)	21.1 ± 0.9 (19.2–22.7)	–	19.8 ± 1.1 (17.6–21.9)

slit length and vulva-anus distance is not confirmed by our observations. All these measurements are within the observed range. Perineal pattern measurements are generally highly variable and a logical reason for Jepson (1987) not to list this type of data when discussing differential characters for the genus *Meloidogyne*.

Reproduction and cytogenetics

The two species descriptions report also on the mode of reproduction and number of chromosomes, both reproduce by mitotic parthenogenesis (= apomixes) and have a somatic chromosome number of 2n = 44–45 for *M. mayaguensis* and 2n = 44–46 for *M. enterolobii*. In conclusion, both species have the same mode of reproduction and somatic chromosome number.

Host plants

Additionally, both species descriptions report in their introduction part some hosts, i.e. they both previously applied the North Carolina differential host test (Hartman and Sasser, 1985). Both species showed the same positive host response for tobacco, pepper, watermelon and tomato and no host response on peanut. Beside this, *M. mayaguensis* did not infest cotton, while *M. enterolobii* moderately infested cotton. As the details of the previously applied host tests have not been described in the material and method part of the species descriptions, we can not explain the reported host response differences on cotton for *M. mayaguensis* and *M. enterolobii*. Interesting is the *M. mayaguensis* study by Brito et al. (2004) with four isolates from Florida (USA). All four isolates, maintained on tomato, reproduced also on cotton, tobacco, pepper and watermelon but not on peanut, i.e. identical to the published results for *M. enterolobii*.

Isozymes

The observed esterase (VS1-S1 type) and malate dehydrogenase (N1a type) isozyme patterns are identical for both species and agrees with previous results (Carneiro et al. 2000; Xu et al. 2004).

Conclusion

In conclusion, the holo- and paratype material of *Meloidogyne mayaguensis* and *M. enterolobii* is morphological and morphometrical identical and it confirms the taxonomical status of *M. mayaguensis* as a junior synonym for *M. enterolobii*.

Acknowledgement

This work was supported by the special fund for agro-scientific research in the public interest of China (grant no. 201103018).

References

- Anonymous (2008) An emerging root-knot nematode, *Meloidogyne enterolobii*: addition to the EPPO Alert List. EPPO Reporting Service 5: 9–10.
- Blok VC, Wishart J, Fargette M, Berthier K, Phillips MS (2002) Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. Nematology 4: 773–781. doi: 10.1163/156854102760402559

- Brito J, Powers TO, Mullin PG, Inserra RN, Dickson DW (2004) Morphological and molecular characterization of *Meloidogyne mayaguensis* isolates from florida. *Journal of Nematology* 36: 232–240.
- Carneiro RMDG, Almeida MRA, Queneherve P (2000) Enzyme phenotypes of *Meloidogyne* spp. populations. *Nematology* 2: 645–654. doi: 10.1163/156854100509510
- Fargette M, Braaksma R (1990) Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 3. A study of some “B” race lines and their taxonomic position. *Revue de Nématologie* 13: 375–386.
- Fargette M, Phillips MS, Blok VC, Waugh R, Trudgill DL (1996) An RFLP study of relationships between species, populations and resistance-breaking lines of tropical species of *Meloidogyne*. *Fundamental and Applied Nematology* 19: 193–200.
- Hartman KM, Sasser JN (1985) Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: Barker KR, Carter CC, Sasser JN (Eds.) *An advanced treatise on Meloidogyne*, vol. 2, Methodology, pp. 69–77, North Carolina State University, Raleigh, USA.
- Jepson SB (1987) Identification of root-knot nematodes (*Meloidogyne* species). CAB International, Wallingford, UK, 265 pp.
- Karssen G (1996) Description of *Meloidogyne fallax* n. sp. (Nematoda: Heteroderidae), a root-knot nematode from The Netherlands. *Fundamental and Applied Nematology* 19: 593–599.
- Karssen G (2002) The plant-parasitic nematode genus *Meloidogyne* Göldi, 1892 (Tylenchida) in Europe. Brill, Leiden, The Netherlands, 157 pp.
- Karssen G, van Hoenselaar T, Verkerk-Bakker B, Janssen R (1995) Species identification of root-knot nematodes from potato by electrophoresis of individual females. *Electrophoresis* 16: 105–109. doi: 10.1002/elps.1150160119
- Kiewnick S, Karssen G, Brito JA, Oggenfuss M, Frey B, Frey J-E (2008) First report of root-knot nematode *Meloidogyne enterolobii* in Switzerland. *Plant Disease* 92: 1370. doi: 10.1094/PDIS-92-9-1370A
- Kiewnick S, Dessimoz M, Franck L (2009) Effects of the *Mi-1* and the *N* root-knot nematode-resistance gene on the infection and reproduction of *Meloidogyne enterolobii* on tomato and pepper cultivars. *Journal of Nematology* 41: 134–139.
- Meng Q, Long H, Xu J (2004) PCR assays for rapid and sensitive identification of three major root-knot nematodes, *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. *Acta Phytopathologica Sinica* 34: 204–210.
- Rammah A, Hirschmann H (1988) *Meloidogyne mayaguensis* n. sp. (Meloidogynidae), a root-knot nematode from Puerto Rico. *Journal of Nematology* 20: 58–69.
- Xu J, Liu P, Meng Q, Long H (2004) Characterisation of *Meloidogyne* species from China using isozyme phenotypes and amplified mitochondrial DNA restriction fragment length polymorphism. *European Journal of Plant Pathology* 110: 309–315. doi: 10.1023/B:EJPP.0000019800.47389.31
- Yang B, Eisenback JD (1983) *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpod tree in China. *Journal of Nematology* 15: 381–391.

A new sexannulate species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from Yakushima Island, Japan

Takafumi Nakano^{1,†}

¹ Department of Zoology, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

[†] [urn:lsid:zoobank.org:author:FE6B9521-5C9E-43C5-B492-08B0D0590E1B](https://zoobank.org/urn:lsid:zoobank.org:author:FE6B9521-5C9E-43C5-B492-08B0D0590E1B)

Corresponding author: Takafumi Nakano (nakano@zoo.zool.kyoto-u.ac.jp)

Academic editor: F. Govedich | Received 13 February 2012 | Accepted 3 April 2012 | Published 6 April 2012

[urn:lsid:zoobank.org:pub:5834B1DE-0092-491F-B9EB-4B8E80E65FA5](https://zoobank.org/urn:lsid:zoobank.org:pub:5834B1DE-0092-491F-B9EB-4B8E80E65FA5)

Citation: Nakano T (2012) A new sexannulate species of *Orobdella* (Hirudinida, Arhynchobdellida, Orobdellidae) from Yakushima Island, Japan. ZooKeys 181: 79–93. doi: 10.3897/zookeys.181.2932

Abstract

A new sexannulate species of the genus *Orobdella* Oka, 1895, *Orobdella mononoke* **sp. n.**, is described on the basis of five specimens collected from Yakushima Island, Japan. *Orobdella mononoke* **sp. n.** differs from other sexannulate *Orobdella* species in its possessing the following combination of characters: dorsal surface bicolor in life, I–XIII, XXVII and caudal sucker grayish purple, XIV–XXVI amber, male gonopore at XI c11/c12, female gonopore at XIII b2, 8 + 1/2 between gonopores, tubular but bulbous at junction with crop gastroporal duct, epididymides in XV–XIX, and atrial cornua ovate. Phylogenetic analyses using nuclear 18S rDNA and histone H3, and mitochondrial COI, tRNA^{Cys}, tRNA^{Met}, 12S rDNA, tRNA^{Val} and 16S rDNA markers show that *O. mononoke* **sp. n.** is closely related to *Orobdella esulcata* Nakano, 2010 from Kyushu, Japan, and two species, *Orobdella dolichopharynx* Nakano, 2011 and *Orobdella shimadae* Nakano, 2011, from the Ryukyu Archipelago, Japan.

Keywords

Hirudinida, Hirudinea, Orobdellidae, *Orobdella*, new species, molecular phylogeny, Japan

Introduction

The genus *Orobdella* Oka, 1895 consists of nine terrestrial gastroporous leeches described from Japan (Nakano 2010, 2011a,b, 2012; Oka 1895; Richardson 1975). The genus *Orobdella* was formerly a member of the family Gastrostomobdellidae

(Oceguera-Figueroa et al. 2011; Richardson 1971, 1975; Sawyer 1986), but a recent molecular phylogenetic study indicated that this genus belongs to the monotypic family Orobdehlidae under Erpobdelliformes (Nakano et al. 2012).

The nine *Orobdehlla* species are split into three groups based on their mid-body somite annulation (Nakano 2012, Nakano et al. 2012): 1) the quadrannulate group containing five species; 2) the sexannulate containing three species; and 3) one octannulate species. Among these groups, the sexannulate *Orobdehlla* species consist of *O. ijimai* Oka, 1895 from Honshu, Japan, and two species, *O. dolichopharynx* Nakano, 2011 and *O. shimadae* Nakano, 2011, from the Ryukyu Archipelago, Japan. Recently, sexannulate *Orobdehlla* specimens were collected from Yakushima Island. These specimens are clearly distinguishable from the other three sexannulate species. *Orobdehlla* leeches from Yakushima Island are thus described as a new species herein. In addition, its phylogenetic position is estimated using nuclear 18S rDNA and histone H3, and mitochondrial COI and tRNA^{Cys}, tRNA^{Met}, 12S rDNA, tRNA^{Val} and 16S rDNA (tRNA^{Cys}–16S) sequence data.

Material and methods

Leeches were collected from Yakushima Island, Japan (Fig. 1), under rocks along mountain or forest trails. Altitude and coordinates for localities were obtained using a Garmin eTrex GPS unit.

Botryoidal tissue was taken from every specimen for DNA extraction, and the rest of the bodies were fixed in 10% formalin and preserved in 70% ethanol. Two measurements were made: body length (BL) from the anterior margin of the oral sucker to the

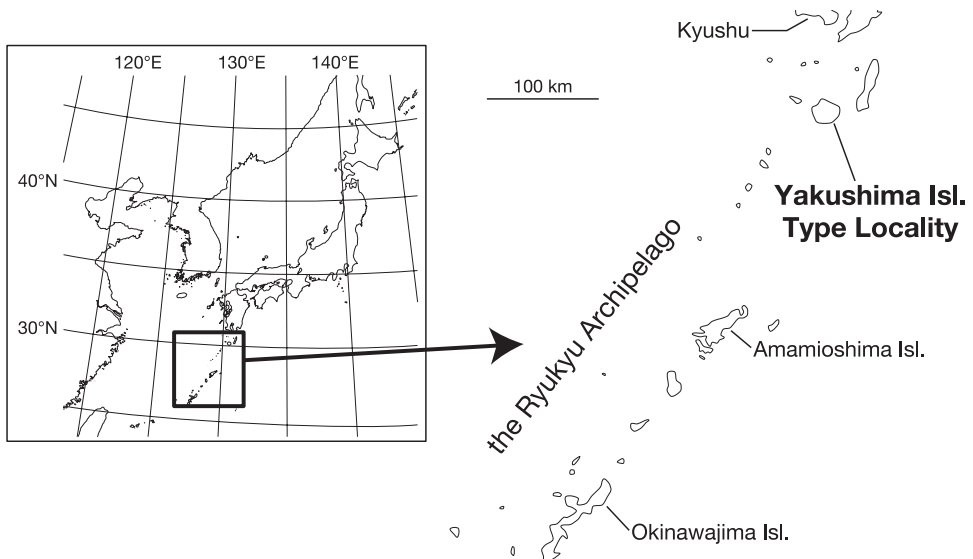


Figure 1. Map showing the northern and the central parts of the Ryukyu Archipelago, Japan.

posterior margin of the caudal sucker, and maximum body width (BW). Examination, dissection, and drawings of the specimens were accomplished under a stereoscopic microscope with a drawing tube (Leica M125). Specimens used in this study have been deposited in the Zoological Collection of Kyoto University (KUZ).

The numbering convention is based on Moore (1927): body somites are denoted by Roman numerals, and annuli in each somite are given alphanumeric designations.

PCR and DNA sequencing

The extraction of genomic DNA followed Nakano (2012). Primer sets used in this study are listed in Table 1: for 18S, A and L, C and Y, and O and B (Apakupakul et al. 1999); for histone H3, H3aF and H3bR (Colgan et al. 1998); for COI, LCO1490 and HCO2198 (Folmer et al. 1994), and LCO-in (Nakano 2012) and HCO-outout; for tRNA^{Cys}, tRNA^{Met}, 12S, tRNA^{Val} and 16S (tRNA^{Cys}–16S), 12SA-out and 12SB-in, and 12SA-in and 12SB-out (Nakano 2012). All amplification reactions were performed using a GeneAmp PCR System 2700 (Applied Biosystems) or a MyCycler (Bi-Rad Laboratories) using an Ex *Taq* Polymerase Kit (Takara Bio Inc.). Only for primer set O and B of 18S, 10% DMSO was included in mixtures. Reaction mixtures were heated to 94°C for 5 min, followed by 35 cycles of 94°C (10 s), 42.5°C for 18S, COI and tRNA^{Cys}-16S or 53°C for histone H3 (20 s), and 72°C (42 s for 18S, 21 s for histone H3, 1 min 13 s for COI, and 1 min for tRNA^{Cys}–16S) and a final extension at 72°C for 6 min. The amplified DNA fragments were purified using polyethylene glycol (20% PEG 6000) precipitation.

Table 1. PCR and cycle sequencing (CS) primers used in this study.

Gene	Primer name	Reaction	Primer sequence (5' → 3')	Source
18S				
1	A	PRC & CS	AACCTGGTTGATCCTGCCAGT	Apakupakul et al. (1999)
	L	PRC & CS	CCAACTACGAGCTTTTAACTG	Apakupakul et al. (1999)
2	C	PRC & CS	CGGTAATTCCAGCTCCAATAG	Apakupakul et al. (1999)
	Y	PRC & CS	CAGACAAATCGCTCCACCAAC	Apakupakul et al. (1999)
3	O	PRC & CS	AAGGGCACCACCAGGAGTGGAG	Apakupakul et al. (1999)
	B	PRC & CS	TGATCCTTCCGCAGGTTACCT	Apakupakul et al. (1999)
Histone H3				
	H3aF	PRC & CS	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (1998)
	H3bR	PRC & CS	ATATCCTTRGGCATRATRGTGAC	Colgan et al. (1998)
COI				
1	LCO1490	PRC & CS	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
	HCO2198	CS	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
2	LCO-in	CS	TCCAGAACGTATTCCATTATTTG	Nakano (2012)
	HCO-outout	PCR & CS	TACACATCTGGATAGTCTGAAT	This study
tRNA ^{Cys} –16S				
1	12SA-out	PCR & CS	TTGATGAACAACATTAAATTGC	Nakano (2012)
	12SB-in	CS	TAAGCTGCACTTTGACCTGA	Nakano (2012)
2	12SA-in	CS	AATTAAACAAGGATTAGATACCC	Nakano (2012)
	12SB-out	PCR & CS	AACCCATAATGCAAAAGGTAC	Nakano (2012)

All samples were sequenced in both directions. Sequencing reactions were performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Each sequencing reaction mixture was incubated at 96°C for 2 min, followed by 40 cycles of 96°C (10 s), 50°C (5 s), and 60°C (42 s for 18S, 21 s for Histone H3, 45 s for COI, and 40 s for tRNA^{Cys}-16S). The products were collected by ethanol precipitation and sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems). The obtained sequences were edited using DNA BASER (Heracle Biosoft S.R.L.). In this study, the following DNA sequences were newly obtained and deposited in GenBank (Table 2): 1) 18S sequences from the holotype (KUZ Z224) of the new species, the holotype (KUZ Z156) of *O. koikei* Nakano, 2012 and the topotype (KUZ Z181) of *O. octonaria* Oka, 1895; 2) histone H3 sequences from ten *Orobiddella* species, *Erpobiddella japonica* Pawłowski, 1962 (Erpobiddellidae), *Gastrostomobiddella monticola* Moore, 1929 (Gastrostomobiddellidae) and *Mimobiddella japonica* Blanchard, 1897 (Salifidae); 3) COI and tRNA^{Cys}-16S sequences from the holotype (KUZ Z224) and two of the paratypes (KUZ Z221, 223) of the new species. Among the new species, DNA sequences of the holotype (KUZ Z224) were analyzed in the present study. The other DNA sequences were taken from GenBank (Table 2).

Table 2. Samples used for the phylogenetic analyses. The information on voucher, collection locality, and GenBank accession numbers is indicated. Acronym: UNIMAS, the Universiti Malaysia Sarawak. Sources: ^aNakano (2012), ^bNakano et al. (2012).

Species	Voucher	18S	Histone H3	COI	tRNA ^{Cys} -16S
<i>Orobiddella esulcata</i>	KUZ Z29 Holotype	AB663655 ^b	AB698873	AB679664 ^a	AB679665 ^a
<i>Orobiddella dolichopharynx</i>	KUZ Z120 Holotype	AB663665 ^b	AB698876	AB679680 ^a	AB679681 ^a
<i>Orobiddella ijimai</i>	KUZ Z110 Topotype	AB663659 ^b	AB698877	AB679672 ^a	AB679673 ^a
<i>Orobiddella kawakatsuorum</i>	KUZ Z167 Topotype	AB663661 ^b	AB698878	AB679704 ^a	AB679705 ^a
<i>Orobiddella koikei</i>	KUZ Z156 Holotype	AB698883	AB698882	AB679688 ^a	AB679689 ^a
<i>Orobiddella mononoke</i> sp. n.	KUZ Z221			AB698862	AB698863
<i>Orobiddella mononoke</i> sp. n.	KUZ Z223			AB698864	AB698865
<i>Orobiddella mononoke</i> sp. n.	KUZ Z224 Holotype	AB698868	AB698869	AB698866	AB698867
<i>Orobiddella octonaria</i>	KUZ Z181 Topotype	AB698870	AB698871	AB679708 ^a	AB679709 ^a
<i>Orobiddella shimadae</i>	KUZ Z128 Holotype	AB663663 ^b	AB698875	AB679676 ^a	AB679677 ^a
<i>Orobiddella tsushimensis</i>	KUZ Z134 Holotype	AB663653 ^b	AB698872	AB679662 ^a	AB679663 ^a
<i>Orobiddella whitmani</i>	KUZ Z45 Topotype	AB663657 ^b	AB698874	AB679668 ^a	AB679669 ^a
<i>Erpobiddella japonica</i>	KUZ Z178	AB663648 ^b	AB698879	AB679654 ^a	AB679655 ^a
<i>Gastrostomobiddella monticola</i>	UNIMAS/A3/ BH01/10	AB663649 ^b	AB698880	AB679656 ^a	AB679657 ^a
<i>Mimobiddella japonica</i>	KUZ Z179	AB663650 ^b	AB698881	AB679658 ^a	AB679659 ^a

Phylogenetic analyses

Histone H3 and COI sequences were aligned by eye since there were no indels. Nuclear 18S and mitochondrial tRNA^{Cys}-16S sequences were aligned using MAFFT X-INS-i (Hofacker et al. 2002; Katoh and Toh 2008; McCaskill 1990; Tabei et al. 2008) taking

into account RNA secondary structure information, and then refined with GBLOCKS (Castresana 2000). The length of aligned sequences of 18S was 1787 bp, that of histone H3 was 327 bp, that of COI was 1266 bp, and that of tRNA^{Cys}-16S was 787 bp. The concatenated sequences thus yielded a total of 4167 bp positions.

Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). ML phylogenies were calculated using TREEFINDER v October 2008 (Jobb et al. 2004) with the tool package PHYLOGEARS v 2.0 (Tanabe 2008), and then non-parametric bootstrapping (Felsenstein 1985) was conducted with 500 replicates. The best-fit models for each partition were selected using the Akaike Information Criterion (Akaike 1974) by using KAKUSAN4 (Tanabe 2011): for 18S, the Jobb 2008 model (J2) with gamma distribution (+G) and proportion of invariant sites (+I) was selected; for the 1st position of histone H3, the Tamura-Nei model (TN93); for the 2nd position of histone H3, the Jukes-Cantor model (JC69); for the 3rd position of histone H3, J2+G; for the 1st position of COI, TN93+G+I; for the 2nd position of COI, the transversion model (TVM)+I; for the 3rd position of COI, the transition model (TIM)+G; and the general time reversal model (GTR)+G was selected for tRNA^{Cys}-16S. BI and Bayesian posterior probabilities (BPPs) were estimated using the MPI version of MRBAYES v 3.1.2 (Altekar et al. 2004; Huelsenbeck et al. 2001; Ronquist and Huelsenbeck 2003). The best-fit models for each partition were identified using the Bayesian Information Criterion (Schwarz 1978) also by using KAKUSAN4: for 18S, the Kimura 1980 model (K80)+I; for histone H3 1st and 2nd position, JC69; for histone H3 3rd position, the Hasegawa-Kishino-Yano model (HKY85)+G; for COI 1st position, GTR+I; for COI 2nd position, the Felsenstein 1981 model (F81)+I; for COI 3rd position, HKY85+G; and for tRNA^{Cys}-16S, GTR+G. Two independent runs for four Markov chains were conducted for 7 million generations and the tree was sampled every 100 generations. Based on checking the parameter estimates and convergence using TRACER v 1.5 (Rambaut and Drummond 2009), the first 15,001 trees were discarded.

The nodes with bootstrap value (BS) higher than 70% were regarded as sufficiently resolved (Hillis and Bull 1993). Nodes with BPP higher than 95% were considered statistically significant (Leaché and Reeder 2002).

Systematics

Genus *Orobdella* Oka, 1895

urn:lsid:zoobank.org:act:FA8333ED-8C17-41FD-AFC1-62A4F98D4AC1

Orobdella mononoke sp. n.

urn:lsid:zoobank.org:act:8B4ED1DA-E1B9-49A8-8B58-014A0921695C

http://species-id.net/wiki/Orobdella_mononoke

Figs 2–5

Diagnosis. In life, dorsal surface of somites I–XIII, XXVII and caudal sucker grayish purple and of somites XIV–XXVI amber, ventral surface grayish white. Somite VI



Figure 2. *Orobdella mononoke* sp. n., holotype, KUZ Z224, taken of live animal, dorsal view.

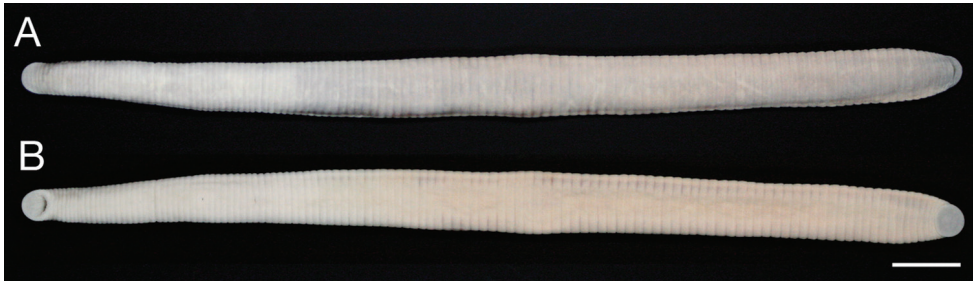


Figure 3. *Orobdella mononoke* sp. n., holotype, KUZ Z224. **A** Dorsal and **B** ventral views. Scale bar, 1 cm.

quadrannulate on dorsal, $b1 = b2 < a2 = a3$, and triannulate on venter, $a1 = a2 = a3$. Somite VII quadrannulate, somites VIII–XXV sexannulate, somite XXVI quinquannulate. Pharynx reaching to XIV. Gastropore conspicuous at XIII b2 (slightly anterior to middle of annulus). Gastroporal duct, winding at junction with gastropore, tubular but slightly bulbous at junction with crop. Male gonopore at XI c11/c12, female gonopore at XIII b2, behind gastropore, gonopores separated by $8 + 1/2$ annuli. Paired epididymides in XV–XIX (approximately four somites). Atrial cornua developed, ovate.

Type materials. KUZ Z224, **holotype**, dissected, collected from under a rock along a mountain trail at Shiratani–unsuikyo, Yakushima, Kagoshima Pref. (Yakushima Island), Japan ($30^{\circ}22.78'N$, $130^{\circ}34.49'E$; Alt. 648 m), by Takafumi Nakano on 29 October, 2011.

Four **paratypes** collected from under rocks along mountain trails in Yakushima, Kagoshima Pref. (Yakushima Island), Japan, by Takafumi Nakano. Two specimens from the type locality: KUZ Z221 ($30^{\circ}22.87'N$, $130^{\circ}34.68'E$; Alt. 649 m), dissected, on 28 October, 2011, and KUZ Z225 ($30^{\circ}22.75'N$, $130^{\circ}34.49'E$; Alt. 646 m), on 29 October, 2011. Two specimens from Kusugawa on 28 October, 2011: KUZ Z222 ($30^{\circ}23.76'N$, $130^{\circ}35.25'E$; Alt. 363 m), and KUZ Z223 ($30^{\circ}23.75'N$, $130^{\circ}35.25'E$; Alt. 363 m), dissected.

Etymology. The specific name is from the Japanese animation movie title ‘Mononoke-hime (Princess Mononoke)’. The type locality of this new species is the origin of an epic forest in that movie. The specific name is a Japanese word, not a Latin or latinized word.

Description of holotype. Body firm, muscular, elongated, gaining regularly in width in caudal direction, dorso-ventral depressed, sides nearly parallel from mid length to point just anterior to caudal sucker, BL 139.3 mm, BW 9.2 mm (Figs 2, 3).

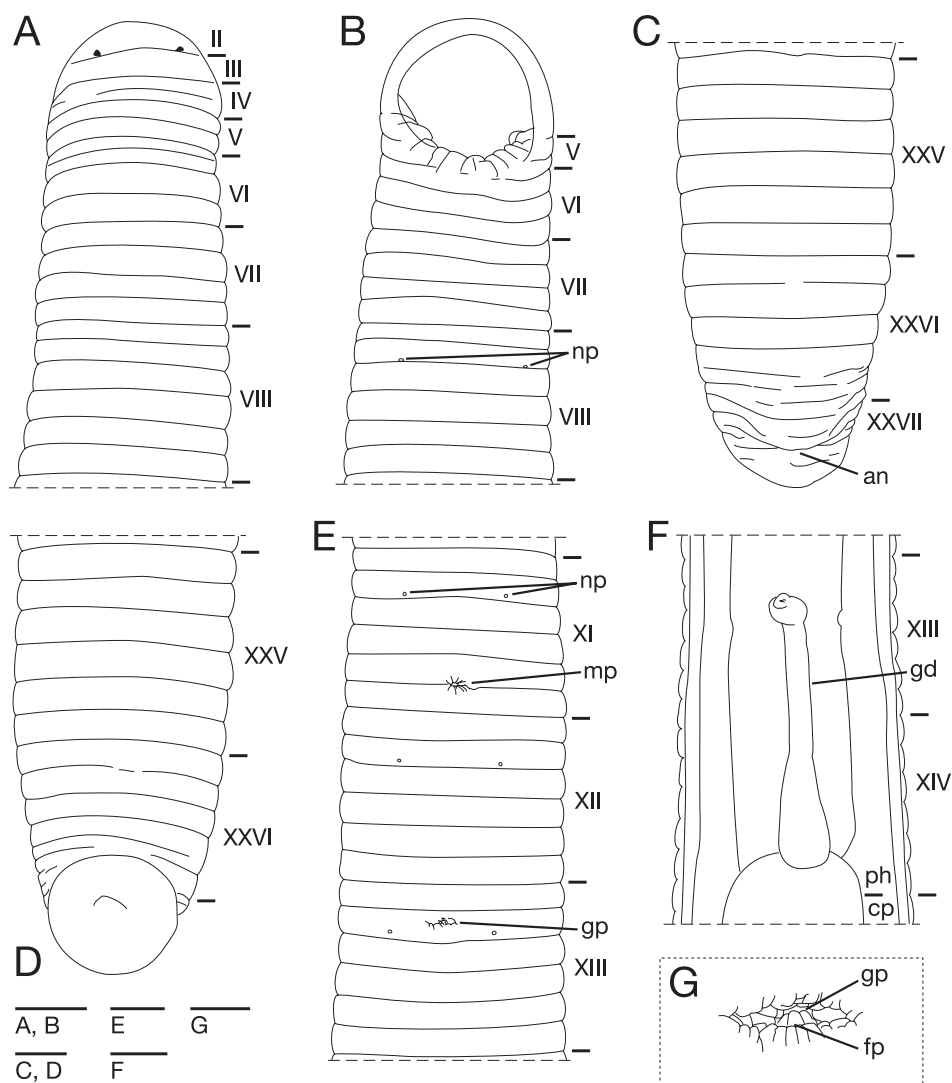


Figure 4. *Orobdella mononoke* sp. n., holotype, KUZ Z224. **A** Dorsal view of somites I–VIII **B** ventral view of somites I–VIII **C** dorsal view of somites XXV–XXVII and caudal sucker **D** ventral view of somites XXV–XXVII and caudal sucker **E** ventral view of somites XI–XIII **F** ventral view of gastroporal duct; and **G** ventral view of gastropore and female gonopore. Scale bars, 2 mm (A–F) and 0.5 mm (G). Abbreviations: an, anus; cp, crop; fp, female gonopore; gd, gastroporal duct; gp, gastropore; mp, male gonopore; np, nephridiopore; and ph, pharynx.

Caudal sucker ventral, oval, its diameter smaller than BW (Figs 3B, 4D). In life, dorsal surface of somites I–XIII, XXVII and caudal sucker grayish purple, and of somites XIV–XXVI amber (Fig. 2), ventral surface grayish white. Color faded in preservative, without any dark lines (Fig. 3).

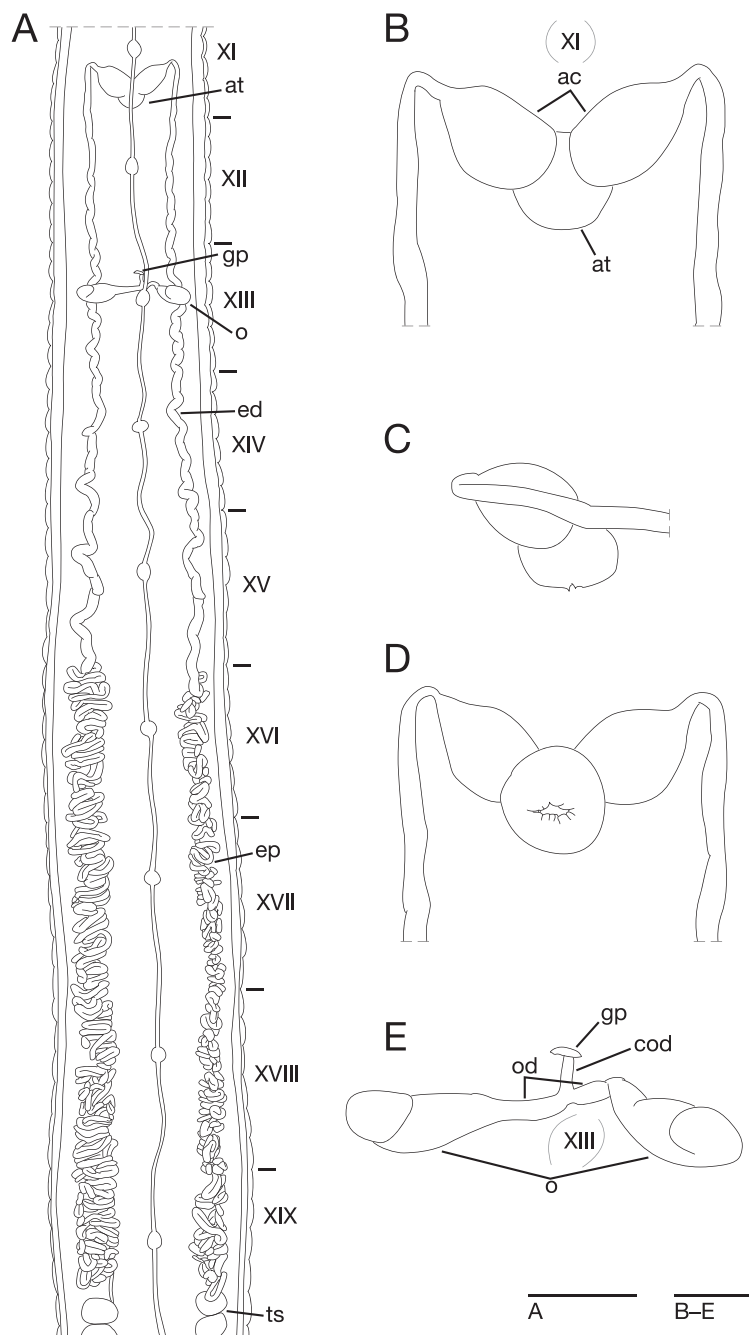


Figure 5. *Orobdella mononoke* sp. n., holotype, KUZ Z224. **A** Dorsal view of reproductive system including ventral nervous system **B** dorsal view of male atrium including position of ganglion XI **C** lateral view of male atrium **D** ventral view of male atrium; and **E** dorsal view of female reproductive system including position of ganglion XIII. Scale bars, 5 mm (**A**) and 1 mm (**B–E**). Abbreviations: ac, atrial cornu; at, atrium; cod, common oviduct; ed, ejaculatory duct; ep, epididymis; gp, gastropore; o, ovisac; od, oviduct; and ts, testisac.

Somite I completely merged with prostomium (Fig. 4A). Somites II and III uniannulate (Fig. 4A). Somites IV and V biannulate, $(a1+a2) = a3$ (Fig. 4A), V $a3$ forming posterior margin of oral sucker (Fig. 4B). Somite VI quadrannulate on dorsal, $b1 = b2 < a2 = a3$, triannulate on venter, $a1 = a2 = a3$ (Fig. 4A–B). Somite VII quadrannulate, $a1 = a2 = b5 = b6$ (Fig. 4A–B). Somites VIII–XXV sexannulate. $b1 = b2 = a2 = b5 = c11 = c12$ (Fig. 4A–E). Somite XXVI quinquannulate, $b1 = b2 = a2 < b5 = b6$, $b5$ and $b6$ with slight furrows on dorsal (Fig. 4C–D), XXVI $b5$ being last complete annulus on venter (Fig. 4D). Somite XXVII comprising a few furrows; anus behind it with no post-anal annulus (Fig. 4C).

Anterior ganglionic mass in VI $a2$ and $a3$. Ganglion VII in $a1$ and $a2$. Ganglia VIII–XV, XXII and XXIII in $a2$ of each somite (Fig. 5A). Ganglia XVI–XXI and XXIV in $b2$ and $a2$ of each somite (Fig. 5A). Ganglion XXV in $b2$. Ganglion XXVI in XXV $c12$ and XXVI $b1$. Posterior ganglionic mass in XXVI $a2$ and $b5$.

Eyes three pairs, first pair dorsally on posterior margin of II (Fig. 4A), second pair dorsolaterally on middle of V ($a1 + a2$). Nephridiopores in 17 pairs, ventrally at posterior margin of $a1$ of each somite of VIII–XXIV (Fig. 4B, E). Papillae numerous, minute, hardly visible, one row on every annulus.

Pharynx agnathous, euthylaematous, reaching to XIV/XV (Fig. 4F). Crop tubular, acaecate, in XIV/XV to XXI $b2/a2$. Gastropore conspicuous, ventral, located slightly anterior to middle of XIII $b2$ (Fig. 4E, G). Gastroporal duct, winding at junction with gastropore, tubular but slightly bulbous at junction with crop, joining with crop in XIV $c11$ (Fig. 4F). Intestine tubular, acaecate, in XXI $b2/a2$ to XXIV $b2/a2$. Rectum, tubular, thin-walled.

Male gonopore in the furrow of XI $c11/c12$ (Fig. 4E). Female gonopore located slightly anterior to middle of XIII $b2$, inconspicuous, located behind gastropore (Fig. 4E, G). Gonopores separated by $8 + 1/2$ annuli (Fig. 4E). Testisacs multiple, one or two testisacs on each side in each annulus, in XIX $c11$ to XXV $b5$ (Fig. 5A). Paired epididymides in XVI $b2$ to XIX $b5$ (Fig. 5A). Ejaculatory bulbs absent. Ejaculatory ducts in XI $b5$ to XVI $b2$, loosely coiled, each winding from each junction with epididymis, narrowing at junction with atrial cornu, then turning sharply inward toward atrial cornu without pre-atrial loop (Fig. 5A–D). Pair of atrial cornua in XI $b5$ and $c11$, muscular, ovate (Fig. 5A–B, D). Atrium short, muscular, globular in XI $c11$ and $c12$ (Fig. 5B–D). Penis sheath and penis absent. Ovisacs one pair, thin-walled, globular, in XIII $a2$ and $b5$ (Fig. 5A, E). Oviducts thin-walled, right oviduct crossing ventrally beneath nerve cord, both oviducts converging into common oviduct in XIII $b2$ (Fig. 5A, E). Common oviduct thin-walled, short, directly ascending to female gonopore (Fig. 5E).

Variation. In life, color generally same as holotype (Fig. 2). Somites III and IV uniannulate. Pharynx reaching to XIV $b5/c11$ –XIV $c11/c12$. Crop reaching to XXI $b2/a2$ –XXI $a2$. Gastroporal duct joining with crop in XIV $b5$; immature specimen (KUZ Z223), simple tubular. Intestine reaching to XXIV $b1$ –XXIV $b5$. Testisacs in XIX $b1$ to XXIV $c11$. Epididymides in XV $a2$ to XVIII $c11$. Immature specimen (KUZ Z223), pair of atrial cornua in XI $c11$, fusiform. Left oviduct crossing ventrally beneath nerve cord.

Distribution. Known from mountainous regions of Yakushima Island, Japan (Fig. 1).

Phylogenetic position. The ML tree with $\ln L = -14306.80$ (Fig. 6) was nearly identical to the obtained BI tree (not shown). Monophyly of the genus *Orobdella* was confirmed (BS = 99 %, BPP = 100 %). The genus *Orobdella* then divided into two clades: clade A (BS = 99 %, BPP = 100 %) consisted of two species from Hokkaido, Japan, *O. kawakatsuorum* Richardson, 1975 and *O. koikei*; and clade B (BS = 98 %, BPP = 100 %) included all the other *Orobdella* species. Clade B comprised two subclades: subclade B1 was *Orobdella tsushimensis* Nakano, 2011 from Tsushima Island, Japan; and subclade B2 (BS = 70 %, BPP = 100 %) was further divided into two subclades. Subclade B2a (BS = 92 %, BPP = 100 %) included *Orobdella mononoke* sp. n., *Orobdella esulcata* Nakano, 2010 from Kyushu, and two *Orobdella* species from the Ryukyu Archipelago, *O. dolichopharynx* and *O. shimadae*. Subclade B2b (BS = 73 %, BPP = 100 %) consisted of three species from Honshu, Japan, *O. whitmani* Oka, 1895, *O. ijimai* and *O. octonaria*.

In subclade B2a, monophyly of *Orobdella dolichopharynx* and *O. shimadae* was well supported (BS = 93 %, BPP = 100 %). However, the precise phylogenetic position of *O. mononoke* sp. n. in the subclade could not be determined. In the ML analysis, *Orobdella mononoke* sp. n. and *O. esulcata* formed a monophyletic clade, but this clade was not supported well (BS = 30 %). In the BI analysis, *Orobdella mononoke* sp. n. and two Ryukyu Archipelago species formed a monophyletic clade, but this relationship was not also supported (BPP = 77 %).

Remarks. *Orobdella mononoke* sp. n. differs from the three other sexannulate congeneric species, *O. ijimai*, *O. dolichopharynx*, and *O. shimadae*, in the following characteristics (Table 3): 1) dorsal surface bicolor, I–XIII, XXVII and caudal sucker grayish purple, XIV–XXVI amber; 2) VI quadrannulate on dorsal; 3) VII quadrannulate; 4) VIII sexannulate; 5) gonopores separated by $8 + 1/2$ annuli; 6) pharynx reaching to XIV; 7) gastroporal duct tubular but bulbous at junction with crop; 8) epididymides in XV–XIX (approximately four somites); and 9) atrial cornua ovate. *Orobdella mononoke* sp. n. is clearly distinguished from *O. esulcata*, *O. kawakatsuorum*, *O. koikei*, *O. tsushimensis*, *O. octonaria* and *O. whitmani*, in having mid-body somites that are sexannulate; they are quadrannulate in *O. esulcata*, *O. kawakatsuorum*, *O. koikei*, *O. tsushimensis* and *O. whitmani*, and octannulate in *O. octonaria*.

The trees obtained in this study are nearly identical to those obtained in other phylogenetic analyses of the genus *Orobdella* (Nakano 2012; Nakano et al. 2012). However, the phylogenetic position of *O. mononoke* sp. n. still remains uncertain. Further taxon samplings will be needed to obtain robust phylogeny of the genus *Orobdella*.

Orobdella mononoke sp. n. inhabits Yakushima Island, which is located in the northern part of the Ryukyu Archipelago (Fig. 1). In the Ryukyu Archipelago, two sexannulate *Orobdella* species have been described: 1) *O. dolichopharynx* from Amami-oshima Island; and 2) *O. shimadae* from Okinawajima Island. These two species have the following characteristics in common: 1) long pharynx, reaching to somite XVI; 2) rudimentary gastroporal duct and absence of gastropore; 3) absence of epididymides;

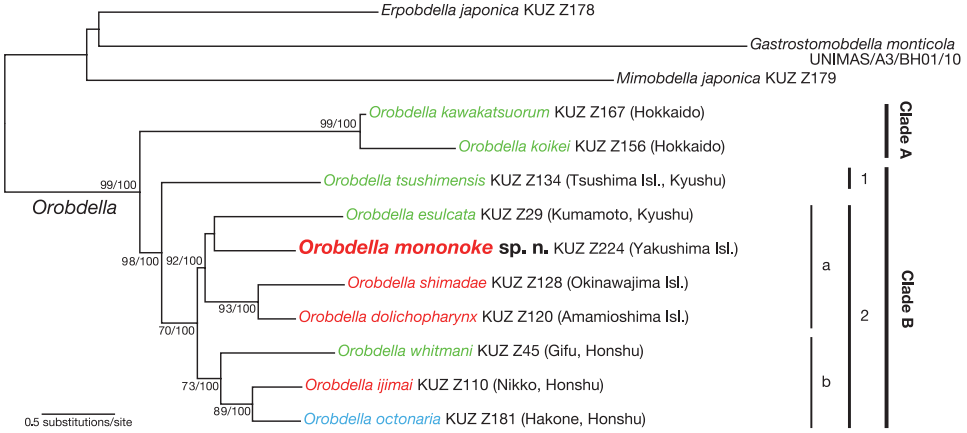


Figure 6. The ML tree of 4167 bp of nuclear 18S rDNA and histone H3 and mitochondrial COI, tRNA-Cys, tRNA^{Met}, 12S rDNA, tRNA^{Val} and 16S rDNA. A species name in green indicates a quadrannulate species; in red, sexannulate; and in blue, octannulate. The numbers associated with the nodes represent the bootstrap values for ML (BS)/ and Baysian posterior probabilities (BPPs). BS higher than 70% and/ or BPP higher than 95% are indicated.

Table 3. Comparisons of morphological characters between *Orobdella mononoke* sp. n. and three sexannulate congeneric species.

Character	<i>O. mononoke</i> sp. n.	<i>O. dolichopharynx</i>	<i>O. ijimai</i>	<i>O. shimadae</i>
Color of dorsal surface	bicolor, I–XIII, XXVII and caudal sucker grayish purple, XIV–XXVI amber	yellowish green	yellowish green	yellowish green
Annulation of VI	quadrannulate on dorsal	triannulate	triannulate	triannulate
Annulation of VII	quadrannulate	quadrannulate	quadrannulate	triannulate
Annulation of VIII	sexannulate	quinnuannulate	sexannulate	quinnuannulate
Number of annuli between gonopores	8 + 1/2	8	1/2 + 7 + 1/2	9
Pharynx	reaching to XIV	reaching to XVI	reaching to XIV	reaching to XVI
Gastroporal duct	tubular, but bulbous at junctions with crop	tubular, reaching to XVI	bulbous	tubular reaching to XV
Epididymides	in XV–XIX (about four somites)	absent	in XVI–XIX (about two and half somites)	absent
Atrial cornua	ovate	absent	ellipsoid	absent

and 4) absence of male atrial cornua. Although *Orobdella mononoke* sp. n. is a sexannulate species, this species does not share such morphological characteristics. *Orobdella mononoke* sp. n. possesses 1) normal length pharynx for the genus *Orobdella*, 2) developed gastroporal duct and conspicuous gastropore, 3) epididymides in XV–XIX, 4) ovate atrial cornua. Molecular phylogenetic analyses in this study also could not show

monophyly of the three species in the Ryukyu Archipelago, *O. mononoke* sp. n., *O. dolichopharynx* and *O. shimadae*. These differences of morphological characteristics and molecular phylogenetic analyses suggest that *Orobodella mononoke* sp. n. is not closely related to *O. dolichopharynx* and *O. shimadae*. In vertebrates, the fauna of the Osumi Islands, in which Yaushima Island is included, is related to that of Kyushu (Toda et al. 2003). In the case of leeches, *Haemadipsa japonica* Whitman, 1886, which inhabits Honshu, Shikoku and Kyushu, Japan, is distributed in Yakushima Island (Itoh 2003). In islands of the Ryukyu Archipelago south of Yakushima Island, however, another species, *Haemadipsa rjukjuana* Oka, 1910, is distributed (Lai et al. 2011). A recent molecular phylogenetic study revealed that *H. japonica* and *H. rjukjuana* are not closely related species (Borda and Siddall 2011). These facts are collateral evidence that *O. mononoke* sp. n. is not very closely related to *O. dolichopharynx* and *O. shimadae*. Whether or not this is true, additional inventory surveys and molecular phylogenetic studies are needed to reveal the phylogenetic relationships within and the biogeographical history of the genus *Orobodella*.

Acknowledgements

The author is grateful to Professor Tsutomu Hikida (Kyoto University; KU) for his helpful comments and suggestions to improve this manuscript. I am also grateful to Koshiro Eto (KU) for his help in collecting specimens in Yakushima Island, to Dr Zainudin Ramlah (UNIMAS) for permitting me to use the *Gastrostomobdella* specimen for molecular analyses, to Dr Elizabeth Nakajima (KU) for checking the English of this text, to two anonymous reviewers and Dr Fredric R. Govedich (Southern Utah University) for their constructive comments on this manuscript, and to Hiroshi Noda (KU) and Eri Kawaguchi (KU) for their technical support. I also express my sincere thanks to Naoki Koike (KU), Dr Kanto Nishikawa (KU) and Taku Shimada (Ant Room) for providing specimens. This study was financially supported in part by a Grant-in Aid for Biodiversity and Evolutionary Research of Global COE (A06) from MEXT, Japan, to Kyoto University.

References

- Akaike H (1974) A new look at the statistical model identification. IEEE Transactions on Automatic Control 19: 716–723. doi: 10.1109/TAC.1974.1100705
- Altekar G, Dwarkadas S, Huelsenbeck JP, Ronquist F (2004) Parallel Metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics 20: 407–415. doi: 10.1093/bioinformatics/btg427
- Apakupakul K, Siddall ME, Bureson EM (1999) Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences. Molecular Phylogenetics and Evolution 12: 350–359. doi: 10.1006/mpev.1999.0639

- Blanchard R (1897) Hirudinées du Musée de Leyde. Notes from the Leyden Museum 19: 73–113.
- Borda E, Siddall ME (2011) Insights into the evolutionary history of Indo-Pacific bloodfeeding terrestrial leeches (Hirudinida :Arhynchobdellida : Haemadipsidae). Invertebrate Systematics 24: 456–472. doi: 10.1071/IS10013
- Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molecular Biology and Evolution 17: 540–552.
- Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. Australian Journal of Zoology 46: 419–437. doi: 10.1071/ZO98048
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791. doi: 10.2307/2408678
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology 42: 182–192. doi: 10.1093/sysbio/42.2.182
- Hofacker IL, Fekete M, Stadler PF (2002) Secondary structure prediction for aligned RNA sequences. Journal of Molecular Biology 319: 1059–1066. doi: 10.1016/S0022-2836(02)00308-X
- Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310–2314. doi: 10.1126/science.1065889
- Itoh T (2003) Class Hirudinea. In: Nishida M, Shikatani N, Shokita S (Eds) The Flora and Fauna of Inland Waters in the Ryukyu Islands. Tokai University Press, Hadano, 197–202.
- Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evolutionary Biology 4: 18. doi: 10.1186/1471-2148-4-18
- Katoh K, Toh H (2008) Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. BMC Bioinformatics 9: 212. doi: 10.1186/1471-2105-9-212
- Lai Y-T, Nakano T, Chen J-H (2011) Three species of land leeches from Taiwan, *Haemadipsa rjukjuana* comb. n., a new record for *Haemadipsa picta* Moore, and an updated description of *Tritetrabdella taiwana* (Oka). ZooKeys 139: 1–22. doi: 10.3897/zookeys.139.1711
- Leaché AD, Reeder TW (2002) Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. Systematic Biology 51: 44–68. doi: 10.1080/106351502753475871
- McCaskill JS (1990) The equilibrium partition function and base pair binding probabilities for RNA secondary structure. Biopolymers 29: 1105–1119. doi: 10.1002/bip.360290621
- Moore JP (1927) The segmentation (metamerism and annulation) of the Hirudinea. In: Harding WA, Moore JP. The Fauna of British India, including Ceylon and Burma Hirudinea. Taylor and Francis, London, 1–12.

- Moore JP (1929) Leeches from Borneo with descriptions of new species. *Proceedings of the Academy of Natural Sciences of Philadelphia* 81: 267–295.
- Nakano T (2010) A new species of the genus *Orobdella* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae) from Kumamoto, Japan, and a redescription of *O. whitmani* with the designation of the lectotype. *Zoological Science* 27: 880–887. doi: 10.2108/zsj.27.880
- Nakano T (2011a) A new species of *Orobdella* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae) from Tsushima Island, Japan. *Species Diversity* 16: 39–47.
- Nakano T (2011b) Redescription of *Orobdella ijimai* (Hirudinida: Arhynchobdellida: Gastrostomobdellidae), and two new species of *Orobdella* from the Ryukyu Archipelago, Japan. *Zootaxa* 2998: 1–15.
- Nakano T (2012) A new species of *Orobdella* (Hirudinida, Arhynchobdellida, Gastrostomobdellidae) and redescription of *O. kawakatsuorum* from Hokkaido, Japan with the phylogenetic position of the new species. *ZooKeys* 169: 9–30. doi: 10.3897/zookeys.169.2425
- Nakano T, Ramlah Z, Hikida T (2012) Phylogenetic position of gastrostomobdellid leeches (Hirudinida, Arhynchobdellida, Erpobdelliformes) and a new family for the genus *Orobdella*. *Zoologica Scripta* 41: 177–185. doi: 10.1111/j.1463-6409.2011.00506.x
- Oceguera-Figueroa A, Phillips AJ, Pacheco-Chaves B, Reeves WK, Siddall ME (2011) Phylogeny of macrophagous leeches (Hirudinea, Clitellata) based on molecular data and evaluation of the barcoding locus. *Zoologica Scripta* 40: 194–203. doi: 10.1111/j.1463-6409.2010.00465.x
- Oka A (1895) On some new Japanese land leeches. (*Orobdella* nov. gen.). *The Journal of the College of Science, Imperial University, Japan* 8: 275–306.
- Oka A (1910) Key to Japanese leeches. *Dobutsugaku Zasshi* 22: 56–64.
- Pawłowski LK (1962) O występowaniu pijawki *Erpobdella octoculata* (L.) w Japonii. *Zeszyty Naukowe Uniwersytetu Łódzkiego Nauki Matematyczno-przyrodnicze Seria II* 12: 127–136.
- Rambaut A, Drummond AJ (2009) Tracer. 1.5: <http://tree.bio.ed.ac.uk/software/tracer/>
- Richardson LR (1971) Gastrostomobdellidae f. nov. and a new genus for the gastroporous *Orobdella octonaria* Oka, 1895, of Japan (Hirudinoidea: Arhynchobdellae). *Bulletin of the National Science Museum (Tokyo)* 14: 585–602.
- Richardson LR (1975) A new species of terricolous leeches in Japan (Gastrostomobdellidae, *Orobdella*). *Bulletin of the National Science Museum Series A (Zoology)* 1: 39–56.
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574. doi: 10.1093/bioinformatics/btg180
- Sawyer RT (1986) *Leech Biology and Behaviour*. Clarendon Press, Oxford, 1065 pp.
- Schwarz G (1978) Estimating the dimension of a model. *The Annals of Statistics* 6: 461–464. doi: 10.1214/aos/1176344136
- Tabai Y, Kiryu H, Kin T, Asai K (2008) A fast structural multiple alignment method for long RNA sequences. *BMC Bioinformatics* 9: 33. doi: 10.1186/1471-2105-9-33
- Tanabe AS (2008) Phylogears. 2.0: <http://www.fifthdimension.jp/>
- Tanabe AS (2011) Kakusan4 and Aminosan: two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data. *Molecular Ecology Resources* 11: 914–921. doi: 10.1111/j.1755-0998.2011.03021.x

- Toda M, Shokita S, Nishida M (2003) Origin of the Ryukyu Biota. In: Nishida M, Shikatani N, Shokita S (Eds) The Flora and Fauna of Inland Waters in the Ryukyu Islands. Tokai University Press, Hadano, 25–32.
- Whitman CO (1886) The leeches of Japan. Quarterly Journal of Microscopical Science New Series 26: 317–416.

