ZooKeys 190: 1–19 (2012)
doi: 10.3897/zookeys.190.3059
www.zookeys.org

RESEARCH ARTICLE

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

Revision of *Ilyphagus* Chamberlin, 1919 (Polychaeta, Flabelligeridae)

Sergio I. Salazar-Vallejo[1]

[1] *El Colegio de la Frontera Sur, CONACYT, Departamento de Ecología Acuática, Chetumal, Quintana Roo, México*

Corresponding author: *Sergio I. Salazar-Vallejo* (ssalazar@ecosur.mx; savs551216@hotmail.com)

Academic editor: *Chris Glasby* | Received 8 March 2012 | Accepted 24 April 2012 | Published 4 May 2012

Citation: Salazar-Vallejo SI (2012) Revision of *Ilyphagus* Chamberlin, 1919 (Polychaeta, Flabelligeridae). ZooKeys 190: 1–19. doi: 10.3897/zookeys.190.3059

Abstract

Ilyphagus Chamberlin, 1919 includes abyssal, fragile benthic species. Most species have large cephalic cages but chaetae are brittle and easily lost which may explain why the original definition included species with a cephalic cage or without it. The type species, *I. bythincola* Chamberlin, 1919, together with another species (*I. pluto* Chamberlin, 1919) were described as lacking a cephalic cage whereas a third species (*I. ascendens* Chamberlin, 1919) was described with one. To clarify this situation, all available type and non-type materials were studied. *Ilyphagus* is redefined to include species with digitiform bodies, abundant filiform papillae and a thin body wall; their neurochaetae are thick, anchylosed aristate spines, and all species have a cephalic cage (in the type species the presence of a cage is inferred from the remaining chaetal scars). *Ilyphagus pluto*, which also lacks a a cephalic cage is determined here to be a holothurian. The redefined genus contains *I. bythincola* (incl. *I. ascendens*), *I. coronatus* Monro, 1939, *I. hirsutus* Monro, 1937, and *I. wyvillei* (McIntosh, 1885).

Keywords

Annelida, *Bradabyssa, Diplocirrus, Therochaeta*, caruncle, branchial filaments, neurochaetae, taxonomy

Introduction

Deep-sea animals are often bizarre. By the end of the XIX century, or during the early XX century, the dredged animals collected during deep-sea expeditions were surprising marine zoologists. Trying to cope with the unexpected body patterns, some general features might have been regarded as diagnostic for grouping species that could be more easily studied. This might explain why Chamberlin (1919) proposed a new name for some polychaetes that were regarded as feeding on mud. By combining the Greek words for mud (*Ilys*) and glutton (*phagos*), he established *Ilyphagus.* For its definition, he relied upon three different species: one with a well-developed cephalic cage, and two others apparently deprived of it. Thus, the generic diagnosis was wide enough to include species in either condition, and that concept prevailed in the current definition (Fauchald 1977). The cephalic cage is a distinctive feature for most flabelligerids. It is made up of long chaetae, usually pointing forward and stemming from at least one of the first few chaetigers. Although it has been a useful diagnostic feature to separate flabelligerid genera, it has been enigmatic how a single genus might contain two distinct morphological patterns; one with a cephalic cage and the other without it. Further, the body shape for *Ilyphagus* has been regarded as depressed or even disc-shaped (Fauchald 1977:117). This, in turn, is explained by the collapse of the body due to compression because the body is subcylindrical in life. Dredging or sieving sediments might distort the body shape, because the thin body wall is easily broken, such that the body becomes depressed, or flat.

Monro (1937:305) made some interesting comments on the genus. He essentially indicated two issues: first, that *Ilyphagus* is an abyssal genus, and second, that there was a single species that included the three described by Chamberlin (*I. bythincola*, *I. pluto*, and *I. ascendens*), and his *I. hirsutus.* Monro was correct on the first statement, because all species do come from deep water habitats; for the second assertion, however, he failed to acknowledge the differences in papillae development and chaetae. Later, he indicated that all species in the genus were described as lacking a cephalic cage (Monro 1939:131), which was not the case, and that the cephalic cage chaetae may be present, but may be broken during collection. The latter is correct and will be discussed later.

Ilyphagus species are poorly known because of their life in deep-water and because they have not been found in hydrothermal vents, cold seeps or whale remains, which have recently received a lot of attention. Further, the species of *Ilyphagus* have very low abundances, most species being known from a single or few specimens. They might live in the sediment-water interface, with the body barely covered by sediment and the very long cephalic cage chaetae are probably used as an anti-predation device.

Because there are problems in the definition for the genus and for the species, the purpose of this revision was to study all material available and currently regarded as belonging in *Ilyphagus.* It was expected that this study would result in a redefinition of the genus, a clarification of the diagnostic features, and probably a modification of the included species. The presence of a cephalic cage is confirmed for the genus and after redefining it, it contains four species.

Methods

All specimens were studied under the stereomicroscope. They were often stained by a few seconds immersion in an oversaturated solution of methyl-green in 70% ethanol which is temporary. Individual chaetae or parapodial rami were observed in compound microscopes. The plates were made by selecting one or by editing several digital pictures of the same objects. The anterior end was dissected to study the head structure and the associated appendages. The materials belong to the following collections.

Museum and collections acronyms

LACM-AHF	Museum of Natural History, Los Angeles, Allan Hancock Foundation Polychaete Collection.
NHML	The Natural History Museum, London.
SIORAS	Shirshov Institute of Oceanology, Russian Academy of Sciences, Moscow.
USNM	National Museum of Natural History, Smithsonian Institution, Washington.
ZIRAS	Zoological Institute, Russian Academy of Sciences, Sankt-Peterburg.
ZMH	Zoologisches Museum und Institut, Universitat Hamburg, Hamburg.

Results

Morphology

Body. The body is sausage-shaped; it may be short and oval, or cigar-shaped, and may even be swollen anteriorly. In some species, juveniles have thinner bodies and may be confused with some species of *Bradabyssa* Hartman, 1967; however, in *Ilyphagus*, the body wall is thin and covered by delicate, long papillae, which are not embedded by the tunic, resulting in a pilose surface. The papillae are filiform, barely swollen distally, if any at all, and are often covered by fine sediment particles. Once the excessive sediment is removed, individual papillae might have a thin or a thick layer of adherent sediment particles. Parapodia are poorly developed and the brittle chaetae emerge from the body wall. Gonopodial lobes are difficult to detect due to the abundant papillae; when they are well developed, they can be visible in chaetigers 5–6.

Cephalic cage. The species belonging in *Ilyphagus* carry very long chaetae in the first 1–2 chaetigers. The first parapodia are markedly displaced dorsally, with both parapodial rami very close to each other and approaching the middorsal line. The second chaetiger has notopodia more dorsally displaced than the first but they are quite separated from the corresponding neuropodia. The second chaetiger's notochaetae are arranged in an oblique line or in a bundle, whereas the first notochaetae are more frontally located. Further, these notochaetae are often the longest, being as long as the whole body, or even longer; this chaetal length is remarkable among the family and among all Polychaeta. Sometimes, these long notochaetae can appear spirally twisted, as in the maldanid polychaete *Nicomache maculata* Arwidsson (Kennedy and Kryvi 1978). However, the chaetae in *Ilyphagus,* instead of being formed by spirally twisted fibers, have parallel microvilli and the external surface has a series of constrictions along the shaft which slowly rotate towards the tip. Nevertheless, these spiral chaetae are brittle and seldom available for observation, such that it is unknown how widespread they are, or if the finding detailed below is based upon accidental growth. All cephalic cage chaetae can be broken off, but their presence can be determined by chaetal scars or holes through the body wall, or by making a longitudinal dissection along the anterior end, if there are several specimens available and papillae cover chaetal scars. A large fan of companion chaetae is visible where the notochaetae of the first chaetiger are present.

Body chaetae. Notochaetae are multiarticulated, at least distally; there are usually 1–3 notochaetae per bundle and often one has long articles, whereas the others may have short articles only basally or throughout the chaeta. Neurochaetae are thicker than notochaetae; those present in the first chaetiger, or first few chaetigers, may be multiarticulated as well. In the former case, there are some chaetigers with transitional chaetae. They have long articles throughout the chaetae in the second chaetiger, but in following chaetigers the articulated region is progressively reduced, such that it becomes restricted to a short distal region. From chaetigers 2–4, anchylosed aristate spines are present. Neurochaetae have basal transverse marks, but these marks become slightly oblique beyond the median region. The distal region of the neurochaetae is delicate, often hyaline, and aristate; in one species, it is hirsute which is due to the rupture and exposure of the abundant oblique fibers .

Anterior end. The anterior end carries two large thick palps and several thick branchial filaments, arranged in 1–2 concentric rows which often resemble a horse-shoe pattern. The nephridial lobes are difficult to find, because they are located on the inner side of the branchial row at about the centre of the branchial plate. The species are apparently devoid of eyes and caruncle; the lack of a caruncle is unexpected because it has been found in all other flabelligerid genera. Although the underlying prostomial wall may be depressed, the ciliary bands may be present; however, because the preservation fluids penetrate slowly, these bands may be difficult to detect. Histological or SEM observations would be required to determine whether the expected bands of cilia are absent, or if they have been reduced.

Systematics

Class Polychaeta Grube, 1850
Order Flabelligerida Pettibone, 1982
Family Flabelligeridae de Saint-Joseph, 1894

Ilyphagus Chamberlin, 1919 restricted

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

Ilyphagus Chamberlin, 1919:402; Hartman 1965:177; Fauchald 1977:117.

Type species. *Ilyphagus bythincola* Chamberlin, 1919, by original designation.

Diagnosis (emended): Body digitiform, rounded at both ends, densely covered by thin, abundant papillae. Body wall thin. Cephalic cage well developed; notochaetae dorsal, arranged in transverse rows. Parapodia biramous, inconspicuous. Notochaetae multiarticulated capillaries; neurochaetae thicker, anchylosed aristate spines.

Remarks. The type species, *I. bythincola* Chamberlin, 1919, originally described as lacking cephalic cage, in reality has one but the chaetae are broken and only their embedded bases or some chaetal scars are left. The other species described as lacking a cephalic cage, *I. pluto* Chamberlin, 1919, is not a polychaete but an abyssal holothurian which belongs to the synallactid genus *Meseres* (identified by the late Dr. Cynthia Ahearn, USNM). The inclusion of *I. pluto* allowed a rather broad concept for body shape and chaetal patterns, because this species is rather cylindrical and completely lacks a cephalic cage. Further, Chamberlin (1919) emphasized the lack of large, wide papillae that resemble tubercles, as those found in some species of *Brada.* Thus, he recognized the difference and restricted the inclusion to those species lacking thick papillae (or tubercles).

Ilyphagus and *Bradabyssa* Hartman, 1967 are closely allied (Salazar-Vallejo *et al.* 2008) because they have anchylosed neurospines which are basally annulated (anchylosed short articles) and distally hyaline, tapering into an arista (hence aristate spines). However, there are four main differences between these genera. First, the relative position of the cephalic cage chaetae: they are arranged as transverse dorsal rows in *Ilyphagus,* whereas in *Bradabyssa* they are lateral, fewer and smaller. Second, the development of the body wall: most *Bradabyssa* species have a thick muscular body wall, whereas in *Ilyphagus* species it is reduced with poorly-developed muscle layers. Third, the branchial features: in *Ilyphagus* there are a few thick branchial filaments arranged in a horse-shoe pattern, whereas in *Bradabyssa* they are abundant and medially separated by the caruncle into two half-moon shaped groups. Fourth, and derived from the latter: the species of *Bradabyssa* have a well-developed caruncle whereas in *Ilyphagus* it is reduced or absent.

Species included. Besides the type species, *I. bythincola* Chamberlin, 1919 from the Eastern Pacific (including *I. ascendens* Chamberlin, 1919), the genus contains *I. coronatus* Monro, 1939 from the Antarctic Ocean, *I. hirsutus* Monro, 1937 from the Central Indian Ocean, and *I. wyvillei* (McIntosh, 1885) from the Antarctic Ocean.

There are several species that have been previously placed in *Ilyphagus* but belong elsewhere. Thus, *I. antarcticus* Hartman, 1978, *I. ilyvestis* Hartman, 1960 and *I. minutus* Amoureux, 1986 belong in *Bradabyssa, I. caudatus* Rioja, 1963 belongs in *Therochaeta* Chamberlin, 1919, and *I. octobranchus* Hartman, 1965 belongs in *Diplocirrus* Haase, 1915 as emphasized elsewhere (Day 1973, Salazar-Vallejo *et al.* 2008, Salazar-Vallejo and Buzhinskaja 2011). Lastly, as indicated above, *I. pluto* Chamberlin, 1919 is not a polychaete but an holothurian.

Distribution. The species of this genus have representatives living in deep to very deep sea sediments (1260–7000 m), from the Pacific, Indian and Antarctic Oceans.

Key to species of *Ilyphagus* Chamberlin, 1919 restricted

1 Body short, about three times longer than wide **2**
– Body cigar-shaped, more than five times longer than wide **3**

2(1) Neurochaetae markedly hirsute subdistally (oblique fibers exposed); chaetiger 1 with 3–4 neurochaetae per side.......................... ***I. hirsutus* Monro, 1937**

– Neurochaetae barely hirsute or smooth subdistally; chaetiger 1 with about 8 neurochaetae per side.................... ***I. bythincola* Chamberlin, 1919 partim**

3(1) Body velvety (papillae short); most neurochaetae smooth or barely hirsute **4**

– Body pilose (papillae long); neurochaetae smooth and hirsute (by fracture); up to 14 branchial filaments .... ***I. coronatus* Monro, 1939**

4(3) Chaetiger 1 with about 8 neurochaetae per side; about 40 branchial filaments .... ***I. bythincola* Chamberlin, 1919 partim**

– Chaetiger 1 with 10–12 neurochaetae per side; about 16 branchial filaments .... ***I. wyvillei* (McIntosh, 1885)**

***Ilyphagus bythincola* Chamberlin, 1919**
http://species-id.net/wiki/Ilyphagus_bythincola
Figures 1, 2

Ilyphagus bythincola Chamberlin, 1919:402–403, pl. 69, Figs 4–9; Hartman 1960:131; Levenstein 1961b:136; Fauchald 1972:224–225.
Ilyphagus ascendens Chamberlin, 1919:403–404; Hartman 1960:131–132.

Type material. Eastern Pacific Ocean. Holotype of *Ilyphagus bythincola* (USNM 19748), one paratype (USNM 19384), off Mexico, R/V Albatross, Stat. 3415 (14°46'N, 98°40'W), 1879 fathoms (3438.6 m), 10 Apr. 1891 (paratype two fragments; may belong to the same organism, not measured). Holotype of *I. ascendens* (USNM 19735), off Hood Island, Galapagos Islands, 12 miles (19.3 km) SE Ripple Point, R/VAlbatross, Stat. 4649 (01°35'S, 89°30'W), 633 fathoms (1158.4 m), 10 Nov. 1904.

Additional material. Eastern Pacific Ocean. One specimen (USNM 49080), off Mexico, R/V Albatross, Stat. 3415 (14°46'N, 98°40'W), 1879 fathoms (3438.6 m), 10 Apr. 1891 complete, K. Fauchald, id. (24 mm long, 9 mm wide, cephalic cage +17 mm long, 22 chaetigers). Two complete specimens (SIORAS-unumb.), R/V Akademik Kurchatov, Stat. 294 (08°23'S, 81°00'W), off Nazca Ridge, 6200–6240 m, Sigsbee trawl, 31 Oct./1 Nov. 1968 (43–75 mm long, 5–8 mm wide, cephalic cage 0–13 mm long (broken), 22–23 chaetigers). Many specimens (SIORAS unnumbered), off Northern Peru, R/V Akademik Kurchatov, Stat. 301 (05°51.7'S, 81°48.8'W), 5300 m, 4 Nov. 1968 (best specimen 48 mm long, 19 mm wide, cephalic cage 21 mm long, 22 chaetigers).

Description. Holotype of *I. bythincola* (USNM-19748) damaged, ovoid, body wall broken by compression, depressed (Fig. 1A); 48 mm long, 26 mm wide, cephalic cage chaetae broken, 21 chaetigers. Holotype of *I. ascendens* (USNM 19735) with body digitate, ovoid, pointed anteriorly, rounded posteriorly (Fig. 1E); 55 mm long, 20 mm wide, cephalic cage 36 mm long (tips broken), 24 chaetigers. Body surface densely papillated, with fine sediment particles trapped between pa-

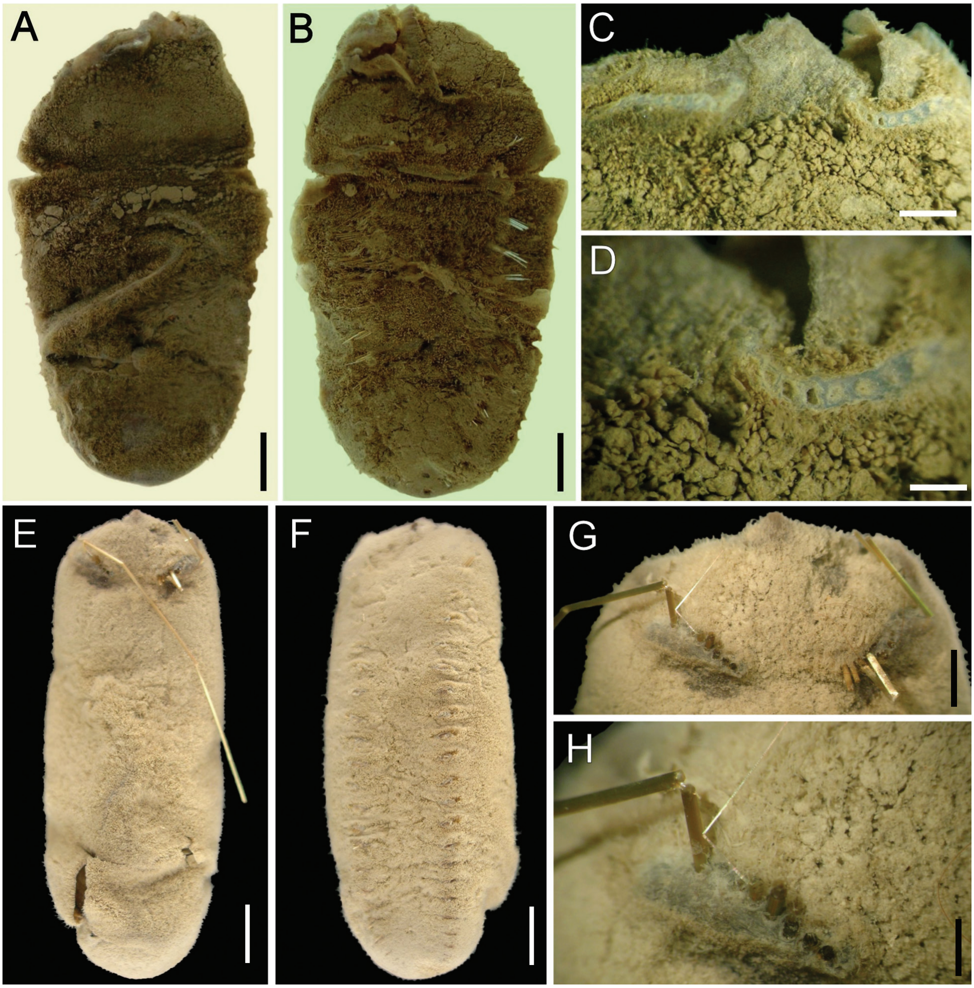


Figure 1. *Ilyphagus bythincola* Chamberlin, 1919. **A** Holotype (USNM 19748) of *I. bythincola*, dorsal view **B** Same, ventral view **C** Same, anterior margin, chaetiger 1 **D** Same, close-up showing chaetal cage scars **E** Holotype (USNM 19735) of *I. ascendens*, dorsal view **F** Same, ventral view **G** Same, anterior end, dorsal view **H** Same, close-up showing chaetal scars and remaining chaetae. Bars.- **A** 5.9 mm **B** 5.6 mm **C** 1.5 mm **D** 0.8 mm **E–F** 6.8 mm **G** 3 mm **H** 1 mm.

pillae (Figs 1B, F, 2A); posterior region with longer papillae; each papillae filiform, most with tips pale, some with black tips.

Cephalic cage chaetae broken; scars present in chaetiger 1, short dorsal transverse row with 8–10 chaetal scars per side (Fig. 1C, D); holotype of *I. ascendens* with chaetae at least twice as long as body width, perhaps as long as body length (Fig. 1E). Chaetiger 1 (and perhaps 2) involved in the cephalic cage, dorsal; most cephalic cage chaetae broken from the base, 8 notochaetae; neurochaetae lateral, 7 per side, arranged in a short row, transverse (oblique in *I. ascendens*, Fig. 1G, H).

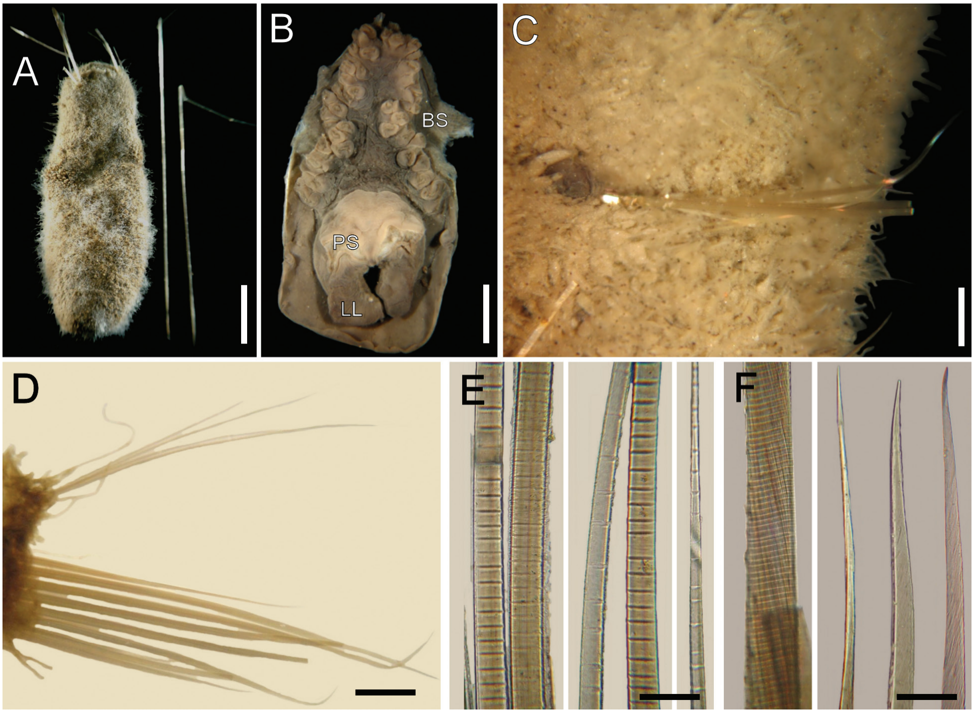


Figure 2. *Ilyphagus bythincola* Chamberlin, 1919, non-type specimens. **A** Complete (USNM-49080), dorsal view **B** Head (SIORAS-unnumb.), frontal view (**BS** branchial scars, **LL** lateral lip, **PS** palp scar) **C** Same, chaetiger 5, ventral view, left neuropodium and gonopodial lobe **D** Same, chaetiger 10, right parapodium **E** Same, basal, medial and distal notochaetal regions **F** Same, median region and tips of neurochaetae. Bars.- **A** 4.9 mm **B** 2.3 mm **C** 0.6 mm **D** 0.7 mm **E** 70 µm **F** 30 µm.

Non-type specimens with chaetae as long as body length (Fig. 2A). Anterior dorsal margin of first chaetiger papillated. Anterior chaetigers without larger papillae. Anterior end observed in a non-type specimen (SIORAS).

Cephalic hood short, not exposed, margin smooth. Prostomium low, as dark as surrounding region; eyes not seen. Caruncle not seen. Palps large, thick, shorter than branchiae; palp lobes reduced. Lateral lips well developed; ventral and dorsal lips reduced.

Branchiae thick, digitate, in different sizes, sessile on branchial plate, in a horseshoe pattern (Fig. 2B), one superior single row with four thick larger filaments, and six pairs of lateral filaments arranged in irregular double rows. Largest branchiae longer than palps. Nephridial lobes in branchial plate not seen. Chaetal transition from cephalic cage to body chaetae abrupt (most chaetae broken); first aristate neurospines in chaetiger 3. Gonopodial lobes not seen in holotype; non-type specimens with dark, digitate, small lobes in chaetiger 5 (Fig. 2C), or in chaetigers 5 and 6.

Parapodia slightly developed (Fig. 2D); notopodia without prominent lobe, chaetae emerge from the body wall. Median neuropodia ventrolateral, projected ridges.

No additional longer papillae associated with chaetal lobes. Noto- and neuropodia lateroventral, very close to each other.

Median notochaetae arranged in oblique rows, as long as one-fourth or one-fifth of body width, 2–3 per bundle; all notochaetae thin, multiarticulated capillaries, with short articles basally, median-sized medially, longer distally (Fig. 2E). Neurochaetae anchylosed aristate spines, 6–8 per bundle; broken, with short anchylosed articles, arranged in an oblique line. Other chaetal features not examined in holotype. Paratype with noto- and neurochaetae broken; non-type specimens with slightly curved, hyaline, smooth tips (Fig. 2F).

Posterior end rounded; pygidium with anus ventral, without anal cirri.

Remarks. The original body shape was digitate rather than sole-like; this distortion was the result of the sudden change of pressure, especially because of the sediment load in the dredge over its body. The damage resulted in the loss of all cephalic cage chaetae, but chaetal scars are visible in the corresponding position. This damage further compressed the body breaking its wall, and making it appear flat. Other specimens (SIORAS) are long, anteriorly swollen, posteriorly tapered; this is more pronounced among juveniles, and although the number of chaetigers is fixed early in development, counting depends on the presence of chaetae and they are often broken off.

Ilyphagus bythincola Chamberlin, 1919 and *I. ascendens* Chamberlin, 1919 are herein regarded as synonyms. The latter has cephalic cage chaetae in an oblique row, rather than in a transverse one as in *I. bythincola*. This displacement results in a larger area between chaetae and the anterior margin of chaetiger 1. However, because the anterior end of *I. bythincola* is severely damaged, and because other body features are similar, there are insufficientdifferences to keep them separate as distinct species.

Ilyphagus bythincola resembles *I. hirsutus* Monro, 1937, but they differ in the relative number of neurochaetae in chaetiger 1 and in the relative smoothness of neurochaetal tips. Thus, *I. bythincola* has about 8 neurochaetae in the first chaetiger, whereas there are 3–4 in *I. hirsutus*, and in the former, the neurochaetal tips are mostly smooth or barely hirsute, whereas in *I. hirsutus* neurochaetae are markedly hirsute. At the same time, *I. bythincola* resembles *I. wyvillei* (McIntosh, 1885), but they differ in the relative number of neurochaetae in chaetiger 1 and in the number of branchial filaments. Thus, in *I. bythincola* there are 8 neurochaetae per side and about 40 branchial filaments, whereas in *I. wyvillei* there are 10–12 neurochaetae and about 16 branchial filaments.

Distribution. Apparently restricted to deep water off southwestern Mexico, to Galapagos and Peru, in 1260–6000 m. There have been two other records for this abyssal species. Levenstein (1961b:137, map), recorded it from the Java Trough, and Kirkegaard (1956:70, Fig. 9) recorded it from the Sunda Trench. The former (ZIRAS-9451) was based on a specimen broken in two, much damaged, collected in 6850 m depth (RV Vitjaz, Stat. 4535, 10°08'S, 107°55'E). It resembles *I. bythincola* but better specimens are needed for a complete identification or description. On the other hand, the specimen from the Sunda Trench belongs to an undescribed species in *Bradabyssa,* and will be described elsewhere.

Ilyphagus coronatus Monro, 1939

http://species-id.net/wiki/Ilyphagus_coronatus
Figure 3

Ilyphagus coronatus Monro, 1939:130–131, fig. 19; Hartman 1966:41, pl. 12, Figs 4–6, Hartman 1967:127; Rozbaczylo 1985:159–160.

Type material. Antarctic Ocean. Three syntypes of *Ilyphagus coronatus* Monro, 1939 (NHML-1941.3.3.99–100), off Princess Elizabeth Land, Stat. 29 (66°28'S, 72°41'E), 1266 m (syntypes complete, one broken in two pieces; the largest one was already dissected by Monro; 23–40 mm long, 5–9 mm wide, cephalic cage (broken) 12–21 mm long, 23–25 chaetigers).

Additional material. Three specimens (USNM-56696, LACM-AHF unnumb.), R/V Eltanin Cruise, Stat. 138 (62°00'S, 61°09'W), 1437 m, 8 Aug. 1962 (slightly damaged; larger specimen (USNM) 52 mm long, 8 mm wide, cephalic cage broken 10 mm long, 22 chaetigers). Three fragments (USNM-56697), R/V Eltanin Cruise, Stat. 480 (58°06'S, 44°56'W), 2800 m, 15 Feb. 1963 (anterior fragment, 20 mm long, 5.5 mm wide, 13 chaetigers). Two specimens (ZMH-24530), Cruise ANT/ XV-3, R/V PolarStern, South of Vestkapp, St. 48-088 (73°28.5'S, 22°30.0'W), 1681 m, 4 Feb. 1998, B. Hilbig, coll. (42–45 mm long, 10–11 mm wide, cephalic cage 7–18 mm long, 24–25 chaetigers).

Description. Largest syntype cylindrical, globose, posteriorly rounded (Fig. 3A); 48 mm long, 8 mm wide, remaining cephalic cage chaetae broken, 6 mm long, 24 chaetigers. Body surface densely papillated (Fig. 3B); papillae long, filiform, tapering, or slightly capitate, with many adherent sediment particles over its basal and medial regions.

Anterior end not exposed; observed by dissection of anterior end in anterior fragment of syntypes, or other non-type specimens (USNM-56698, USNM-56696). Prostomium low cone, without eyes. No caruncle (Fig. 3D). Palps massive, as long as branchiae; palp lobes low. Lateral and dorsal lips fused; ventral lip reduced. Branchiae digitate, 14 filaments, arranged in three irregular rows: superior one with 4 filaments, two lateral groups medially placed each with 3 filaments, and two lateral basal ones with 2 filaments each; largest branchiae as long as palps.

Cephalic cage chaetae mostly broken; size relationships with body length or width unknown; syntypes with chaetae at least as long as body width and one with chaetae almost as long as body length; one very long chaetae straight, with successive constrictions but anchylosed articles perpendicular to the main shaft. Chaetiger 1 involved in cephalic cage; notochaetae of chaetiger 2 very long, thin. Chaetiger 1 with 8 notochaetae in transverse short dorsal row; neurochaetae in C-pattern, opening towards the posterior end, looking like two series, with 8 neurochaetae.

Anterior dorsal margin of first chaetiger papillated, projected anteriorly, conical, continued with the longitudinal body opening; anterior chaetigers without especially long papillae. Chaetigers 1–3 of about same length. Chaetal transition from cephalic cage to body chaetae abrupt; aristate neurospines from chaetiger 2. Gonopodial lobes in chaetiger 5, as long as neuropodial width (Fig. 3C), dark in syntypes (paler in other specimens), digitate.

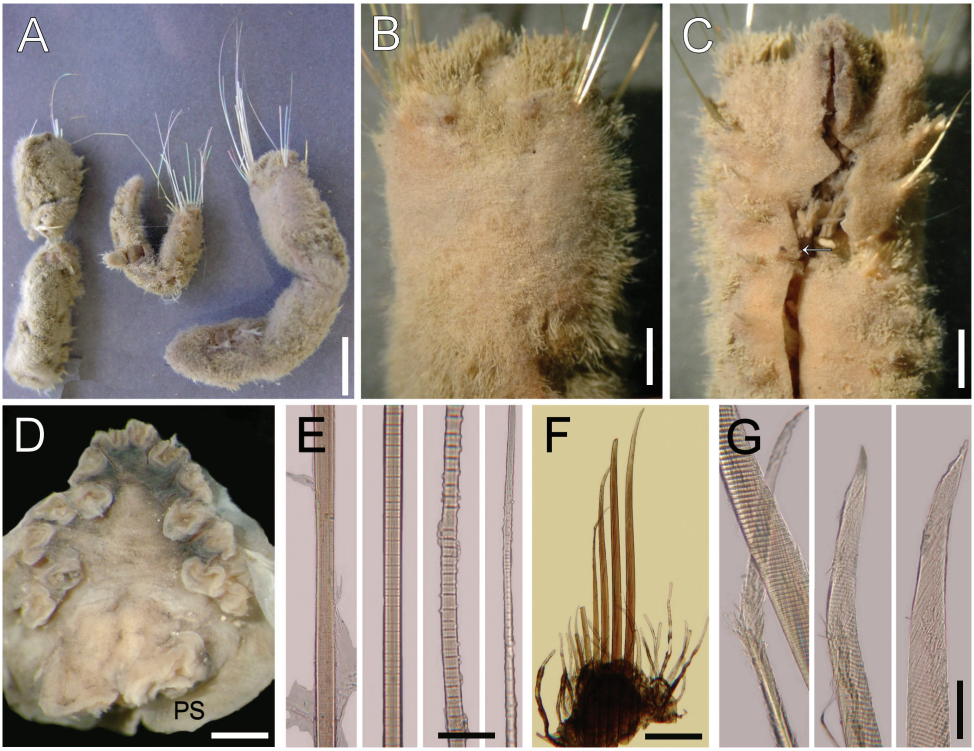


Figure 3. *Ilyphagus coronatus* Monro, 1939. **A** Syntypes (NHML-1941.3.3.99-100) **B** Larger syntype, anterior end, dorsal view **C** Same, anterior end, ventral view (arrow points gonopodial lobe) **D** Non-type specimen (USNM-56696), head, frontal view, palp scars and mouth directed downwards (PS: palp scar) **E** Smaller syntype, chaetiger 9, notochaetal regions **F** Same, chaetiger 5, neuropodium **G** Same, neurochaetal tips. Bars.- **A** 7 mm **B** 1.8 mm **C** 2 mm **D** 0.7 mm **E** 190 µm **F** 0.5 mm **G** 60 µm.

Parapodial development difficult to detect due to papillae cover (USNM-56698); notopodia not detected; chaetae stem from long, rounded neuropodial lobes. Parapodia lateral; median neuropodia ventrolateral. Noto- and neuropodia close to each other.

Median notochaetae arranged in short longitudinal rows, as long as half body width, about 2 per ramus; all notochaetae multiarticulated capillaries, articles very short along most of the chaeta, distally difficult to see (Fig. 3E), hyaline. Neurochaetae anchylosed aristate spines from chaetiger 2, arranged in transverse rows, 4–5 or up to 7–8 per ramus (Fig. 5F). Both noto- and neurochaetae (USNM-56697) with distal portions rough; fibers are individually and irregularly broken off from the main axis (Fig. 3G), not hirsute.

Posterior end (USNM-56696) rounded, pygidium with anus terminal, without cirri.

Remarks. *Ilyphagus coronatus* Monro, 1939 can be separated from other cigar-shaped species because of the relative size of body papillae, which appear pilose, and because its neurochaetae, despite possibly appearing hirsute due to fracture, are mostly smooth.

Distribution. Only known from two localities around Antarctica, in 1200–3500 m.

Ilyphagus hirsutus Monro, 1937

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

Figure 4

Ilyphagus hirsutus Monro, 1937:304–305, textfig. 22.

Type material. Central Indian Ocean, South Arabian Sea. Holotype (NHML-1937.9.2.455), John Murray Expedition, H.E.M.S. Mabahiss, Stat. 133 (01°25'54"S, 66°34'12"E → 01°19'42"S, 66°35'18"E), 15 Feb. 1934, 3385 m (station data after Sewell 1935).

Description. Holotype pale, globose, widened in the posterior half (Fig. 4A, B); 37 mm long, 10 mm wide, cephalic cage chaetae broken, 19 chaetigers. Body surface densely papillated, with abundant fine sediment particles; papillae long, cylindrical, each covered by a thin layer of fine sediment particles.

Cephalic hood not exposed; specimen not dissected to avoid further damage. Cephalic cage chaetae length unknown. Chaetiger 1 involved in the cephalic cage; chaetae arranged in short rows, notochaetae dorsal, 5–7 per bundle (bases damaged, difficult to count); neurochaetae ventrolateral, 3–4 per bundle.

Anterior dorsal margin of first chaetiger projected anteriorly (Fig. 4C), large rounded lobe, bent ventrally. Anterior chaetigers without especially long papillae. Chaetiger 1 largest, chaetigers 2–3 of about the same size. Chaetal transition from cephalic cage to body chaetae abrupt; chaetiger 2 with shorter neurochaetae, directed ventrolaterally. Gonopodial lobes present in chaetiger 5, low rounded dark, displaced ventrally, and positioned towards posterior segmental margin.

Parapodia lateral; median neuropodia ventrolateral. Notopodia scarcely noticed; neuropodia thick low muscular lobes, without especially longer papillae. Noto- and neuropodia close to each other.

Median notochaetae mostly broken; one anterior notopodia with 3 notochaetae, arranged in a tuft, median notochaetae as long as 1/5 body width, about 3 per fascicle; all notochaetae multiarticulated capillaries, articles short basally, medium-sized medially, long distally (Fig. 4D). Neurochaetae probably multiarticulated capillaries in chaetiger 1; from chaetiger 2, anchylosed aristate spines (Fig. 4E), arranged in transverse rows, in two series, with about 8 chaetae per fascicle. Neurospines basally cylindrical with very short articles, medially flat, distally tapering with slightly longer articles; neurospines basally smooth, subdistally and distally with fibers separated from the main stem, giving hirsute appearance to chaetal surface (Fig. 4F).

Posterior end globose, damaged; pygidium with anus terminal, without anal cirri.

Remarks. *Ilyphagus hirsutus* Monro, 1937 resembles *I. bythincola* because of their dense coverage with fine, long papillae. They differ because *I. hirsutus* has a projected lobe in the first neuropodia, which is not present in *I. bythincola,* and because the neurochaetae of the former are markedly hirsute distally, whereas those in *I. bythincola* are either distally hyaline or slightly hirsute.

Distribution. Originally described from the Central Indian Ocean, in deep water (3385 m); it has not been recorded since.

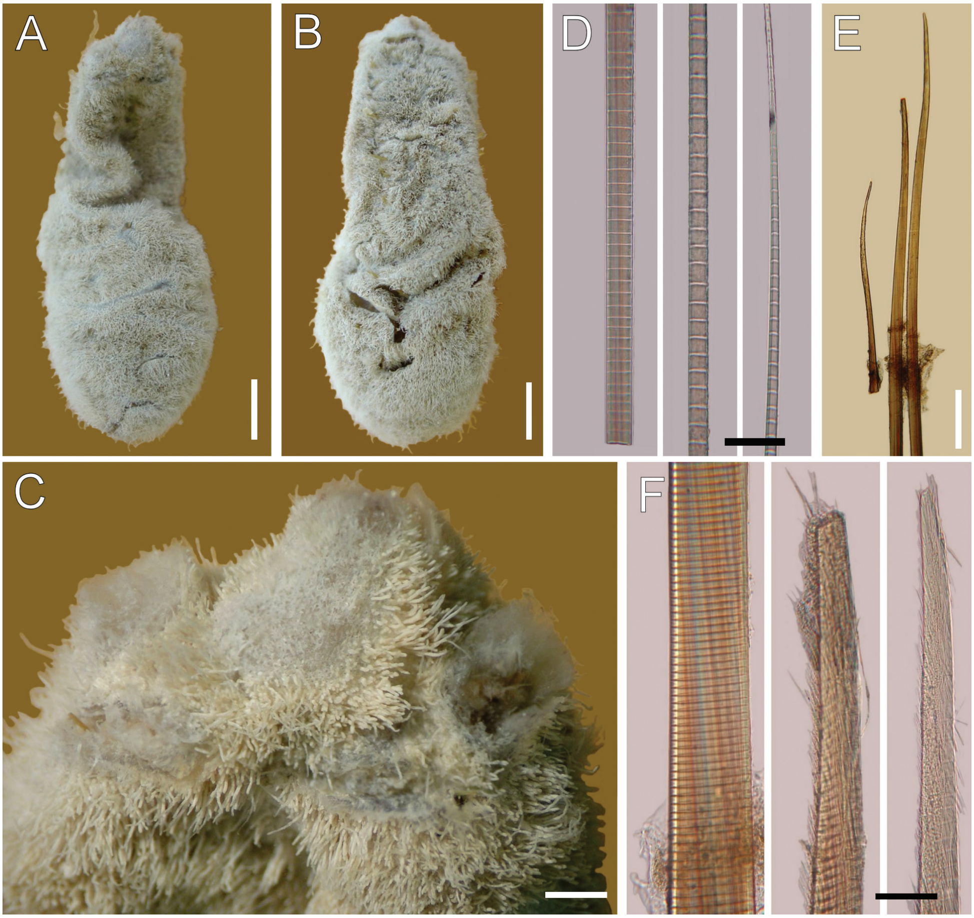


Figure 4. *Ilyphagus hirsutus* Monro, 1937. Holotype (NHML-1937.9.2.455). **A** Dorsal view **B** Ventral view **C** Anterior end, dorsal view **D** Chaetiger , basal, medial and distal notochaetal regions **E** Chaetiger , neurochaetae **F** Same, basal region and two hirsute tips. Bars.- **A, B** 5 mm **C** 0.2 mm **D** 45 µm **E** 0.4 mm **F** 65 µm.

Ilyphagus pluto Chamberlin, 1919

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

Ilyphagus pluto Chamberlin, 1919:403; Hartman 1960:132.

Material examined. Off Peru. Holotype (USNM 19721), 88 miles (141.7 km) SW Palominos Light House, R/V Albatross, Stat. 4672 (13°11'30"S, 78°18'00"W), 2845 fathoms (5206.4 m), 21 Nov. 1904.

Remarks. This is a holothurian. The stout cylindrical processes forming a 'complete closed circle'from the original description are actually tentacles surrounding the mouth. Each tentacle is short and branched, but each branch is like a wart, making them apparently crenulated. The long, typical reddish brown chaetae found penetrating the body belong to other, deep-water polychaetes, such as the aphroditid *Laetmonice,* which during dredging, frequently loose their chaetae. The holothurians belongs in the genus *Meseres*, currently in the family Synallactidae (O'Loughlin and Ahearn 2005); after O'Loughlin (2002), two species have been described from the same region: *M. torvus* (Théel, 1886) and *M. macdonaldi* Ludwig, 1894; however, the former species has an uncertain generic placement, whereas the second is retained in its genus (O'Loughlin and Ahearn 2005).

Ilyphagus wyvillei **(McIntosh, 1885)**

http://species-id.net/wiki/Ilyphagus_wyvillei
Figure 5

Trophonia wyvillei McIntosh, 1885:366–370, pl. 44, fig. 6, pl. 23A, figs 11–14, pl. 36A, figs 5–7, pl. 37A, fig. 1.

Ilyphagus wyvillei: Hartman 1966:41–43, pl. 12, figs 7, 8 (n. comb.); Levenstein 1975:133; Detinova 1993:100–101.

Brada gravieri McIntosh, 1922:7–8, pl. 1, figs 4–6, pl. 3, fig. 1; Hartman 1966:33, pl. 9, figs 1, 2; Hartman 1978:173.

Type material. Southeastern Pacific Ocean. Holotype (NHML-85.12.1.261), R/V Challenger Expedition, Stat. 157 (53°55'S, 108°35'E), dredged, 1950 fathoms (3568.5 m), diatom ooze, 3 Mar. 1874.

Additional material. Antarctic Ocean. Several specimens (SIORAS-unnumb.), R/V Akademik Kurchatov, Stat. 914 (56°21'S, 50°48'W), 5650–6070 m, 14 Dec. 1971 (best specimen 49 mm long, 10 mm wide, cephalic cage 24 mm long (chaetiger 2 notochaetae 18 mm long), 22 chaetigers; 11 notochaetae in chaetiger 1; two anterior fragments dissected).

Description. Holotype pale brown (Fig. 5A), completely dissected mid-ventrally (Fig. 5B), internal organs and most anterior end appendages previously removed (now lost). Body sausage-shaped, anteriorly truncate, medially widened, posteriorly rounded (confirmed in non-type specimen, Fig. 5E); 63 mm long, about 30 mm wide, cephalic cage 27 mm long, 19 chaetigers. Body surface papillated; papillae abundant, cylindrical, very long, sediment particles along papillae, more abundant basally.

Anterior end dissected, most appendages now lost. Cephalic hood short, margin smooth. Prostomium flat, without eyes. No caruncle. Palps very large; one remains attached to anterior end fragment (Fig. 5C), with a distal parasite (Fig. 5D); other palp loose in container , longer than branchiae (longest remaining detached branchia 5 mm long), expanded, with a median furrow; palp lobes reduced. Branchiae cirriform, distally colorless, sessile on branchial plate, arranged in single row, in horse-shoe pattern (Fig. 5F), with 16 filaments (perhaps other four, much smaller, filaments distally, would make a secondary distal row).

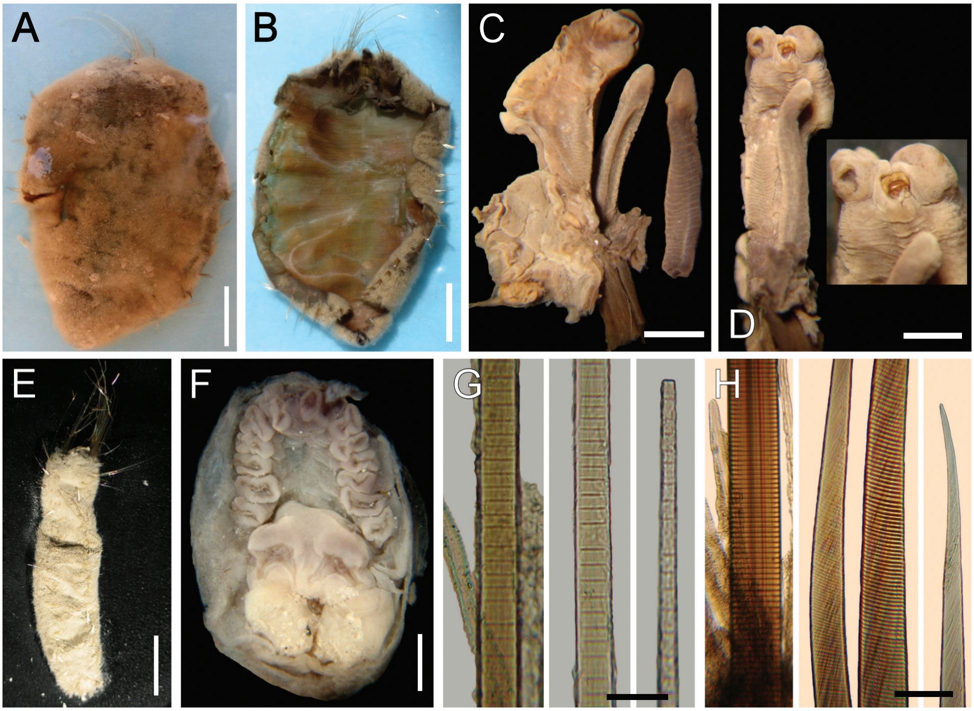


Figure 5. *Ilyphagus wyvillei* (McIntosh, 1885). **A** Holotype (NHML-85.12.1.261), dorsal view **B** Same, ventral view, body completely dissected and inner organs removed **C** Same, left palp and two branchial filaments **D** Same, palp and branchia, with parasite and parasite scar on palp (insert: close-up of palp tip) **E** Non-type specimen (SIORAS-unnumb.), ventral view **F** Same, head, frontal view, palps and branchiae removed **G** Holotype (NHML-85.12.1.261), chaetiger 11, basal, medial and distal notochaetal regions **H** Same, chaetiger 10, basal, medial and distal neurochaetal regions. Bars.- **A, B:** 13 mm **C** 1 mm **D** 1.7 mm **E** 10 mm **F** 2.3 mm **G** 70 µm **H** 140 µm.

Cephalic cage chaetae as long as half body length, or about as long as body width. Chaetigers 1–2 involved in the cephalic cage, chaetiger 1 with 8–9 notochaetae in a single transverse row and 11–12 neurochaetae arranged in a C-pattern, opening to posterior region; chaetiger 2 with 5–6 noto- and 9–10 neurochaetae.

Anterior dorsal margin of first chaetiger truncate, papillated; anterior chaetigers without especially long papillae. Chaetigers 1–3 becoming progressively longer. Chaetal transition from cephalic cage to body chaetae abrupt; aristate neurospines from chaetiger 3. Gonopodial lobes in chaetiger 5, short, dark digitate, mostly covered by papillae.

Parapodia poorly developed, chaetae emerge from the body wall. Parapodia lateral, median neuropodia ventrolateral. Noto- and neuropodia close to each other, without especially longer papillae, some slightly thicker papillae bordering chaetae.

Median notochaetae arranged in short transverse rows, most notochaetae broken, length relationships with body width unknown, 1–3 per ramus; all multiarticulated capillaries, articles short basally, slightly longer medially, long subdistally (tips unknown, Fig. 5G). Neurochaetae multiarticulated capillaries in chaetigers 1–2; aristate neurospines from chaetiger 3, arranged in transverse rows, 7–8 per bundle. Each neurospine with very short articles basal- and medially (Fig. 5H); distally hyaline, smooth.

Posterior end rounded; pygidium with anus ventro-terminal, without anal cirri.

Remarks. *Ilyphagus wyvillei* (McIntosh, 1885) resembles *I. bythincola* because they both have globose bodies with distally smooth aristate neurospines. They differ because *I. wyvillei* has comparatively shorter cephalic cage chaetae than *I. bythincola,* and because in *I. wyvillei* there are only 16 branchiae, whereas in *I. bythincola* there are about 40. On the other hand, *I. wyvillei* resembles *I. coronatus* Monro, 1939, because in their first chaetiger, neurochaetae are arranged in a C-pattern, opening to the posterior region, and by having distally smooth neurospines. However, they differ because *I. wyvillei* has fewer chaetigers (19–22 *vs* 23–25) and a more globose body but these differences might be modified after more specimens are studied. Two other differences are probably more relevant and must be emphasized:the relative number of neurochaetae in the first chaetiger (11–12 in *I. wyvillei*, 8 in *I. coronatus*) , and the start of the aristate neurospines (chaetiger 2 in *I. wyvillei*, chaetiger 3 in *I. coronatus*).

The presence of parasitic copepods in the branchial bases of *I. wyvillei* cannot be confirmed due to the state of the anterior end; however, one portion of a parasite is visible at one of the palps tip, and there is another deep scar in the same palp. McIntosh might have confused the attachment site, because he dissected the anterior end and branchial scars could be confused with these parasite attachment sites.

Brada gravieri McIntosh, 1922 might belong to the same species. There is no type material available; it is probably lost. However, the original illustrations and description noticed the lack of the cephalic cage chaetae, whereas the neurochaetae (pp 7–8) were described as translucent, smooth, devoid of transversal marks. The distal part of neurochaetae is often smooth, hyaline, but the rest of the chaetae have anchylosed articles or transverse markings throughout it. They were collected from relatively close localities but fresh material needs to be examined to clarify this .

Distribution. Originally described from the Antarctic Ocean, it has been found in abyssal depths off Western South America (Levenstein, 1975). The Bering Sea records by Levenstein (1961a:160, 1966:46), cannot be confirmed because the specimens were not found.

Acknowledgments

The generous support by several researchers and collection managers is deeply appreciated: Andrey Gebruk and Natasha Budaeva (SIORAS), Emma Sherlock and Miranda Lowe (NHML), Galina Buzhinskaja (ZIRAS), Kristian Fauchald and Goeff Keel (USNM), Leslie Harris, Kathy Omura and Kirk Fitzhugh (LAMNH), and Tarik Meziane (MNHN). For my research visit in Russia, Anna Zhadan (Lomonosov State University, Moscow) and Galina Buzhinskaja (ZIRAS) were very helpful with some paper work for the visa and finding some housing facilities in Moscow and Sankt-Peterburg. The late Cynthia A.

Ahearn (USNM) kindly confirmed that what has been regarded as a flabelligerid species was actually a holothurian. The fast and kind help by José Santos Gómez, our librarian in Chetumal, was fundamental to find some uncommon publications. The careful reading by Pat Hutchings, Teresa Darbyshire and Chris Glasby helped a lot for improving this final version. Additional funds came from the U.S.A. Department of the Interior, Fulbright Commission, MNHN, and from the Université Catholique de l´Ouest, Angers, France.

References

Amoureux L (1986) Annélides polychètes abyssaux de la campagne Abyplaine au large de Madère. Bulletin du Museum National d'Histoire Naturelle, Paris, 4e série, section A 8: 591–615.

Chamberlin RV (1919) The Annelida Polychaeta of the *Albatross* Tropical Pacific Expedition, 1891–1905. Memoirs of the Museum of Comparative Zoology, Harvard College 48: 1–514.

Day JH (1973) New Polychaeta from Beaufort, with a key to all species recorded from North Carolina. National Oceanographic and Atmospheric Administration, Technical Report of the National Marine Fisheries Service, Circular 375: 1–153.

de Saint-Joseph A (1894) Les annélides polychètes des côtes de Dinard, troisième partie (Nephtydiens – Serpuliens). Annales des Sciences Naturelles, Zoologie, 7ème série 17: 1–395.

Detinova NN (1993) Mnogoshchetinkovye chervi Orkneiskogo zheloba. Trudy Instituta Okeanologiya P.P. Shirshova, Rossiya Akademi Nauk 127: 97–106.

Fauchald K (1972) Benthic polychaetous annelids from deep waters off Western Mexico and adjacent areas in the Eastern Pacific Ocean. Allan Hancock Monographs in Marine Biology 7: 1–575.

Fauchald K (1977) The polychaete worms: Definitions and keys to the orders, families and genera. Natural History Museum of Los Angeles County, Science Series 28: 1–190.

Grube AE (1850) Die Familien der Anneliden. Archiv für Naturgeschichte, Berlin 16: 249–364.

Haase P (1915) Boreale und arktisch Chloraemiden. Wissenschaftliche Meeresuntersuchungen der Kommission zur Wissenschaftlichen Untersuchung der Deutschen Meere, Neue Folge, Kiel 17: 169–228.

Hartman O (1960) Systematic account of some marine invertebrate animals from the deep basins of Southern California. Allan Hancock Pacific Expeditions 22: 69–215.

Hartman O (1965) Deep-water benthic polychaetous annelids off New England to Bermuda and other North Atlantic areas. Allan Hancock Foundation, Occasional Papers 28: 1–378.

Hartman O (1966) Polychaeta Myzostomidae and Sedentaria of Antarctica. Antarctic Research Series 7: 1–158. doi: 10.1029/AR007

Hartman O (1967) Polychaetous annelids collected by the USNS *Eltanin* and *Staten Islands* Cruises, chiefly from Antarctic seas. Allan Hancock Monographs in Marine Biology 2: 1–387.

Hartman O (1978) Polychaeta from the Weddell Sea Quadrant, Antarctica. Antarctic Research Series 26: 125–222. doi: 10.1029/AR026p0125

Kennedy B, Kyrvi H (1978) The ultrastructure of the spiral notosetae of *Nicomache maculata* Arwidsson (Polychaeta, Maldanidae). Experientia 34: 460–461. doi: 10.1007/BF01935926

Kirkegaard JB (1956) Benthic Polychaeta from depths exceeding 6,000 meters. Galathea Reports 2: 63–78.

Levenstein RY (1961a) Mnogotschetinkovye tchervi glubokovodnoi tchasti Beringova morya. Akademiya Nauk SSSR, Trudy Instituta Okeanology 46: 147–178.

Levenstein RY (1961b) Novye dannye o mnogoshchetinkovykh chervyakh (Polychaeta) Yavanskoi vpadiny. Okeanologiya 1: 136–139.

Levenstein RY (1966) Mnogotshchetinkov'e chervi (Polychaeta) zapadnoi tasti Beringova morya. Akademiya Nauk SSSR, Trudy Instituta Okeanologii 81: 3–131.

Levenstein RY (1975) Mnogotschetinkovye chervi (Polychaeta) zhelobov Atlantitcheskogo sektora Antarktiki. Akademiya Nauk SSSR, Trudy Instituta Okeanologii 103: 119–142.

Ludwig H (1894) Reports on an exploration off the west coasts of Mexico, Central and South America, and off the Galapagos Islands, in charge of Alexander Agassiz, by the U.S. Fish Commission Steamer *Albatross* during 1891, 12. The Holothuroidea. Memoirs of the Museum of Comparative Zoology, Harvard University 17(3): 1–183.

McIntosh WC (1885) Report on the Annelida Polychaeta collected by H.M.S. *Challenger* during the years 1873–76. Challenger Reports 12: 1–554.

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 British marine Polychaeta (continued). Annals and Magazine of Natural History, series 9 9: 1–30.

Monro CCA (1937) Polychaeta. Scientific Reports of the John Murray Expedition 1933–34 4(8): 243–321.

Monro CCA (1939) Polychaeta. Reports of the British Antarctic Research Expedition 1929–1931, series B 4(4): 87–156.

O'Loughlin PM (2002) Report on selected species of BANZARE and ANARE Holothuroidea, with reviews of *Meseres* Ludwig and *Heterocucumis* Panning (Echinodermata). Memoirs of Museum Victoria 59: 297–325.

O'Loughlin PM, Ahearn C (2005) A review of pygal-furrowed Synallactidae (Echinodermata: Holothuroidea), with new species from the Antarctic, Atlantic and Pacific Oceans. Memoirs of Museum Victoria 62: 147–179.

Pettibone MH (1982) Annelida. In: Parker SP (Ed) Synopsis and Classification of Living Organisms. McGraw Hill, New York. 43pp.

Rioja E (1963(1962)) Estudios anelidológicos, 26. Algunos anélidos poliquetos de las costas del Pacífico de México. Anales del Instituto de Biología, México 33: 131–229.

Rozbaczylo N (1985) Los anélidos poliquetos de Chile: Indice sinonímico y distribición geográfica de especies. Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Monografías Biológicas 3: 1–284.

Salazar-Vallejo SI, Buzhinskaja G (2011) Revision of *Diplocirru*s Haase, 1915, including *Bradiella* Rullier, 1965 and *Diversibranchius* Buzhinskaja, 1993 (Polychaeta, Flabelligeridae). ZooKeys 106: 1–45. doi: 10.3897/zookeys.106.795

Salazar-Vallejo SI, Carrera-Parra LF, Fauchald K (2008) Phylogeny of Flabelligeridae (Annelida: Polychaeta). Journal of Systematic Zoology and Evolutionary Research 46: 203–215. doi: 10.1111/j.1439-0469.2008.00464.x

Sewell RBS (1935) Introduction and list of stations. The John Murray Expedition 1933–34, Scientific Reports 1(1): 1–43.

Théel H (1886) Report on the Holothuroidea dredged by H.M.S. *Challenger* during the years 1873–1876. Challenger Reports 14(39): 1–290.

ZooKeys 190: 21–31 (2012)
doi: 10.3897/zookeys.190.2924
www.zookeys.org

RESEARCH ARTICLE

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

Two new spider species of the genus *Chrysso* O. P.-Cambridge, 1882 (Araneae, Theridiidae) in Hainan Island, China

Bao-Shi Zhang[1,†], Feng Zhang[2,‡]

1 *Baoding University, Baoding, 071051, P. R. China* **2** *College of Life Sciences, Hebei University, Baoding, Hebei 071002, P. R. China*

† *urn:lsid:zoobank.org:author:484F2B50-D862-476D-9651-B09A81534ECC*
‡ *urn:lsid:zoobank.org:author:904C4445-AAEA-40BC-B418-502053EF39B8*

Corresponding author: *Feng Zhang* (dudu06042001@163.com)

Academic editor: *D. Dimitrov* | Received 12 february 2012 | Accepted 27 April 2012 | Published 4 May 2012

urn:lsid:zoobank.org:pub:DE2AD1BC-7A09-4B30-BED0-D5A868EC1EA8

Citation: Zhang B-S, Zhang F (2012) Two new spider species of the genus *Chrysso* O. P.-Cambridge, 1882 (Araneae, Theridiidae) in Hainan Island, China. ZooKeys 190: 21–31. doi: 10.3897/zookeys.190.2924

Abstract

Two new spider species of the genus *Chrysso* O. P.-Cambridge, 1882 are reported from Hainan Island, China, *Chrysso bifurca* **sp. n.** (male, female) and *C. bicuspidata* **sp. n.** (male, female). *C. bimaculata* Yoshida, 1998 is recorded from China for the first time.

Keywords

Chrysso, taxonomy, new species, newly recorded, China

Introduction

The genus *Chrysso* was erected by O. P.-Cambridge (1882). It was regarded as a junior synonymy of *Theridion* Walckenaer, 1805, and removed by Bryant (1940). Levi and Levi (1962) considered this genus as a senior synonym of *Arctachaea* Levi, 1957, *Argyroaster* Yaginuma, 1958 and *Meotipa* Simon, 1894, but Deeleman-Reinhold (2009) removed the genus *Meotipa* from the synonymy of *Chrysso*. Until now no large revision of this genus has been done. However, Agnarsson (2004) listed 10 autapomorphies of the *Chrysso* in his phylogeny, these characteristics are putative *Chrysso* synapomorphies.

Currently 62 *Chrysso* species are reported, mostly from America and Asia (Levi 1957; Levi and Levi 1962; Barrion and Litsinger 1995; Miller and Agnarsson 2005; Gonzaga et al. 2006; Yoshida 2009; Siliwal 2009; Platnick 2012), among them, 22 species are known from China. 94 species out of 24 genera are known from Hainan Island, China (Zhu 1998; Song et al. 1999; Yoshida et al. 2000; Tang et al. 2003; Song et al. 2006), including six *Chrysso* species: *C. cyclocera* Zhu, 1998, *C. trispinula* Zhu, 1998, *C. trimaculata* Zhu, Zhang & Xu, 1991, *C. scintillans* (Thorell, 1895), *C. pulcherrima* (Mello-Leitão, 1917) and *C. nigra* (O. P.-Cambridge, 1880).

During the examination of spider specimens collected from 2007 to 2009 in Hainan Island, China, two new species, *Chrysso bifurca* sp. n. and *C. bicuspidata* sp. n. were recognized and are described here. *C. bimaculata* Yoshida, 1998, known from Japan previously, is newly recorded from Hainan, China.

Material and methods

All specimens were kept in 75% ethanol and examined, drawn and measured under a Tech XTL-II stereomicroscope equipped with an Abbe drawing device. Carapace length was measured medially from the anterior margin to the rear margin of the carapace. Eye sizes were measured as the maximum diameter of the lens in dorsal or frontal view. MOA length was measured medially from the anterior margin to the rear margin of MOA. Leg measurements are given as: total length (femur, patella, tibia, metatarsus, tarsus). Epigynes were cleared in warm potassium hydroxide (KOH) and transferred to 75% ethanol for drawing. The labeling of the palpal sclerites is adopted following Agnarsson (2004). All measurements are in millimeters. All specimens studied are deposited in the Museum of Hebei University (MHBU), Baoding, China.

Abbreviations

AER	anterior eye row
ALE	anterior lateral eyes
AME	anterior median eyes
C	conductor
CD	copulatory ducts
E	embolus
FD	fertilization ducts
MA	median apophysis
MOA	median ocular area
PER	posterior eye row
PLE	posterior lateral eyes
PME	posterior median eyes
S	spermathecae

ST	subtegulum
T	tegulum
TTA	theridiid tegular apophysis

Taxonomy

Chrysso bifurca sp. n.

urn:lsid:zoobank.org:act:5B93397C-1884-4E05-9738-5B5A003645A6
http://species-id.net/wiki/Chrysso_bifurca
Figs 1–6

Type material. Holotype ♂, CHINA, Hainan Island: Jianfengling Mountain (19°07'N, 109°13'E), 29 May 2009, C. Zhang leg. **Paratypes:** 2 ♀, same data as holotype; 1 ♀, Limu Mountain (19°10'N, 109°39'E), 20 August 2007, C. Zhang leg.; 3 ♂, 12 ♀, Limu Mountain, 20 November 2008, G.X. Han leg.

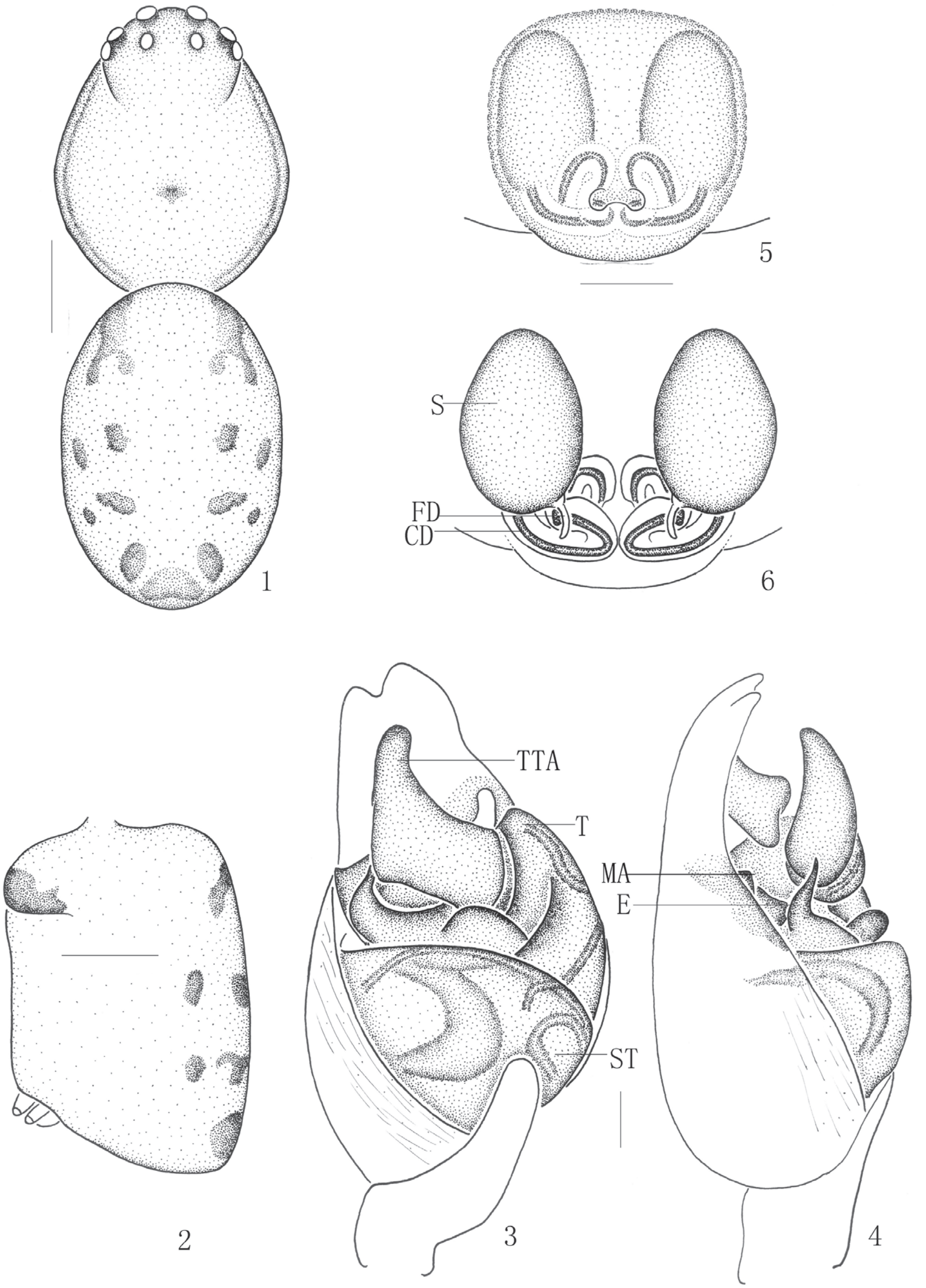
Diagnosis. Males can be distinguished from other *Chrysso* species by the following characters: apex of cymbium bifurcate; embolus short and thin; theridiid tegular apophysis erect, with obtuse apex (Figs 3–4). Females differ from other *Chrysso* species by the bigger and oval spermathecae, and the longer and winding copulatory ducts, differ from *C. octomaculata* (Bösenberg & Strand, 1906) by the process of copulatory ducts (Fig. 6).

Etymology. The species name refers to bifurcate apex of cymbium.

Description. Male (holotype). Total length 2.70: cephalothorax 1.26 long, 1.08 wide; abdomen 1.44 long, 0.99 wide, 0.90 high. Carapace yellow, longer than wide, lateral margin with thin black striations. Only anterior part with several gray setae. Cervical groove distinct. Median furrow almost triangle, yellowish. Each eye with a black ring. Both the eye rows recurved from dorsal view (Fig. 1). Diameters of eyes: AME 0.12, ALE 0.10, PME 0.10, PLE 0.10. Interdistances of eyes: AME–AME 0.10, AME–ALE 0.08, ALE–ALE 0.64, PME–PME 0.10, PME–PLE 0.14, PLE–PLE 0.66, ALE and PLE closed to each other. MOA long 0.29, front width 0.34, back width 0.31. Clypeus 0.32 high, yellow and furnished with few short gray setae. Chelicerae armed with sparse gray setae, promargin with 2 teeth, fangs short and thin. Anterior margin of endites armed with gray scopula. Labium armed with sparse long black setae. Sternum furnished with sparse black setae, its anterior margin lightly procurved. Legs yellow, patella, metatarsus and the base of femur and tibia reddish-brown. Measurements of legs: leg I 9.44 (2.52, 0.59, 2.55, 2.88, 0.90), II 4.38 (1.49, 0.45, 1.13, 0.90, 0.41), III 3.16 (0.90, 0.36, 0.59, 0.90, 0.41), IV 5.09 (1.58, 0.36, 1.17, 1.44, 0.54). Leg formula: 1423.

Abdomen oval, longer than wide and armed with brown setae. Dorsum yellowish, armed with six pairs of irregular black patches, posterior with a median black patch (Figs 1–2). Venter yellowish, with a black median patch. Anal tubercle yellow. Spinnerets yellow.

Male palp with long cymbium, apical part of cymbium bifurcate; embolus short, base with a lunate process, distal part thin; theridiid tegular apophysis erect, with wide base, apex thin and obtuse; conductor lying behind theridiid tegular apophysis (Figs 3–4).



Figures 1–6. *Chrysso bifurca* sp. n., **1–4** male holotype **1** body, dorsal view **2** abdomen, lateral view **3** male left palp, ventral view **4** same, prolateral view **5–6** female paratype **5** epigynum, ventral view **6** vulva, dorsal view. Scale bars: 0.5 mm (**1**–**2**); 0.1 mm (**3**–**6**).

Female (one paratype from Jianfengling Mountain) total length 3.33: cephalothorax 0.95 long, 0.94 wide; abdomen 2.34 long, 1.62 wide, 1.58 high. Diameters of eyes: AME 0.12, ALE 0.10, PME 0.10, PLE 0.10. Interdistances of eyes: AME–AME 0.13, AME–ALE 0.09, ALE–ALE 0.59, PME–PME 0.14, PME–PLE 0.16, PLE–PLE 0.61, ALE and PLE closed to each other. MOA long 0.27, front width 0.30, back width 0.31. Clypeus 0.32 high. Measurements of legs: leg I10.81 (3.24, 0.63, 2.70, 3.29, 0.95), II 6.44 (2.03, 0.54, 1.44, 1.80, 0.63), III 3.52 (1.13, 0.41, 0.63, 0.90, 0.45), IV 7.08 (1.94, 0.54, 2.21, 1.80, 0.59). Leg formula: 1423. Other characters as in holotype.

Female genitalia lightly sclerotized, posterior part with a kidney-shaped atrium; spermathecae big, oval; copulatory ducts long, thick, winding and connected with spermathecae from posterior part (Figs 5–6).

The new species with putative *Chrysso* synapomorphies as follow: (1) carapace pars stridens irregular; (2) abdomen extending beyond spinnerets; (3) cymbial hood of male palp broad; (4) median apophysis of male palp with distinct apophysis; (5) palpal claw of female dentition sparse.

Variation. The lateral part of dorsal abdomen of some females examined with eight to eleven pairs of irregular black patches. Males total body length from 2.64–2.80, female total length from 3.28–3.35.

Distribution. China (Hainan).

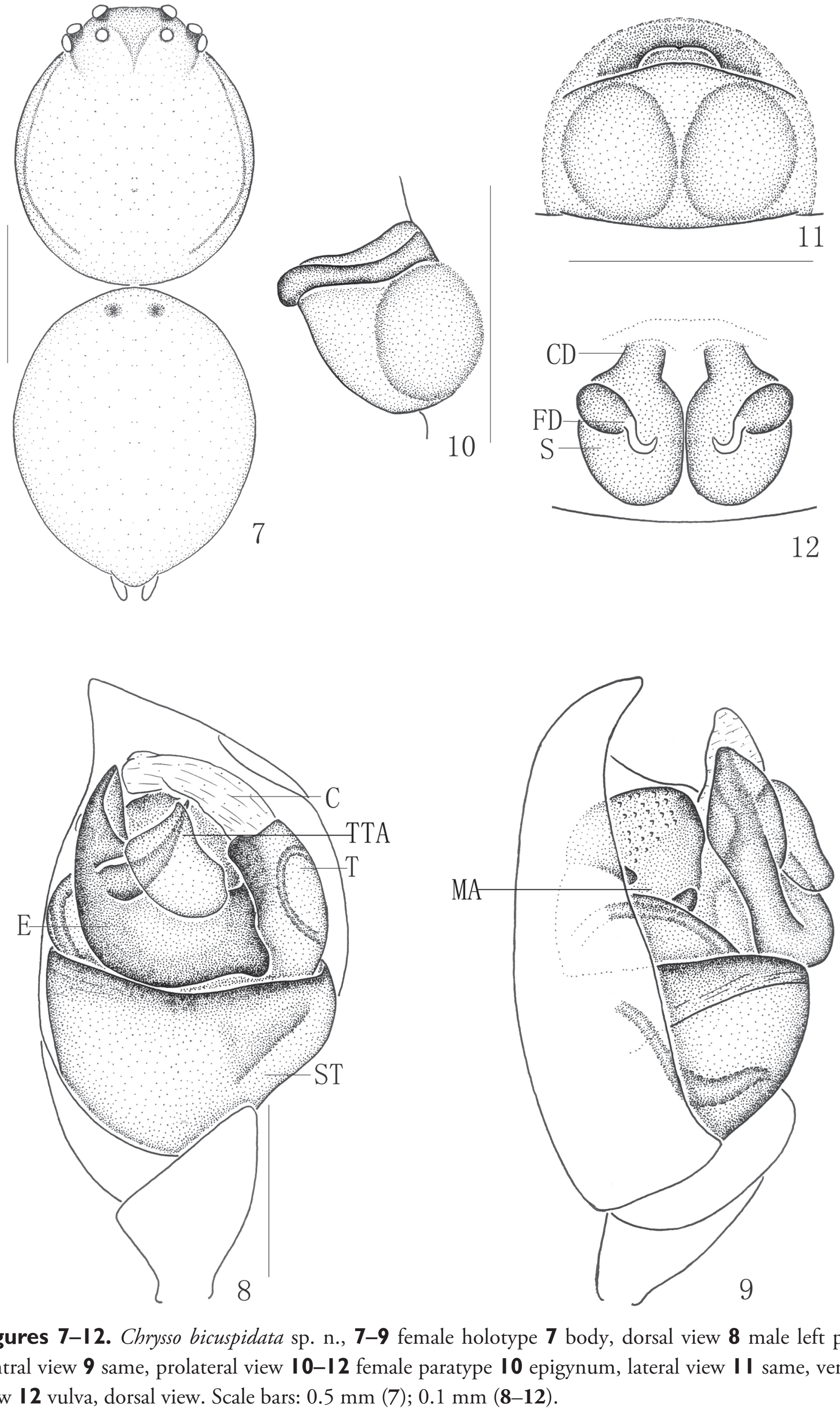
Chrysso bicuspidata sp. n.

urn:lsid:zoobank.org:act:660AA46B-0F72-4257-A1FC-8B734804876B
http://species-id.net/wiki/Chrysso_bicuspidata
Figs 7–12

Type material. Holotype ♂, CHINA, Hainan Island: Jianfengling Mountain, 29 May 2009, C. Zhang leg. **Paratypes:** 2 ♀, same data as holotype; 3 ♀, Jianfengling Mountain, 12 November 2008, G.X. Han leg.; 3 ♂, 1 ♀, Bawangling Mountain (19°07'N, 109°04'E), 25 May 2009, C. Zhang leg.; 5 ♀, Bawangling Mountain, 7 November 2008, G.X. Han leg.; 2 ♂, 1 ♀, Diaoluo Mountain (18°45'N, 109°45'E), 6 June 2009, C. Zhang leg.; 1 ♂, 2 ♀, Limu Mountain, 19 August 2007, F. Zhang leg.

Diagnosis. Males can be distinguished from most *Chrysso* species by membranous conductor, wider embolus and acuate apex of cymbium. And it resembles *C. cyclocera* Zhu, 1998 and *C. oxycera* Zhu & Song, 1993 in the general shape of the palpal organ, but differs from them by the wider embolus (Figs 8–9). Females differ from all other *Chrysso* species except *C. viridiventris* Yoshida, 1996 by the epigynum with a big atrium and a large posterior lobe. It differs from *C. viridiventris* by the bigger spermathecae and shorter copulatory ducts (Figs 10–12).

Etymology. The species name refers to the shape of embolic tip.



Figures 7–12. *Chrysso bicuspidata* sp. n., **7–9** female holotype **7** body, dorsal view **8** male left palp, ventral view **9** same, prolateral view **10–12** female paratype **10** epigynum, lateral view **11** same, ventral view **12** vulva, dorsal view. Scale bars: 0.5 mm (**7**); 0.1 mm (**8–12**).

Description. Male (holotype). Total length 1.84: cephalothorax 0.92 long, 0.71 wide; abdomen 0.92 long, 0.82 wide, 0.92 high. Carapace longer than wide, yellowish, lateral margin with thin black striations, anterior median with a triangular black patch. Only anterior part with several gray setae. Cervical groove yellowish. Median furrow yellowish, almost triangle. Each eye with a black ring. Both the eye rows recurved from dorsal view (Fig. 7). Diameters of eyes: AME 0.05, ALE 0.04, PME 0.04, PLE 0.04. Interdistances of eyes: AME–AME 0.10, AME–ALE 0.03, ALE–ALE 0.33, PME–PME 0.10, PME–PLE 0.08, PLE–PLE 0.38, ALE and PLE closed to each other. MOA long 0.13, front width 0.18, back width 0.18. Clypeus 0.20 high and furnished with few short gray setae. Chelicerae armed with sparse gray setae, promargin with 2 teeth, fangs short and thin. Endites, labium and sternum yellowish. Anterior margin of endites armed with gray scopula. Sternum furnished with sparse gray setae. Legs yellowish, the end of tibia with gray spots. Measurements of legs: leg I 7.14 (1.94, 0.41, 1.94, 1.63, 1.22), II 3.69 (1.33, 0.31, 0.82, 0.82, 0.41), III 2.14 (0.71, 0.20, 0.31, 0.61, 0.31), IV 3.86 (1.12, 0.31, 0.82, 1.20, 0.41). Leg formula: 1423.

Abdomen oval, longer than wide and armed with brown setae. Dorsum yellowish, anterior part with a pair of black patches (Fig. 7). Venter yellowish. Spinnerets yellowish.

Apical cymbium of male palp acuate; embolus big, thick, and end with a thin ramus; conductor membranous, falciform from ventral view; apex of median apophysis with some small tubers (Figs 8–9).

Female (one paratype from Limu Mountain) total length 2.32: cephalothorax 0.92 long, 0.71 wide; abdomen 1.43 long, 1.22 wide, 1.43 high. Diameters of eyes: AME 0.05, ALE 0.05, PME 0.03, PLE 0.05. Interdistances of eyes: AME–AME 0.05, AME–ALE 0.02, ALE–ALE 0.30, PME–PME 0.09, PME–PLE 0.08, PLE–PLE 0.33, ALE and PLE closed to each other. MOA long 0.13, front width 0.15, back width 0.15. Clypeus 0.30 high. Measurements of legs: leg I 7.75 (2.24, 0.51, 1.94, 2.35, 0.71), II 3.59 (1.24, 0.31, 0.71, 0.92, 0.41), III 2.04 (0.71, 0.20, 0.31, 0.51, 0.31), IV 3.79 (1.24, 0.31, 0.81, 1.02, 0.41). Leg formula: 1423. Dorsal abdomen yellowish. Other characters as in holotype.

Female genitalia lightly sclerotized, and with a circular atrium and a large posterior lobe; spermathecae big, oval; copulatory ducts short, thick and connected with spermathecae from anterior part; each fertilization duct with a global head (Figs 10–12).

The new species with putative *Chrysso* synapomorphies as follow: (1) carapace pars stridens smooth; (2) abdomen extending beyond spinnerets; (3) cymbial hood of male palp broad; (4) subconductor of male palp present; (5) median apophysis of male palp with apophysis; (6) palpal claw of female dentition sparse; (7) anterior margin of female genital atrium medially acute.

Variation. The anterior part of dorsal abdomen of some species examined with a pair of black patches, some species without. Males total body length from 1.73–1.88, females total length from 2.22–2.35.

Distribution. China (Hainan).

Chrysso bimaculata Yoshida, 1998

http://species-id.net/wiki/Chrysso_bimaculata
Figs 13–17

Chrysso bimaculata Yoshida, 1998: 105, f. 1–6; Yoshida 2003: 125, f. 330–335; Yoshida 2009: 378, f. 203–204.

Material examined. CHINA, Hainan Island: 1 ♂, 1 ♀, Jianfengling Mountain, 31 May 2009, C. Zhang leg.; 1 ♀, Bawangling Mountain, 6 November 2008, G.X. Han leg.; 1 ♀, Bawangling Mountain, 25 May 2009, C. Zhang leg.; 5 ♀, Limu Mountain, 29 August 2007, G.X. Han leg.; 1 ♂, 3 ♀, Limu Mountain, 21 November 2008, G.X. Han leg.

Description. Male (one specimen from Jianfengling Mountain) total length 1.40: cephalothorax 0.59 long, 0.54 wide; abdomen 0.81 long, 0.59 wide, 0.54 high. Carapace longer than wide, yellowish. Cervical groove yellowish. Median furrow yellowish and almost triangle. Each eye with a red ring. AER recurved and PER procurved from dorsal view (Fig. 13). Diameters of eyes: AME 0.08, ALE 0.07, PME 0.07, PLE 0.07. Interdistances of eyes: AME–AME 0.08, AME–ALE 0.07, ALE–ALE 0.34, PME–PME 0.08, PME–PLE 0.06, PLE–PLE 0.36, ALE and PLE closed to each other. MOA long 0.18, front width 0.18, back width 0.17. Clypeus 0.16 high and furnished with few short gray setae. Chelicerae armed with sparse black setae, promargin with 2 teeth. Endites, labium and sternum yellowish. Anterior margin of endites armed with gray scopula. Sternum furnished with sparse black setae. Legs yellow, the end of tibia yellow brown. Measurements of legs: leg I 5.10 (1.44, 0.36, 1.22, 1.58, 0.50), II 3.07 (0.99, 0.32, 0.63, 0.77, 0.36), III 1.81 (0.50, 0.23, 0.45, 0.36, 0.27), IV 3.20 (1.17, 0.27, 0.63, 0.77, 0.36). Leg formula: 1423.

Abdomen oval, armed with gray setae. Dorsum yellowish, posterior part with two pairs of white patches, lateral part armed with three pairs of black patches and several black stripes (Fig. 13). Venter yellowish. Spinnerets yellowish.

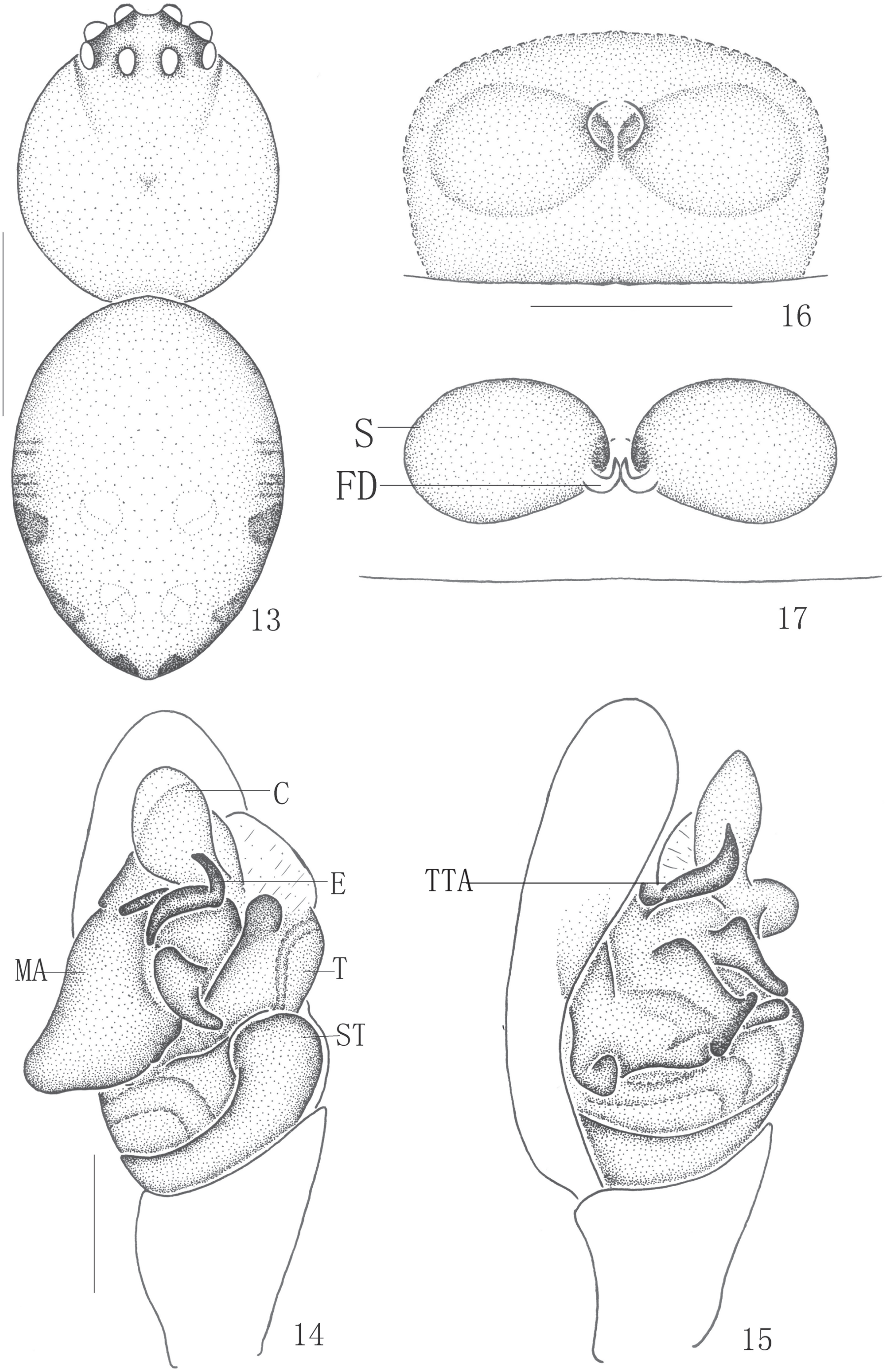
Embolus of male palp small, thin and wind; conductor wind, membranous; median apophysis big, posterior part with a small tuber from prolateral view (Figs 14–15).

Female (one specimen from Jianfengling Mountain) total length 1.80: cephalothorax 0.63 long, 0.54 wide; abdomen 1.08 long, 0.95 wide, 1.04 high. Diameters of eyes: AME 0.08, ALE 0.07, PME 0.07, PLE 0.07. Interdistances of eyes: AME–AME 0.09, AME–ALE 0.03, ALE–ALE 0.35, PME–PME 0.10, PME–PLE 0.07, PLE–PLE 0.38, ALE and PLE closed to each other. MOA long 0.17, front width 0.17, back width 0.20. Clypeus 0.16 high. Measurements of legs: leg I 4.73 (1.35, 0.27, 1.17, 1.44, 0.50), II 2.81 (0.90, 0.23, 0.59, 0.77, 0.32), III 1.95 (0.63, 0.18, 0.32, 0.50, 0.32), IV 2.94 (0.95, 0.27, 0.59, 0.77, 0.36). Leg formula: 1423. Other characters as in holotype.

Female genitalia lightly sclerotized, median part with a circular atrium; spermathecae oval; the copulatory ducts very short (Figs 16–17).

Variation. The amount of white patches and black patches of dorsal abdomen variated from two pairs to three pairs. Males total body length of some species examined from 1.40–1.55, females total length from 1.68–1.82.

Distribution. China (Hainan), Japan.



Figures 13–17. *Chrysso bimaculata* Yoshida, 1998 **13–15** male **13** body, dorsal view **14** male left palp, ventral view **15** same, prolateral view **16–17** female **16** epigynum, ventral view **17** vulva, dorsal view. Scale bars: 0.5 mm (**13**); 0.1 mm (**14–17**).

Acknowledgements

We are grateful to Dr. Dimitar Dimitrov, Dr. Ingi Agnarsson and an anonymous referee for valuable suggestions. We are also grateful to Chao Zhang and Guangxin Han for the collection of specimens. This study was supported by the Ministry of Science and Technology of the People's Republic of China (2006FY110500), the National Natural Science Foundation of China (No. 31071885, 30970325, 31093430), and partly by the Education Office of Hebei Province, China (No. Z2009101), and the Natural Science Foundation of Baoding University (No. 2010Z01).

References

Agnarsson I (2004) Morphological phylogeny of cobweb spiders and their relatives (Araneae, Araneoidea, Theridiidae). Zoological Journal of the Linnean Society 141: 447–626. doi: 10.1111/j.1096-3642.2004.00120.x

Barrion AT, Litsinger JA (1995) Riceland Spiders of South and Southeast Asia. CAB International, Wallingford, UK, 700 pp.

Bryant EB (1940) Cuban spiders in the Museum of Comparative Zoology. Bulletin of the Museum of Comparative Zoology 86: 247–554.

Cambridge OP (1882) On new genera and species of Araneidea. Proceedings of the Zoological Society of London, 423–442.

Deeleman-Reinhold CL (2009) Spiny theridiids in the Asian tropics. Systematics, notes on behaviour and species richness (Araneae: Theridiidae: *Chrysso*, *Meotipa*). Contributions to Natural History 12: 403–436.

Gonzaga MO, Leiner NO, Santos AJ (2006) On the sticky cobwebs of two theridiid spiders (Araneae: Theridiidae). Journal of Natural History 40: 293–306. doi: 10.1080/00222930600703433

Levi HW (1957) The spider genera *Chrysso* and *Tidarren* in America. Journal of the New York Entomological Society 63: 59–81.

Levi HW, Levi LR (1962) The genera of the spider family Theridiidae. Bulletin of the Museum of Comparative Zoology at Harvard University 127: 1–71.

Miller J, Agnarsson I (2005) A redescription of *Chrysso nigriceps* (Araneae, Theridiidae) with evidence for maternal care. The Journal of Arachnology 33: 711–714. doi: 10.1636/H04-10.1

Platnick NI (2012) The world spider catalog, version12.5.American Museum of Natural History, online at http://research.amnh.org/entomology/spiders/catalog/index.html [accessed 31 December, 2011]

Siliwal M (2009) A new species of the genus *Chrysso* O. P.-Cambridge (Araneae: Theridiidae) from Arunachal Pradesh, India. Biosystematica 3: 5–9.

Song DX, Zhang JX, Zhu MS (2006) Araneae. In: Li ZZ, Jin DC (Eds) Insects from Fanjingshan Landscape. Guizhou Science and Technology Publishing House, Guiyang, 656–690.

Song DX, Zhu MS, Chen J (1999) The Spiders of China. Hebei Science & Technology Publishing House, Shijiazhuang, 640 pp.

Tang G, Yin CM, Peng XJ (2003) Two new species of the genus *Chrysso* from Hunan Province, China (Araneae, Theridiidae). Acta zootaxonomica Sinica 28: 53–55.

Yoshida H (2009) Uloboridae, Theridiidae, Ctenidae. In: Ono H (Ed) The Spiders of Japan with keys to the families and genera and illustrations of the species. Tokai University Press, Kanagawa, 142–147, 356–393, 467–468.

Yoshida H, Tso IM, Severinghaus LL (2000) The spider family Theridiidae (Arachnida: Araneae) from Orchid Island, Taiwan: Descriptions of six new and one newly recorded species. Zoological Studies 39: 123–132.

Zhu MS (1998) Fauna Sinica: Arachnida: Araneae: Theridiidae. Science Press, Beijing, 436 pp.

ZooKeys 190: 33–54 (2012)
doi: 10.3897/zookeys.190.3276
www.zookeys.org

RESEARCH ARTICLE

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

New species of *Prosopodesmus* Silvestri, 1910 (Diplopoda, Polydesmida, Haplodesmidae) from Queensland, Australia

Robert Mesibov[1,†]

1 *Queen Victoria Museum and Art Gallery, Launceston, Tasmania 7250, Australia*

† *urn:lsid:zoobank.org:author:24BA85AE-1266-494F-9DE5-EEF3C9815269*

Corresponding author: *Robert Mesibov* (mesibov@southcom.com.au)

Academic editor: *S. Golovatch* | Received 24 April 2012 | Accepted 25 April 2012 | Published 4 May 2012

urn:lsid:zoobank.org:pub:99E9D244-588D-4A68-AEF5-0129A8411449

Citation: Mesibov R (2012) New species of *Prosopodesmus* Silvestri, 1910 (Diplopoda, Polydesmida, Haplodesmidae) from Queensland, Australia. ZooKeys 190: 33–54. doi: 10.3897/zookeys.190.3276

Abstract

Prosopodesmus crater **sp. n.**, *P. kirrama* **sp. n.** and *P. monteithi* **sp. n.** are described from the Wet Tropics of north Queensland. The hothouse species *P. panporus* Blower & Rundle, 1980 is recorded from rainforest on Queensland's Cape York Peninsula, where it is likely to be native.

Keywords

Diplopoda, Polydesmida, Haplodesmidae, millipede, Australia, Queensland

Introduction

Prosopodesmus Silvestri, 1910 is a small genus of tropical haplodesmid Polydesmida with three transverse rows of large tubercles on the metatergites and downward-bending paranota (Golovatch et al. 2009). The unbranched, sickle-shaped gonopod telopodites normally lie parallel to the main body axis in a hollow formed by ventromedial concavities on the gonocoxae.

Two species have been described in *Prosopodesmus*. The type species *P. jacobsoni* Silvestri, 1910 is a pantropical tramp first collected at Jakarta ('Batavia') on Java. It has since been found in Florida and Puerto Rico (USA), Haiti (as the synonymous

Homodesmus parvus Chamberlin, 1918), St Eustatius (Leeward Islands, West Indies), Panama, Brazil, Zanzibar, India, New Caledonia and the Galapagos Islands (Hoffman 1999). It has recently been confirmed as present on Taiwan (Golovatch et al. 2011) and there are *P. jacobsoni* records from Louisiana (USA) (Shelley and Golovatch 2000), Christmas Island (Indian Ocean) (Jeekel 2006) and Fiji (Akkari and Enghoff 2011). The native range of *P. jacobsoni* is unknown.

P. panporus Blower & Rundle, 1980 was described from tropical plant hothouses at the Royal Botanic Gardens at Kew, England (Blower and Rundle 1980). The describers did not suggest a native range for the species: "Presumably *P. panporus* lurks in some unworked tropical habitat." (Blower and Rundle 1980, p. 32).

Two more species were added to *Prosopodesmus* when the Japanese genus *Rhipidopeltis* Miyosi, 1958 was made a junior synonym (Golovatch et al. 2009, Golovatch et al. 2010). *P. sinuatus* (Miyosi, 1958) is known only from far southern Honshu and *P. similis* (Haga, 1968) from Kyushu, to the south of Honshu across the Kanmon Strait.

While sorting museum samples of Pyrgodesmidae from eastern Australia, I found four species of pyrgodesmid-like *Prosopodesmus* in Berlese samples from rainforest in tropical north Queensland. Three of the species are new, while the fourth is *P. panporus*.

Methods

'Male' and 'female' in the text refer to adult individuals. All specimens are stored in 75–80% ethanol in their respective repositories.

Whole, selected specimens and rings 7 of selected males were cleared in 80% lactic acid and temporarily mounted in 60% lactic acid for optical microscopy. Preliminary gonopod drawings were traced from digital images acquired with an eyepiece video camera temporarily fitted to an optical microscope. Body parts of uncleared specimens were temporarily mounted in a 1:1 glycerol:water mixture for examination and measurement. Photomicrographs were taken with a Canon EOS 1000D digital SLR camera mounted on a Nikon SMZ800 binocular dissecting microscope equipped with a beam splitter. Specimens for scanning electron microscopy were briefly air-dried, temporarily fixed to a stub with double-sided adhesive tape or a sticky carbon pad, examined uncoated with a FEI Quanta 600 operated in low-vacuum mode, then returned to alcohol. Images and drawings were prepared for publication using GIMP 2.6. The map figure was generated using ArcView GIS 3.2.

The specimen localities given below are also listed in Appendix 2 in table form. Latitude and longitude (WGS84 datum) are from museum collection databases. The uncertainty for each locality (in square brackets) is the radius of a circle around the stated position, and is my own estimate.

Abbreviations: ANIC = Australian National Insect Collection, Canberra, Australian Capital Territory; IEA = Instituto di Entomologia Agraria, Portici, Italy; NHM = Natural History Museum, London, UK; Qld = Queensland; QM = Queensland Museum, Brisbane, Qld; VMNH = Virginia Museum of Natural History, Martinsville, Virginia, USA.

Results

Order Polydesmida Pocock, 1887
Suborder Polydesmidea Pocock, 1887
Family Haplodesmidae Cook, 1895

Prosopodesmus Silvestri, 1910

Prosopodesmus Silvestri 1910: 360. Attems 1914: 177, 1926-1930: 136, 1940: 292. Brölemann 1916: 570, 1920: 225. Jeekel 1971: 348. Blower and Rundle 1980: 27. Hoffman 1980: 174, 1999: 432. Golovatch et al. 2009: 2, 43. Golovatch et al. 2010: 33.

Type species. *P. jacobsoni* Silvestri, 1910, by original designation.

Other included species. *P. crater* sp. n., *P. kirrama* sp. n., *P. monteithi* sp. n., *P. panporus* Blower and Rundle, 1980, *P. similis* (Haga, 1968), *P. sinuatus* (Miyosi, 1958).

Homodesmus Chamberlin 1918: 222. Attems 1926-1930: 141, 1940: 294. Loomis 1950: 166 (synonymised with *Prosopodesmus*). Jeekel 1971: 331. Hoffman 1980: 174, 1999: 432. Golovatch et al. 2009: 40, 43.

Type species. *H. parvus* Chamberlin, 1918. (Synonymised with *P. jacobsoni* in Loomis 1950: 166)

Rhipidopeltis Miyosi 1958: 297. Hoffman 1980: 174. Shelley et al. 2000: 127. Golovatch et al. 2009: 2, 43 (synonymised with *Prosopodesmus*). Golovatch et al. 2010: 33.

Type species. *R. sinuata* (recte *sinuatus*) Miyosi, 1958.

Prosopodesmus crater sp. n.

urn:lsid:zoobank.org:act:D20FAE64-B7BC-4A68-9733-4EB33AC6CAAF
http://species-id.net/wiki/Prosopodesmus_crater
Figs 1A, 2A, 3A, 5A

Holotype. Male, Eacham National Park, Qld, 17°18'S, 145°37'E [±2 km], 760 m, 28 June 1971, R.W. Taylor and J. Feehan, berlesate 344, rainforest, ANIC 64-000213.

Paratypes. 1 male, 2 stadium 7 males, 2 stadium 7 females, 1 stadium 6 male, details as for holotype, ANIC 64–000212; 1 male, details as for holotype but 1–7 October 1972, R.W. Taylor, berlesate 428, ANIC 64–000214; 1 male, 2 females, Cathedral Fig, Qld, 17°10'52"S, 145°39'26"E [±500 m], 720 m, 7 February 1996, G. Monteith, berlesate 907, rainforest, sieved litter, QM S37593; 1 male, 1 female, Downey Creek, 25 km SE of Millaa Millaa, Qld, 17°40'48"S, 145°46'58"E [±500 m], 400 m, 7 December 1988, G. Monteith and G. Thompson, berlesate 813, rainforest, sieved litter, QM S91625.

Other material. 1 male, Cammoo Caves near Rockhampton, Qld, 23°10'S, 150°28'E [±2 km], 25 October 1976, R.W. Taylor and T.A. Weir, berlesate 535, dense, low, closed forest, ANIC 64–000215.

Diagnosis. Males and females with head + 20 rings; adults 7–8 mm long; midbody metatergites typically with 3 transverse rows of 10 large tubercles; posterior por-

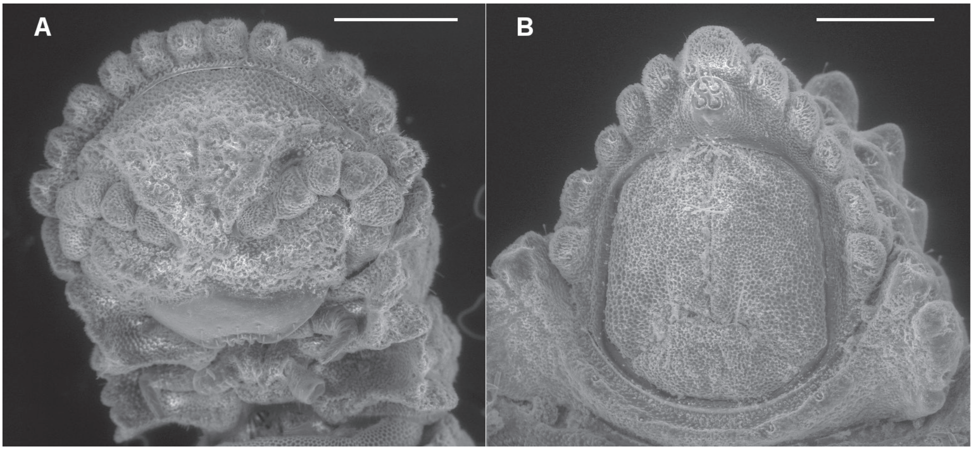


Figure 1. A Ventral view of head of *P. crater* sp. n., paratype, ANIC 64–000212, showing 12 lobes on anterior edge of collum, antennae retracted below edges of collum and ring 2 tergite, and textured frons with smooth clypeus. **B** Ventral view of telson of *P. monteithi* sp. n., QM S91632, showing 5+5 lobe pattern on edge of preanal ring and apical epiproct.

tion of prozonite with small disks, no microtubercles; ozopores not on porosteles; gonopod telopodite with single posteriad bend, near tip.

Description. Male with head + 20 rings, ca 7 mm long. Colour in alcohol pale yellow; lightly encrusted with fine soil particles. Ring 12 with maximum vertical diameter 0.7 mm; maximum width (including paranota) 0.9 mm and 1.6X prozonite width; paranotal length ca 1/2 of total ring length.

Head (Fig. 1A) facing ground, covered by anterior collum edge in dorsal view; vertex microtuberculate; frons with large, irregular tubercles with microvillose texture; clypeus smooth, sparsely setose ventrally. Antennal sockets separated by slightly more than a socket diameter. Retracted antennae with distalmost antennomeres held in groove formed by lateral edge of frons anteriorly and confluent lateral collum and ring 2 tergite edges posteriorly. Antennomere relative widths (5,6)>(2,3,4), relative lengths 6>5>(2,3,4).

Collum with 12 lobes along anterior edge (Fig. 1A). Collum, ring 2 tergite and metatergites 5–15 about equally wide; rings 3,4 slightly narrower; rings16–18 progressively narrowing. Collum, tergites and metatergites textured with large, low tubercles, each with irregular, roughened, microvillose fine structure; 3 transverse rows of typically 10+10+10 tubercles on midbody metatergites. Dorsal and lateral setae on collum, tergites and metatergites sparse, bisegmented, the distal portion flattened, slightly flared at tip and minutely toothed along distal edge. Anterior portion of prozonite (Fig. 2A) cellular, posterior portion irregularly covered with small, more or less round, variably sized, convex disks. Limbus a smooth, straight-edged lamella.

Ring 2 tergite edge lower than collum edge and ring 3 tergite edge. Paranota set low on body and declined at about 45°, subquadrate in dorsal view; lateral margin notched into 3 lobes at ca 1/8 and 1/2 the paranotal length.

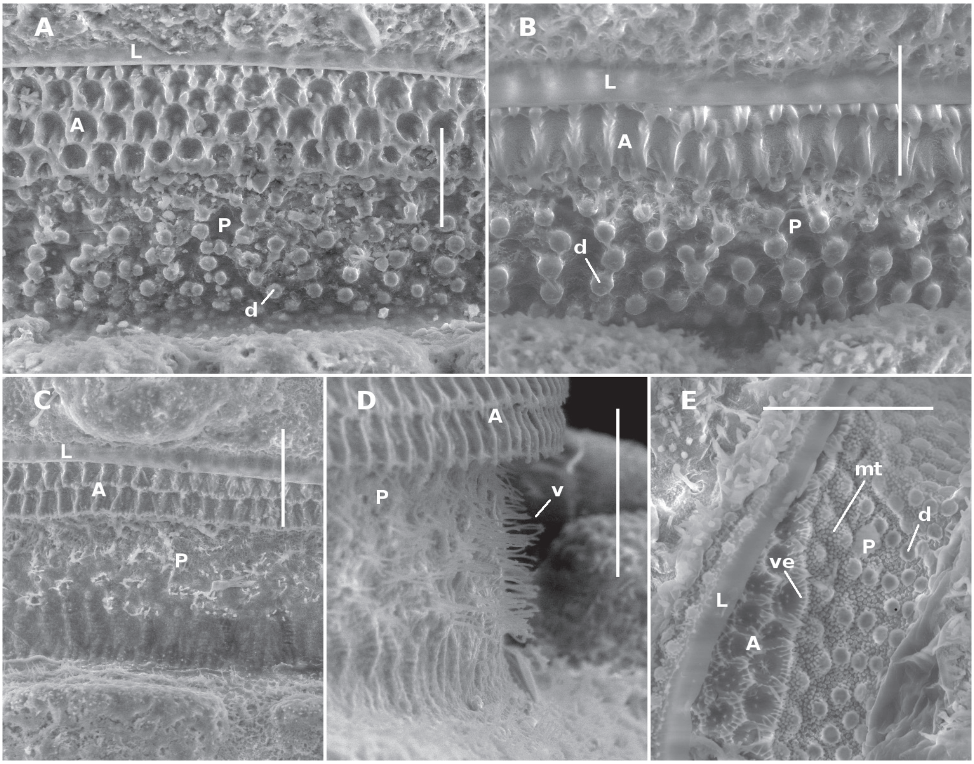


Figure 2. Midbody limbus and prozonite of *Prosopodesmus* species, dorsal views. **A** *P. crater* sp. n., paratype, QM S37593. **B** *P. kirrama* sp. n., paratype, QM S91627. **C, D** *P. monteithi* sp. n., QM S91632. **E** *P. panporus* Blower and Rundle, 1980, ANIC 64–000118. **L** limbus **A** anterior portion of prozonite **P** posterior portion of prozonite **d** disk **mt** microtubercles **v** villi **ve** microvillose extensions. Scale bars: **A, B, E** = 0.05 mm, **C, D** = 0.1 mm.

Ozopore not on porostele, inconspicuous near posterolateral corner of paranotum; pore formula 5, 7, 9, 10, 12, 13, 15–19.

Sternites as wide as long. Legs short, hidden by paranota in dorsal view. Relative podomere lengths (femur, tarsus)>prefemur>(postfemur, tibia).

Spiracles not evident.

Telson (as for *P. monteithi* sp. n in Fig. 1B) facing ground; preanal ring with 5 lobes on each side and 1 larger lobe (epiproct) apically. Spinnerets recessed in individual chambers, basal sheaths with unnotched distal edges; setal shafts smooth. Paraprocts more or less flat, rounded-rectangular; paraproctal setae close to and equidistant from margin. Hypoproct trapezoidal, hypoproctal setae not on raised tubercles.

Gonopore inconspicuous on leg 2 coxa. Ring 6 metatergite with slight medial excavation on posterior margin ventrally, accommodating tips of retracted gonopod telopodites. Aperture ovoid, as wide as ring 7 prozonite and extending anteriorly to occupy ventral portion of prozonite. Gonocoxae (Fig. 3A) massive, slightly tapering

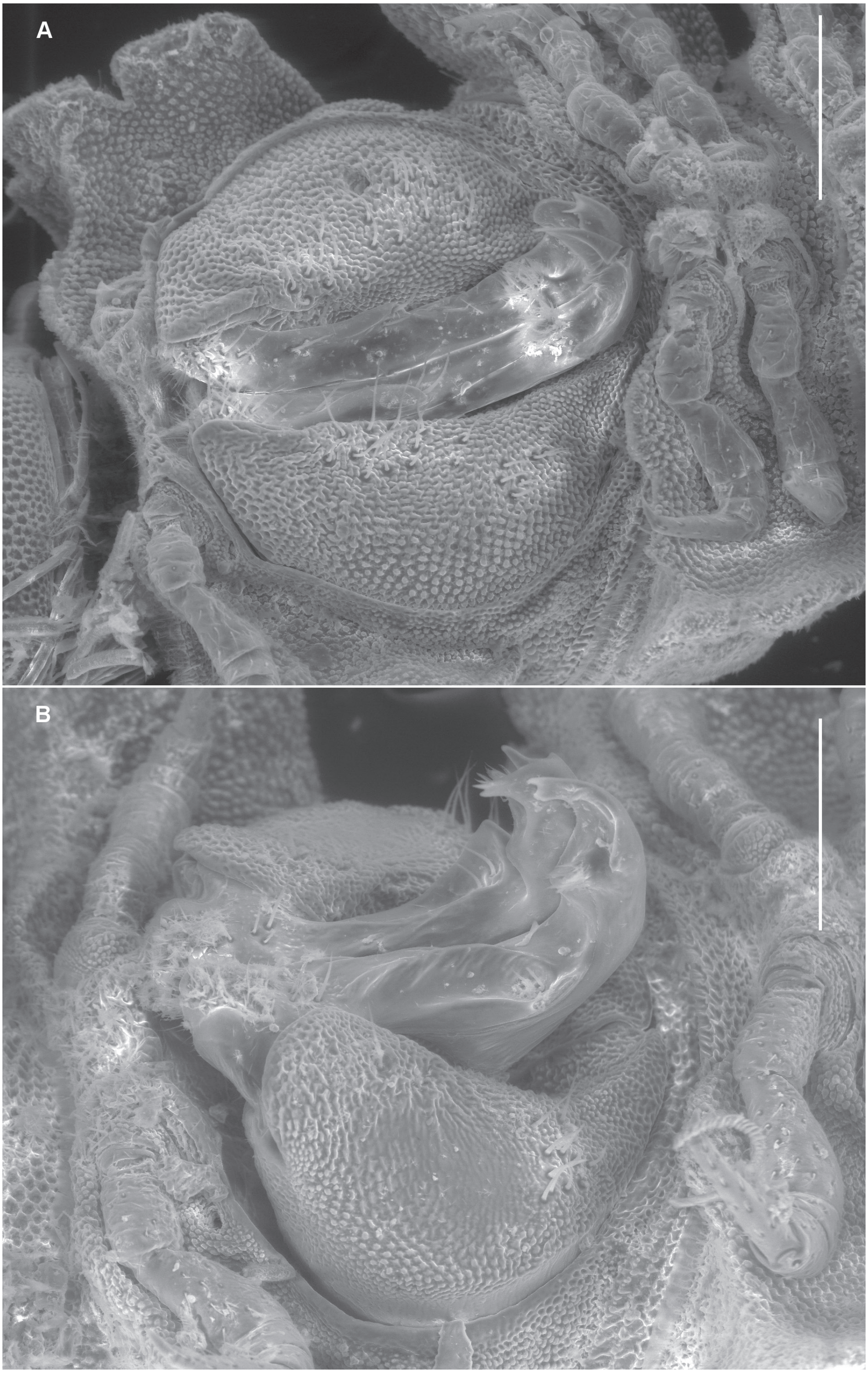


Figure 3. Left ventrolateral views of gonopods of *Prosopodesmus* species. **A** *P. crater* sp. n., paratype, ANIC 64–000212. **B** *P. kirrama* sp. n., paratype, QM S91627. Tip of left gonopod telopodite of *P. crater* is broken. Scale bars = 0.2 mm. Image contrast is low because specimens are uncoated.

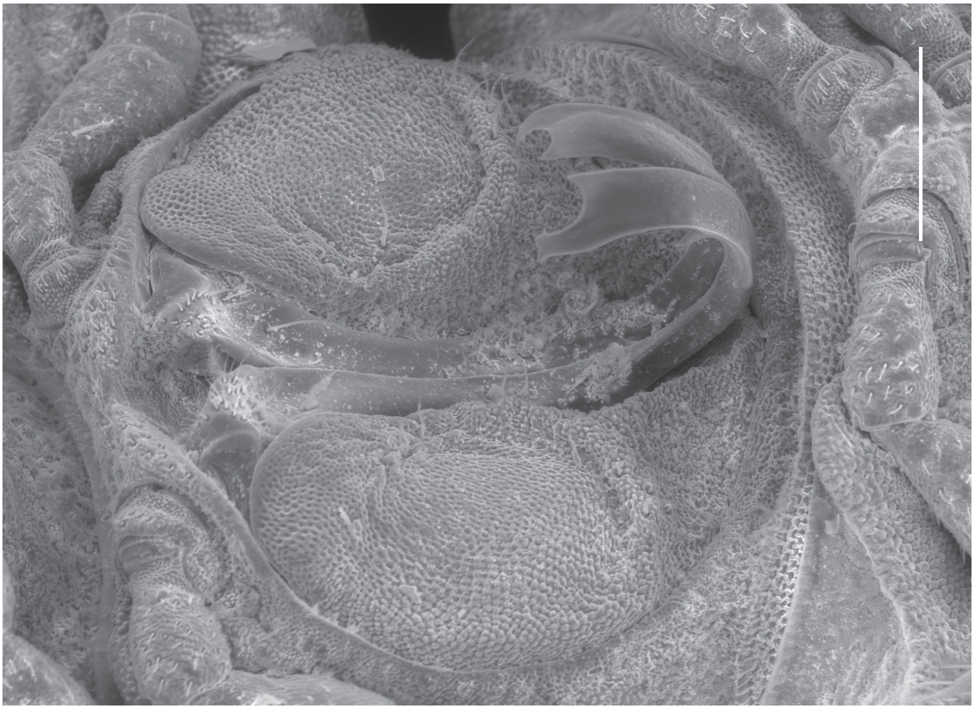


Figure 4. Left ventrolateral view of gonopods of *Prosopodesmus monteithi* sp. n., QM S91632. Scale bar = 0.25 mm. Image contrast is low because specimen is uncoated.

posteroventrally, surface densely microtuberculate; flat ventrally with a few setae near medial side; medial side slightly concave.

Telopodite base in shallow recess at posteroventral corner of gonocoxa. Telopodites (Figs 3A, 5A) slender, straight, more or less uniformly wide, parallel, just touching when retracted, slightly convex anteriorly, slightly concave ventrally; bent posteriorly at tip; with thin, rounded tabs projecting posteriorly from lateral edge at between 1/4 and 1/2 the telopodite length and just basal to the apical bend; with thin, rounded tab similarly extending the medial edge just distal to the bend. Telopodites with a few larger setae to about 1/2 telopodite length on posterior surface laterally; with numerous fine setae around and just inside basal concavity into which the prominent cannula inserts. Prostatic groove running straight to small, low mound on middle of posterior telopodite surface at about 3/4 telopodite length; mound covered with 'hairpad' of slender, pointed villi.

Female with head + 20 rings; a little larger than male, ca 8 mm long. Epigyne ca 1/3 ring width, slightly raised, rounded rectangular with straight distal edge; cyphopods not examined.

Distribution. Rainforest on the Atherton Tableland southwest from Cairns, Queensland, with a known north-south range of ca 40 km (Fig. 8). There is also one questionable record from the Cammoo Caves area in central coastal Queensland. (See Remarks.)

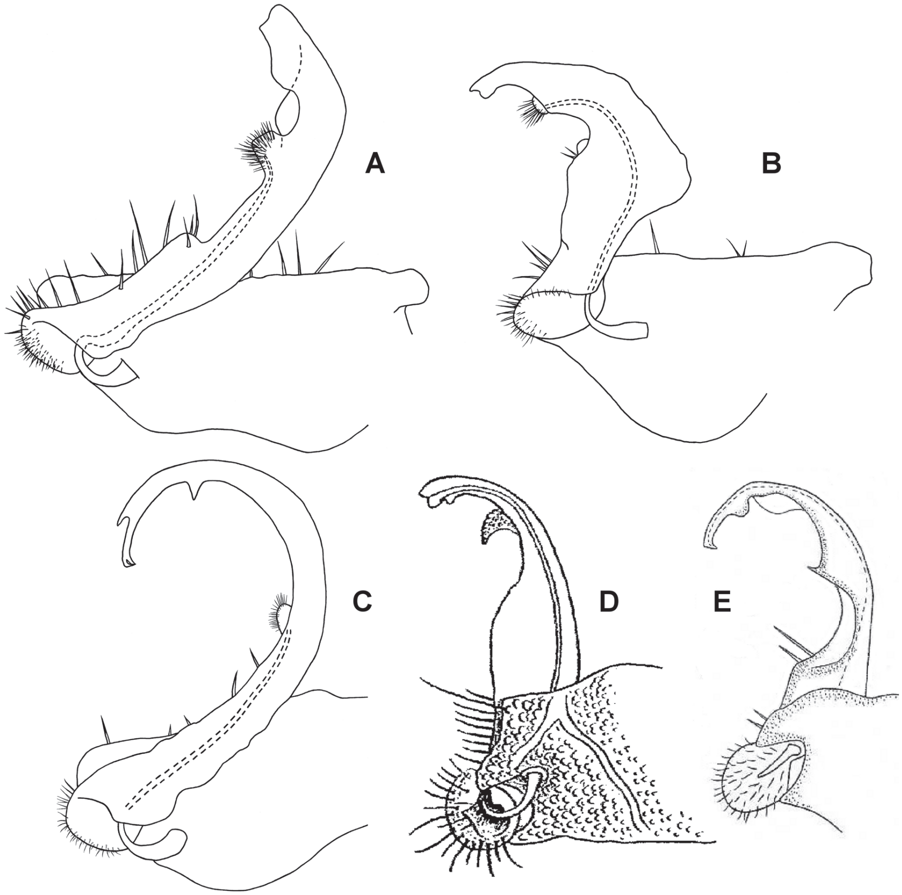


Figure 5. Medial views of right gonopod of *Prosopodesmus* species, not to same scale. **A** *P. crater* sp. n., paratype, ANIC 64–000214. **B** *P. kirrama* sp. n., paratype, QM S91626. **C** *P. monteithi* sp. n., QM 91635. **D** *P. sinuatus* (Miyosi, 1958), holotype, drawing scanned and modified from Fig. 1G in Miyosi (1958). **E** *P. similis* (Haga, 1968), holotype, drawing scanned and modified from Fig. 12B in Haga (1968).

Etymology. Latin *crater*, 'cup'. The type locality surrounds Lake Eacham, a crater lake on the Atherton Tableland in far north Queensland.

Remarks. The male in 'Other material' is from a site nearly 800 km south and east from the nearest other *P. crater* locality. This specimen is from the same ANIC berlesate that indicated a similar 800+ km disjunction in the range of the unrelated millipede *Asphalidesmus magnus* Mesibov, 2011 (Mesibov 2011). Although the Cammoo Caves *P. crater* is very slightly different in gonopod details from those collected on the Atherton Tableland, the most likely explanation is that the Cammoo Caves specimens of *P. crater* and *A. magnus* were actually collected in the Wet Tropics, and that the locality labels are incorrect.

Prosopodesmus kirrama **sp. n.**

urn:lsid:zoobank.org:act:308DBF9D-BF94-4705-9F0B-15F44ECFD6BD
http://species-id.net/wiki/Prosopodesmus_kirrama
Figs 2B, 3B, 5B

Holotype. Male, Douglas Creek Road, Kirrama Range, Qld, 18°13'30"S, 145°48'13"E [±500 m], 800 m, 10 December 1986, G. Monteith and G. Thompson, berlesate 731, rainforest, sieved litter, QM S91629.

Paratypes. 1 male, 2 stadium 7 males, details as for holotype, QM S91628; 2 males, 3 females, 1 stadium 6 male, near Yuccabine Creek, Kirrama Range, Qld, 18°12'21"S, 145°45'47"E [±500 m], 700 m, 10 December 1986, G. Monteith and G. Thompson, berlesate 732, QM S91626; 2 males, Kirrama Range, Qld, 18°12'57"S, 145°47'15"E [±500 m], 700 m, 9 December 1986, G. Monteith and G. Thompson, berlesate 730, QM S91627.

Other material. 1 female, Upper Broadwater valley, Cardwell Range, Qld, 18°19'15"S, 145°58'34"E [±500 m], 800 m, 16 January 1987, S. Hamlet, berlesate 759, rainforest, sieved litter, QM S91631; 3 stadium 7 females, 1 stadium 6 female, same details but 700 m, 20 December 1986, G. Monteith, G. Thompson and S. Hamlet, berlesate 745, QM S91630.

Diagnosis. Males and females with head + 20 rings; adults 8–9 mm long; midbody metatergites typically with 3 transverse rows of 12 large tubercles; posterior portion of prozonite (Fig. 2B) with small disks, no microtubercles; ozopores not on porosteles; gonopod telopodite with two posterior bends, one at about midlength and one near tip, the more basal bend marked by strong anterior production of telopodite.

Description. As for *P. crater*, differing in the following details:

Male/female lengths ca 8/9 mm, respectively. Ring 12 with maximum vertical diameter 0.8 mm; maximum width (including paranota) 1.2 mm and 1.7X prozonite width. Ring 2 slightly narrower than collum. 3 transverse rows of typically 12+12+12 tubercles on metatergites.

Gonocoxae (Fig. 3B) massive, strongly tapering anteroventrally and posteroventrally. Telopodite (Figs 3B, 5B) strongly produced anteriorly at about midlength, bending posteriorly there and again near tip. Hairpad mound medial on posterior surface midway between second bend in telopodite and tip. Thin, rounded tab on medial edge of telopodite just below level of hairpad; thin, rounded tabs on lateral edge at level of first bend and just distal to hairpad; narrow longitudinal ridge medially on posterior telopodite surface from near base to near first bend.

Distribution. Rainforest in the mountains southwest from Tully and northwest from Ingham, Queensland, with a known north-south range of ca 25 km (Fig. 8).

Etymology. For the type locality, the Kirrama Range.

Prosopodesmus monteithi **sp. n.**

urn:lsid:zoobank.org:act:1E427018-6159-407B-9178-C6AA835A723F
http://species-id.net/wiki/Prosopodesmus_monteithi
Figs 1B, 2C, 2D, 4, 5C, 6

Holotype. Male, 2 km SE of Mt Spurgeon via Mt Carbine, Qld, 16°27'17"S 145°12'26"E [±500 m], 1100 m, 20–21 December 1988, G. Monteith and G. Thompson, ex QM S18018, QM S91641.

Paratypes. 1 female, 1 stadium 7 male, 1 stadium 5 male, details as for holotype but 20 December 1988, berlesate 825, rainforest, sieved litter, QM S91640; 1 male, 1 female, 7 km N of Mt Spurgeon, Qld, camp 2, 16°22'31"S 145°12'49"E [±500 m], 1200 m, 17–19 October 1991, G. Monteith, H. Janetzki, D. Cook and L. Roberts, QM S91639.

Other material. 1 male, Alexandra Bay, Qld, 16°12'S 145°26'E [±2 km], <50 m, 24 June 1971, R.W. Taylor and J. Feehan, berlesate 331, rainforest, ANIC 64–000211; 1 male, 1 female, 1 stadium 7 male, Bargoo Creek, Windsor Tableland, 35 km NNW of Mt Carbine, Qld, 16°14'51"S 145°04'08"E [±500 m], 850 m, 18 April 1982, G. Monteith, D. Yeates and D. Cook, berlesate 397, rainforest, sieved litter, QM S91638; 1 male, 4.0 km W of Mt Tribulation, Qld, site 8, 16°04'44"S 145°25'59"E [±500 m], 720 m, 2 January 1983, G. Monteith, berlesate 503, rainforest, sieved litter, QM S91636; 1 male, 4.5 km W of Cape Tribulation, Qld, site 9, 16°04'41"S 145°25'46"E [±500 m], 760 m, January 1983, G. Monteith and D. Yeates, berlesate 531, rainforest, sieved litter, QM S91634; 2 males, 4 females, same details but G. Monteith, berlesate 515, QM S91635; 1 stadium 7 female, 2.5 km N of Mt Lewis via Julatten, Qld, 16°33'49"S 145°15'51"E [±500 m], 1040 m, D. Yeates and G. Thompson, berlesate 611, rainforest, sieved litter, QM S91642; 1 male, 2 females, North Bell Peak, Qld, 17°05'06"S 145°52'00"E [±500 m], 600 m, 22 November 1990, G. Monteith and G. Thompson, berlesate 845, rainforest, sieved litter, QM S91643; 1 male and 1 female in copula, 1 stadium 7 male, 1 stadium 7 female, Roaring Meg valley, Qld, 16°03'45"S 145°25'06"E [±500 m], 720 m, 22 November 1993, G. Monteith, H. Janetzki, L. Roberts and D. Cook, QM S91633; 2 males, 3 females, 1 stadium 7 male, Mt Halcyon, Qld, 16°03'16"S 145°25'16"E [±500 m], 870 m, 22–24 November 1993, G. Monteith, H. Janetzki, D. Cook and L. Roberts, QM S91632; 2 males, 2 females, 1 stadium 7 male, Mt Hemmant, Qld, 16°06'44"S 145°24'58"E [±500 m], 1050 m, 27 November 1993, G. Monteith and H. Janetzki, berlesate 865, rainforest, sieved litter, QM S91637.

Diagnosis. Males and females with head + 20 rings; adults 14–15 mm long; midbody metatergites typically with 3 transverse rows of 10 large tubercles; posterior portion of prozonite microvillose, without small disks or microtubercles; ozopores not on porosteles; gonopod telopodite slender, curved smoothly in J-shape.

Description. As for *P. crater*, differing in the following details:

Male/female lengths ca 14/15 mm, respectively; adults light to medium brown (Fig. 6A, 6B). Ring 12 with maximum vertical diameter 1.2 mm; maximum width (including paranota) 2.0 mm and 1.8X prozonite width. Antennomere relative widths

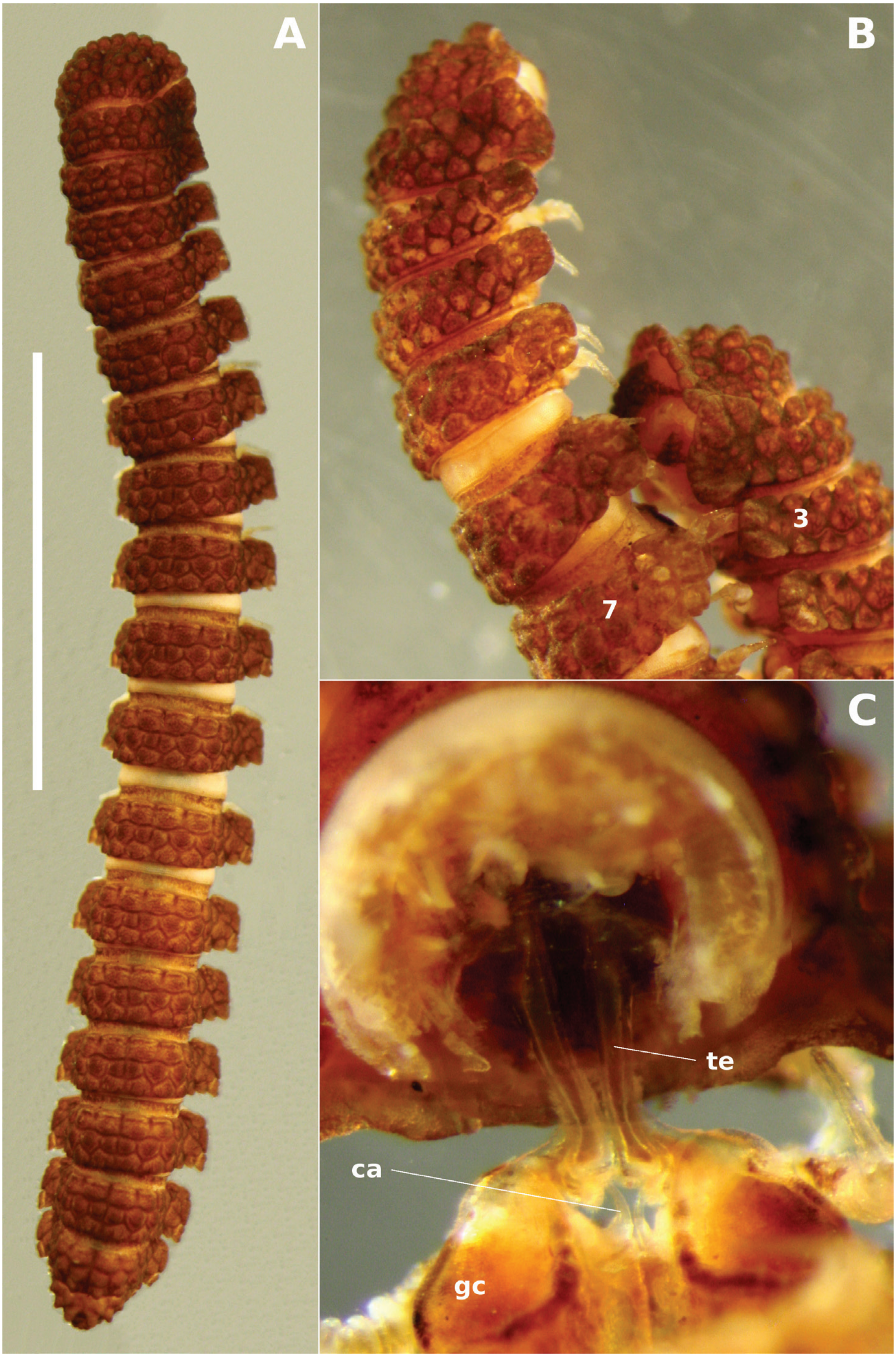


Figure 6. *Prosopodesmus monteithi* sp. n. **A** Adult female ex QM S91632; scale bar = 5 mm. **B** Mating pair ex QM S91633 with male ring 7 and female ring 3 labelled. **C** Partial dissection of the mating pair in **B ca** cannula, **gc** gonocoxa, **te** telopodite.

5>6>(2,3,4), relative lengths (2,6)>(3,4,5). Collum, tergite and metatergite tubercles polygonal, closely fitted. Posterior portion of prozonite (Figs 2C, 2D) irregularly rugose and finely microvillose, without disks or microtubercles. Posterior notch on paranota at ca 2/3 paranotal length, anterior notch sometimes indistinct; paranota declined at ca 30°.

Telopodite (Figs 4, 5C) slender, smoothly curving in J-shape; hairpad mound at about midlength; a small triangular tab directed basally near curved-over tip; tip apically slightly excavate, the lateral side extended and terminating in 3 minute, finger-like processes.

Distribution. Rainforest from Daintree National Park west of Cape Tribulation to the Malbon Thompson Range on the coast southeast from Cairns in Queensland, a north-south range of ca 125 km (Fig. 8).

Etymology. For Geoff Monteith, former curator of insects at the Queensland Museum. Geoff and his colleagues collected most of the specimens of the three new *Prosopodesmus* species described in this paper.

Remarks. *P. monteithi* is the largest known *Prosopodesmus* and the striking dorsal macrosculpture is easily visible to the unaided eye (Fig. 6A). One of the Queensland Museum samples contained a mating pair (Fig. 6B) which I partially dissected (Fig. 6C). As expected, the telopodites were rotated 90° out of the gonocoxal cavity in which they normally lie. The curved distal portion of each telopodite (Fig. 5C) was fully inserted into the cavity anterior to the epigyne, but how much of the curve was actually in contact with the cyphopod could not be seen, and would be better investigated with fixed, sectioned material.

Prosopodesmus panporus Blower & Rundle, 1980

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

Figs 2E, 7

Prosopodesmus panporus Blower and Rundle, 1980: 27; figs 1-3, 6-8; table 1. Golovatch et al. 2009: 3.

Holotype. Male, Palm House, Royal Botanic Gardens, Kew, London, UK, 16 May 1976, A.J. Rundle, extracted by hand from leaf litter in bed No. 1, slide-mounted in balsam, NHM. (Not examined.)

Paratypes. 1 male, details as for holotype, VMNH; 2 males, details as for holotype, IEA; 1 male and 1 female found in copula, details as for holotype but 15 April 1976, bed No. 2, NHM; 75 males, 41 females, 204 juveniles, details as for holotype but including Tullgren-extracted specimens, NHM. (Not examined.)

[Type details from Blower and Rundle (1980).]

Material examined. 9 males, 4 females, 9 km ENE of Mt Tozer, Qld, 12°43'S 143°17'E [±2 km], 5–10 July 1986, T. Weir, ANIC berlesate 1057, rainforest litter, ANIC 64-000118; 15 males, 1 female, same details but ANIC berlesate 1059, ANIC 64-000210.

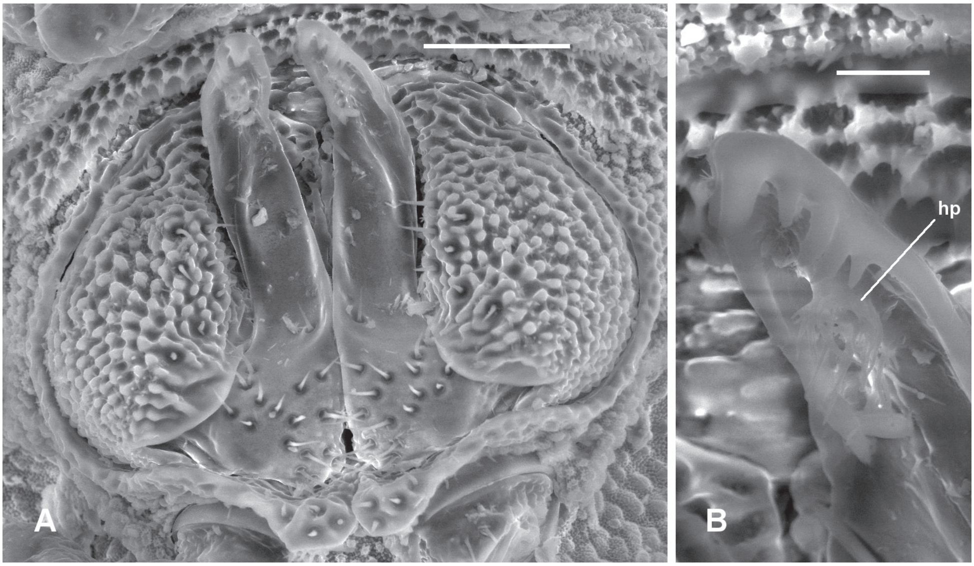


Figure 7. *Prosopodesmus panporus* Blower and Rundle, 1980, male ex ANIC 64–000210. **A** Gonopods in situ, ventral view **B** Close-up of left gonopod tip, showing hairpad **hp**. Image contrast is low because specimen is uncoated. Scale bars: **A** = 0.05 mm, **B** = 0.01 mm.

Diagnosis. Males with head + 19 rings, females with head + 20; adults ca 3.5-4 mm long; midbody metatergites typically with 3 transverse rows of 10-12 large tubercles; posterior portion of prozonite with small disks and microtubercles; ozopores on porosteles on all podous rings beginning with ring 5; gonopod telopodite bent posteriorly at midlength, lateral edge near tip with several rounded teeth.

Description. The excellent description and illustrations of Blower and Rundle (1980) are reproduced below in Appendix 1. Here I add a few details:

Antennal sockets separated by ca 1X a socket diameter. Antennomere relative widths (5,6)>(2,3,4); relative lengths 6>5>(2,3,4). Head with vertex and frons microtuberculate; clypeus smooth, sparsely setose dorsally. Dorsal and lateral setae on collum, tergites and metatergites sparse, bisegmented, the tips flared and minutely toothed along distal edge. Anterior portion of prozonite with cellular structure, the cell walls with microvillose extensions (Fig. 2E; see also Fig. 3 of Blower and Rundle (1980) in Appendix 1); posterior portion of prozonite more or less uniformly microtuberculate, raised into low mounds each topped with a more or less round, convex disk 3-4X the diameter of a microtubercle. Limbus a thin, straight-edged lamella (Fig. 2E). Sternites as wide as long. Podomere relative lengths femur>(prefemur, femur)>(postfemur, tibia). 5+5 lobes along posterior edge of telson in male examined with SEM (including bilobed epiproct), rather than 6+6 as noted by Blower and Rundle (1980). Spinnerets (the *four prominent setae...housed in a collar at the apex of the telson* of Blower and Rundle (1980, p. 29)) recessed in individual chambers, basal sheaths with unnotched distal edges.

Gonopore midventral on leg 2 coxa, opening on low, truncate conical process. Prostatic groove ending in hairpad in posterior concavity of telopodite (Fig. 7).

Distribution. So far known in Queensland from a single site in rainforest in the Iron Range Resource Reserve, ca 10 km northwest of Lockhart on the Cape York Peninsula (Fig. 8).

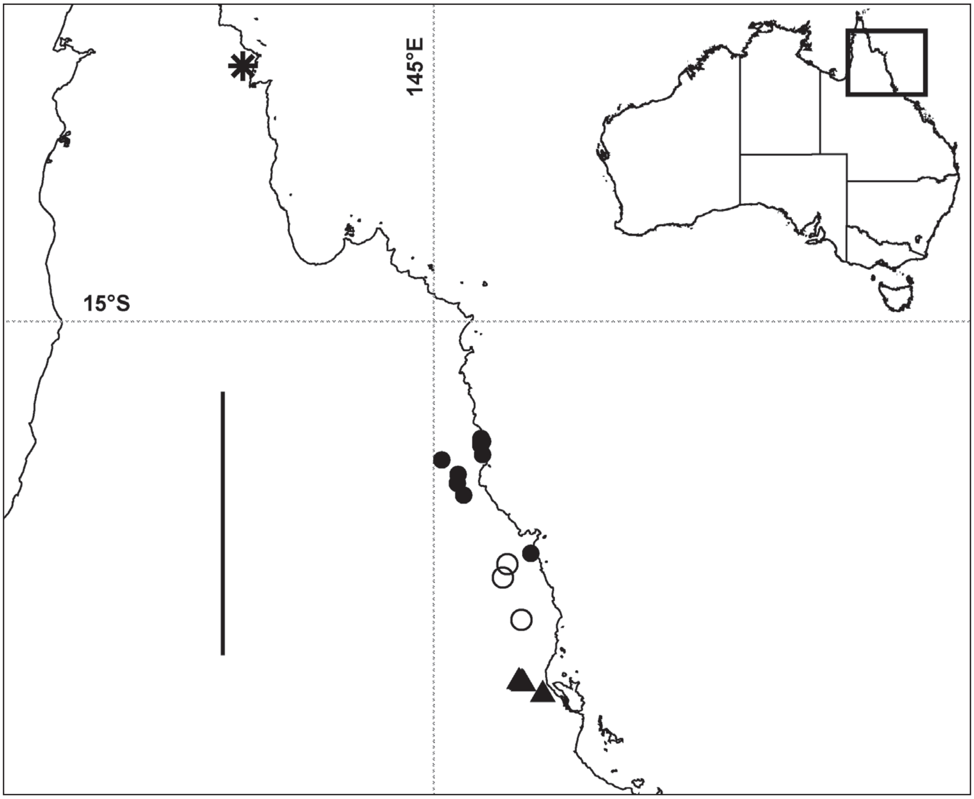


Figure 8. Localities in tropical north Queensland for *Prosopodesmus panporus* Blower and Rundle, 1980 (star), *P. monteithi* sp. n. (filled circles), *P. crater* sp. n. (open circles) and *P. kirrama* sp. n. (triangles). The questioned locality for *P. crater* (see text) is ca 700 km to the south of the furthest south *P. kirrama* locality and is not shown here. Geographic projection; scale bar = ca 250 km. Inset map of Australia shows location of main map.

Discussion

Is *P. panporus* native to Queensland?

The Queensland *P. panporus* site is several kilometres inside a block of tropical rainforest several hundred square kilometres in extent. What is now the Old Coen Track, off Portland Roads Road, was used for vehicle access (Tom Weir, in litt., 24 April 2012). The site's remoteness is evidence that *P. panporus* is native there rather than introduced. A second reason to think that *P. panporus* is native to Queensland is that three of its congeners, described in this paper, were collected in little-disturbed tropical rainforest ca 450 km to the south.

The case for native status would be even stronger if the Kew hothouses where *P. panporus* was first collected in 1975-76 were known to have contained plants imported from the Cape York Peninsula. I queried the Gardens but was told that glasshouse records for that period were not detailed enough to answer this question (Roxana Glenn, in litt., 11 April 2012).

It seems very likely, but not certain, that *P. panporus* is native to the Cape York Peninsula. Sequencing of a marker such as mitochondrial COI, from the Kew population and from specimens collected across the range of the species in Queensland, might help to locate the source or sources of the hothouse millipedes in the wild.

Notes on morphology

The close resemblance of *Rhipidopeltis sinuata* to the *Prosopodesmus* species known at the time led Golovatch et al. (2009) to synonymise the two genera. They were aware that Miyosi (1958) had shown the prostatic groove terminating at the tip of the telopodite (Fig. 5D), rather than at a subapical hairpad. Golovatch et al. (2009) considered this difference to be only species-specific. Haga (1968) illustrated the gonopod of *R. similis* with the prostatic groove ending, again, at the telopodite tip (Fig. 5E), and with no hairpad or other projection, only a tooth-like tab at midlength on the telopodite's medial edge. The two species groups (with and without a hairpad termination for the prostatic groove) are clearly very closely related and I leave them here in *Prosopodesmus*.

The fine structure of the prozonite of *P. panporus* (Fig. 2E) corresponds exactly to that of *P. jacobsoni* as illustrated in Akkari and Enghoff (2011; Fig. 4, p. 7): anteriorly with cellular chambers whose walls are extended with microvilli, posteriorly with slightly elevated convex disks ('subspherical knobs' of Akkari and Enghoff) on a ground covered with microtubercles. These are not genus-level character states, since the other three Australian *Prosopodesmus* species lack microvillose extensions anteriorly and microtubercles posteriorly, and the posterior prozonite in *P. monteithi* lacks disks and is irregularly rugose with microvilli (Figs 2A–D).

I could not find spiracular openings above the legbases in any of the Australian *Prosopodesmus* species. Although this is consistent with the apparent lack of spiracles in two other Australian haplodesmids, *Agathodesmus johnsi* Mesibov, 2009 and *A. steeli* Silvestri, 1910 (Mesibov 2009), it is very puzzling. Spiracles are clearly visible in all known species of *Asphalidesmus* Silvestri, 1910 (Dalodesmidea) and in the Australian Pyrgodesmidae I have examined. Both groups are similar to *Prosopodesmus* species in body form, size range and cryptic habits in rainforest. Unless the two haplodesmid genera lack tracheae, which seems to me improbable, then their tracheal systems must have spiracular entrances. A histological study of freshly collected and suitably prepared *Prosopodesmus* specimens, e.g. the large *P. monteithi*, might solve this puzzle.

Acknowledgements

For specimen loans and registration numbers I thank Beth Mantle (ANIC) and Wendy Hebron (QM). Digital copies of hard-to-find references were kindly provided by Sergei Golovatch (Russian Academy of Sciences), Zoltan Korsós (University of the Ryukyus) and Hans Reip (Jena). Richard Hoffman (VMNH), editor of *Myriapodologica*, gave me permission to reproduce information from Blower and Rundle (1980). Tom Weir (CSIRO Ecosystem Sciences) kindly checked his field notes from the 1986 trip that yielded *P. panporus*. SEM images were acquired with the help of Karsten Goemann (Central Science Laboratory, University of Tasmania). I also thank ZooKeys editor Sergei Golovatch for helpful comments on a draft of the paper. This project was assisted by 2011–12 Capacity Building Grant CN211-01 from the National Taxonomy Research Grant Program of the Australian Biological Resources Study.

References

Akkari N, Enghoff H (2011) On some surface structures of potential taxonomic importance in families of the suborders Polydesmidea and Dalodesmidea (Polydesmida, Diplopoda). ZooKeys 156: 1–24. (Online through *ZooKeys* website) doi: 10.3897/zookeys.156.2134

Attems C (1914) Die indo-australischen Myriopoden. Archiv für Naturgeschichte (A) 80(4): 1–398.

Attems C (1926–1930) Myriopoda. In: Kükenthal W, Krumbach T (Eds) Handbuch der Zoologie. Eine Naturgeschichte der Stämme des Tierreiches. 4(1). Progoneata. Chilopoda. Insecta I. Walter de Gruyter & Co., Berlin, 1–402.

Attems C (1940) Das Tierreich. 70. Polydesmoidea. III. Fam. Polydesmidae, Vanhoeffeniidae, Cryptodesmidae, Oniscodesmidae, Sphaerotrichopidae, Peridontodesmidae, Rhachidesmidae, Macellolophidae, Pandirodesmidae. Walter de Gruyter and Co., Berlin, 577 pp.

Blower JG, Rundle AJ (1980) *Prosopodesmus panporus*, an interesting new species of polydesmoid millipede from the Royal Botanic Gardens, Kew, England. Myriapodologica 1(4): 27–34. doi: 10.1515/9783111609645

Brölemann HW (1916) Essai de classification des Polydesmiens (Myriapodes). Annales de la Société Entomologique de France 84: 523–608. (Online in Biodiversity Heritage Library)

Brölemann HW (1920) Voyage de Ch. Alluaud et R. Jeannel en Afrique Orientale (1911–1912). Résultats scientifiques. Myriapodes. III. Diplopoda. L'Homme, Paris, 298 pp, pls 1–18.

Chamberlin RV (1918) The Chilopoda and Diplopoda of the West Indies. Bulletin of the Museum of Comparative Zoology at Harvard College 62(5): 151–262.

Golovatch SI, Geoffroy J-J, Mauriès J-P, VandenSpiegel D (2009) Review of the millipede family Haplodesmidae Cook, 1895, with descriptions of some new or poorly-known species (Diplopoda, Polydesmida). ZooKeys 7: 1–53. (Online through *ZooKeys* website) doi: 10.3897/zookeys.7.117

Golovatch SI, Mikhaljova EV, Chang, H-W (2011) The millipede families Cryptodesmidae, Haplodesmidae, Pyrgodesmidae, Opisotretidae and Xystodesmidae in Taiwan (Diplopoda, Polydesmida). Tropical Natural History 11(2): 119–134.

Golovatch SI, Mikhaljova EV, Korsós Z, Chang, H-W (2010) The millipede family Haplodesmidae (Diplopoda, Polydesmida) recorded in Taiwan for the first time, with the description of a new species. Tropical Natural History 10(1): 27–36.

Haga A (1968) [Japanese Millipedes] Privately published, 11 pp. [In Japanese]

Hoffman RL (1980) ['1979'] Classification of the Diplopoda. Muséum d'Histoire Naturelle, Genève, 237 pp.

Hoffman RL (1999) Checklist of the millipeds of North and Middle America. Virginia Museum of Natural History Special Publication No. 8: 1–584.

Jeekel CAW (1971) Nomenclator generum et familiarum Diplopodorum: a list of the genus and family-group names in the Class Diplopoda from the 10th edition of Linnaeus, 1758, to the end of 1957. Monografieën van de Nederlandse Entomologische Vereniging 5: i–xii, 1–412.

Jeekel CAW (2006) Some notes on Diplopoda from Christmas Island (Indian Ocean). Myriapod Memoranda 9: 59–63.

Loomis HF (1950) Synonymy of some native American and introduced millipeds. Journal of the Washington Academy of Sciences 40: 164–166.

Mesibov R (2009) Revision of *Agathodesmus* Silvestri, 1910 (Diplopoda: Polydesmida: Haplodesmidae). ZooKeys 12: 87–110. (Online through *ZooKeys* website) doi: 10.3897/zookeys.12.206

Mesibov R (2011) New species of *Asphalidesmus* Silvestri, 1910 from Australia (Diplopoda: Polydesmida: Dalodesmidea). ZooKeys 93: 43–65. (Online through *ZooKeys* website) doi: 10.3897/zookeys.93.1255

Miyosi Y (1958) Beiträge zur Kenntnis japanischer Myriopoden. 25. Aufsatz: Über eine neue Gattung und eine neue Art von Diplopoden. Zoological Magazine, Tokyo 67(10): 297–300. [In Japanese with German summary]

Shelley RM, Golovatch SI (2000) The milliped family Haplodesmidae in the Hawaiian Islands, with records of *Prosopodesmus jacobsoni* from Florida and Louisiana (Diplopoda: Polydesmida). Bishop Museum Occasional Papers 64: 48–49.

Shelley RM, Sierwald P, Kiser SB, Golovatch S.I. (2000) Nomenclator generum et familiarum Diplopodorum II. A list of the genus and family-group names in the Class Diplopoda from 1958 through 1999. Pensoft, Sofia, 167 pp.

Silvestri F (1910) Descrizioni preliminari di nuovi generi di Diplopodi. I. Polydesmoidea. Zoologischer Anzeiger 35: 357–364. (Online in Biodiversity Heritage Library).

Appendix I

Original description and illustrations of *P. panporus*, from Blower and Rundle (1980), pp. 27–31. [Reproduced with permission]

Prosopodesmus panporus sp. n.

The following is a composite description based on all the material in our possession. Specific reference to one or another of the types is made in the legends to the figures.

Body almost pigmentless, white to cream-white. Males with 19 segments, 3.3–3.8 mm long, 0.45–0.50 mm broad; females with 20 segments, 3.8–4.3 mm long, 0.50–0.51 mm broad.

Head almost completely covered by a fan shaped (sectorial) collum; metazonite of second ring broader than the metazonites of all subsequent rings, the paranota forwardly directed, embracing the head and collum, their lateral edges confluent with the sides of the collum and continuing the lines of same; the division between the collum and second ring in life is not immediately evident in dorsal view.

The antennae are inserted close together on the vertex; when at rest, they occupy deep grooves diverging dorsally and then passing posteriorly, their distal antennomeres overhung by the sides of the collum and the paranota of the second ring. The forehead triangle enclosed within the diverging antennal bases and the anterior edge of the collum is raised into seven tubercles, one at the apex, two in the next row and four forming the base beneath the collum edge. The sixth antennomere is the longest and broadest; the fifth is slightly shorter. Each of these two larger antennomeres carries a group of sensory cones on their distal dorsal extremities.

The dorsal surface of the collum is raised into an even pavement of tubercles; those of the outermost row are flatter than the remainder and form the incised anterior edge of twelve lobes; these are followed by four rows of more convex tubercles. The succeeding metazonites are strongly arched and are raised into three transverse rows of regular tubercles uniform with those of the collum. The metazonites of the third and fourth rings are much less broad than those of the second ring and slightly less broad than the fifth and all subsequent rings except the telson. The paranota are placed slightly below the mid-lateral level and are incised into three lobes; the middle lobe is slightly broader but not more protrusive; the posterior lobe is more clearly separated from the other two and is directed slightly postero-laterally.

Ozopores occur on all diplopodous segments, 5–17 in the male and 5–18 in the female and there is a pair of rudimentary pores on the single apodous ring (ring 18 of the male and 19 of the female). The pores are borne on the outermost pair of tubercles, of the middle row of the fifth and of the posterior row of the sixth to the last podous ring. The poriferous tubercles of the fifth metazonite obscure the middle lobe of the paranotal edge in dorsal view; those of the remaining diplopodous rings obscure the posterior paranotal lobes. The poriferous tubercles are directed slightly posteriorly, a

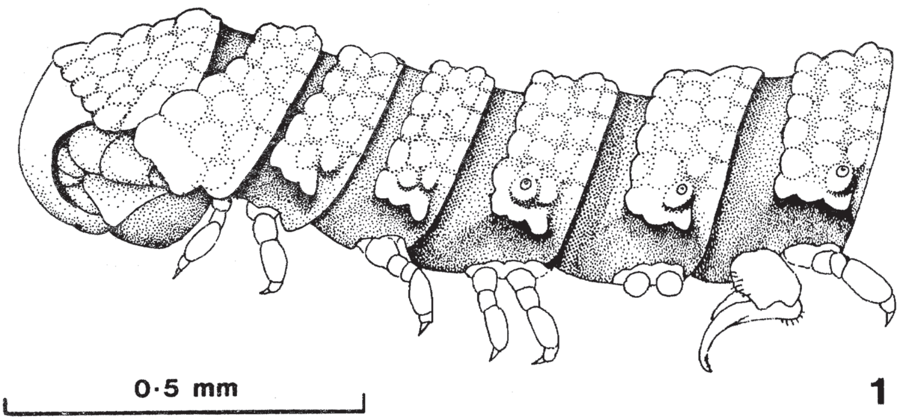


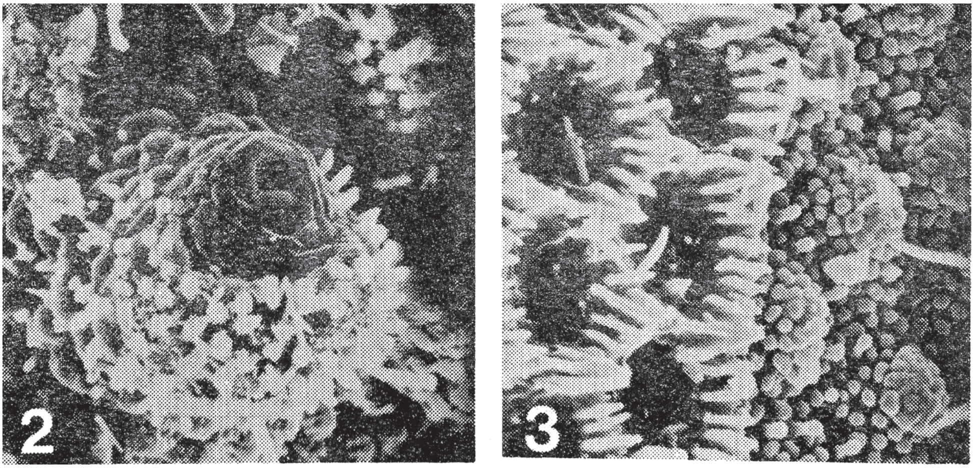
Figure 1. *Prosopodesmus panporus*, n. sp. Lateral view of the anterior part of the male of a mating pair collected in the Palm House, 15 April 1976 (paratype 5). [Reproduced with permission from Blower and Rundle (1980), p. 28]

tendency most obviously developed on the last two or three diplopodous rings where they project posterolaterally beyond the posterior limit of the metazonite.

The anterior row of tubercles on the metazonites slightly overhang the anterior edge. The third row of tubercles stop just short of the posterior edge which is incised into flat fields corresponding in number to the tubercles; these flat posterior portions of the metazonite could just conceivably be described as a fourth row of rather flat tubercles. There are eight tubercles in the first and second rows of the anterior rings, passing to ten on the sixth and eventually twelve from the eighth onwards. The posterior row on rings one to five include 9 tubercles increasing to 11 from the sixth to the last ring, the outermost pair carrying the ozopores, which on the fifth ring are borne on the outermost pair of tubercles of the second row. The telson carries the same three rows of tubercles although the numbers in the second and third rows naturally diminish towards the apex. The tubercles along the posterior edge of the telson overhang as 6+6 lobes.

The entire surface of the tubercles and the region between them is raised into microtubercles and micropapillae, as also is the surface of the prozonites (figs 2, 3). The only macrosetae are carried on the telson, anal valves and scale; there are 4+4 inserted just beneath the twelve-lobed posterior edge of the telson, level with the dorsalmost 3+3 of these lobes. A further four prominent setae are housed in a collar at the apex of the telson just below the medianmost lobes. Each of the anal valves carries 2+2 setae and a further pair are carried by the trapezoidal anal scale. None of these macrosetae is visible from the dorsal side due to the ventrally directed curvature of the telson.

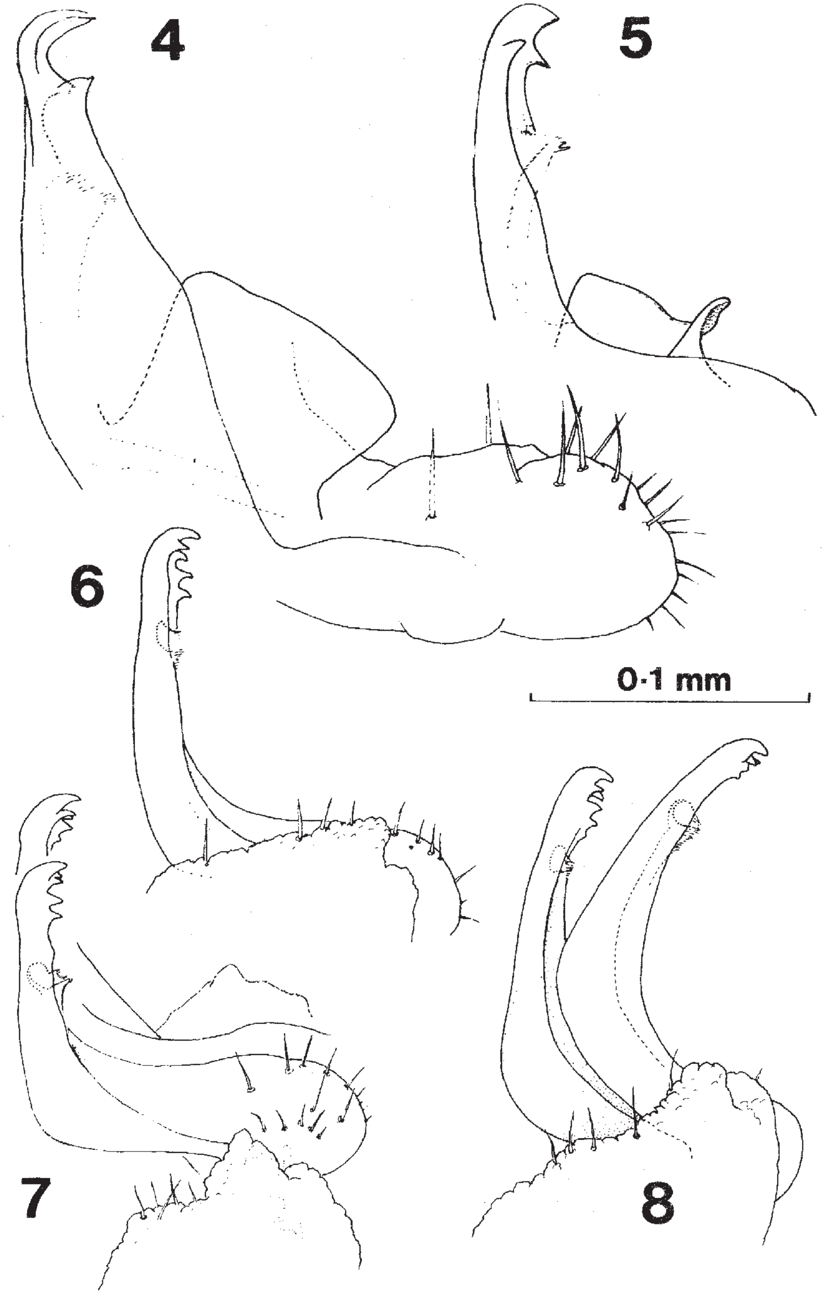
The gonopods are shown in figs 6–8 along with those of *P. jacobsoni*. In *P. panporus* they are simple uniramous structures terminating in prominent teeth, one apical, two subapical arising at about the same level and a further two (sometimes a third rudimentary tooth) along the external edge of the ramus just below the first sub-apical pair. Figs. 6–8 indicate different appearances of these teeth when viewed from different aspects.



Figures 2–3. 2 *Prosopodesmus panporus*, n. sp. Electron scan photograph of the porostele and ozopore on the last podous segments of a female, X 775 **3** Detail of the junction of prozonum and metazonum of the 14th ring of the same female, prozonum on the right, X 1,500. [Reproduced with permission from Blower and Rundle (1980), p. 29]

Table 1. Comparison of *Prosopodesmus panporus* sp. n. with *P. jacobsoni* Silvestri.

Prosopodesmus jacobsoni	***Prosopodesmus panporus***
1. Males and females with 20 segments	Males with 19, females with 20 segments
2. Red-brown, 6.0 × 0.71 mm (nominate form) Yellowish, 7.7 × 0.95 mm (subsp. *hilaris* Brolemann)	Cream-white, males 3.3–3.8 mm × 0.45–0.50 mm Females 3.8–4.3 × 0.50–0.51 mm
3. Ozopores on segments 5, 7-–19	Ozopores on segments 5–17(18) males, 5–18(19) females
4. First row of metazonal tubercles largest, third row smallest (a fourth row in *P. j. hilaris*)	Tubercles of all three rows of equal size (posterior edge of metazonite could be regarded as a fourth row of flatter tubercles).
5. Posterior edge of telson with ten lobes	Posterior edge of telson with 12 lobes.
6. Gonopods with a large lateral lamella on proximal half of telopodite. Two subapical teeth (Figs. 4 & 5).	No lateral lamella on telopodite. Four (sometimes a fifth) subapical teeth (Figs. 6, 7, 8).



Figures 4–8. Gonopods of *Prosopodesmus* **4** *P. jacobsoni* Silvestri, right gonopod in external profile, drawn from holotype **5** Right gonopod, redrawn from 7 of the original description (Silvestri, 1910) **6** *P. panporus* n. sp. External profile view of right gonopod of paratype 2 (the specimen is mounted with left side uppermost, but the drawing is reversed to compare more easily with the other two) **7** *P. panporus*, gonopods of the holotype *in situ*, viewed from the right and slightly ventrally **8** *P. panporus*, gonopods of paratype 1 *in situ*, viewed from the right. [Reproduced with permission from Blower and Rundle (1980), p. 31]

Appendix 2

Records of species of *Prosopodesmus* Silvestri, 1910 in Australia.

Explanation note: Specimen records for *Prosopodesmus* species in Australia are available on the ZooKeys website as a CSV file (doi: 10.3897/zookeys.190.3276.app2).

Citation: Mesibov R (2012) New species of *Prosopodesmus* Silvestri, 1910 (Diplopoda, Polydesmida, Haplodesmidae) from Queensland, Australia. ZooKeys 190: 33–54. doi: 10.3897/zookeys.190.3276.app2

ZooKeys 190: 55–79 (2012)
doi: 10.3897/zookeys.190.2555
www.zookeys.org

RESEARCH ARTICLE

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

Morphological and molecular evidence for cryptic species of springsnails [genus *Pseudamnicola* (*Corrosella*) (Mollusca, Caenogastropoda, Hydrobiidae)]

Diana Delicado[1,†], Marian A. Ramos[1,‡]

1 *Museo Nacional de Ciencias Naturales (CSIC), José Gutiérrez Abascal 2, 28006-Madrid, Spain*

† *urn:lsid:zoobank.org:author:52A050CB-4AE4-4EEF-8D5E-B508A0EC5AB2*
‡ *urn:lsid:zoobank.org:author:C108BACD-06A7-4C6D-8466-2A1CFE349DEB*

Corresponding author: *Marian A. Ramos* (m.ramos@mncn.csic.es); *Diana Delicado* (dideig@hotmail.com)

Academic editor: *E. Gittenberger* | Received 16 December 2011 | Accepted 23 April 2012 | Published 4 May 2012

urn:lsid:zoobank.org:pub:49700A29-5ADB-4D52-AFAA-DE05BC3CF246

Citation: Delicado D, Ramos MA (2012) Morphological and molecular evidence for cryptic species of springsnails [genus Pseudamnicola (Corrosella) (Mollusca, Caenogastropoda, Hydrobiidae)]. ZooKeys 190: 55–79. doi: 10.3897/zookeys.190.2555

Abstract

Several *Pseudamnicola* (*Corrosella*) populations of the central and eastern Iberian Peninsula have been ascribed to *P.* (*C.*) *astieri* (Dupuy, 1851), though recent evidence demonstrates the species could be endemic to the departments of Var and Alpes-Maritimes in France. Through the identification of cryptic species using a combined morphological and phylogenetic approach, this paper provides a detailed morphological description of *P.* (*C.*) *astieri*, clarifying its taxonomic boundaries and confirming it as a French endemic. In parallel, by comparing *Pseudamnicola* (*Corrosella*) populations from the provinces of Castellón and Valencia in Eastern Spain, it was observed that rather than *P.* (*C.*) *astieri* they represented a new species here described as *P.* (*C.*) *hauffei* **sp. n.** Among other characters, the two species show marked differences in shell shape, male and female genital systems, radular formula and concentration of the nervous system. *P.* (*C.*) *hauffei* **sp. n.** was also compared morphologically to another two *Pseudamnicola* (*Corrosella*) species living in nearby areas [*P.* (*C.*) *hinzi* Boeters, 1986 and *P.* (*C.*) *navasiana* (Fagot, 1907)], molecularly to *P.* (*C.*) *falkneri* (Boeters, 1970), the type species of the subgenus, and to the rest of the *Pseudamnicola* (*Corrosella*) species described so far. Morphological differentiation between the species is supported by a genetic divergence of 7.4% inferred from a partial sequence (658 bp) of the mitochondrial gene cytochrome c oxidase subunit I (COI). On the basis of an average 8% (5.39 to 11.15%) divergence estimated for the COI gene in other *P.* (*Corrosella*) species reported in GenBank, the existence of two specific entities is here proposed, which will have impact on conservation policies both in France and in Spain.

Keywords
Hydrobiidae, *Pseudamnicola* (*Corrosella*), *P.* (*C.*) *astieri* (Dupuy, 1851), *P.* (*C.*) *hauffei* sp. n., France, Spain, Iberian Peninsula, taxonomy, COI, cryptic species, conservation

Introduction

The Mediterranean basin, and within it the Iberian Peninsula, has been identified as a biodiversity hotspot for animal species including those of hydrobiid gastropods (Arconada and Ramos 2003). The Peninsula's hydrobiid fauna comprises a large number of endemic genera and species with restricted distribution areas, in addition to those showing a typically circummediterranean distribution. Many hydrobiid species and populations are threatened, and in some cases, in danger of extinction due to the fragile nature of the ecosystems they inhabit. The freshwater genus *Pseudamnicola* Paulucci, 1878, among the most cumbersome in terms of taxonomy, is one of the largest and most diverse groups of Hydrobiidae Stimpson, 1965, with around 85 nominal species (Boeters 1976, Ghamizi et al. 1997, Glöer et al. 2010, Glöer and Pesic 2009, Fauna Europaea 2011). However, many of these taxa require confirmation of their taxonomic status since they have yet to be morphologically well characterized. Delimiting species is essential both to assess diversity and to inform conservation agencies about possible strategies to preserve this sensitive group of molluscs and their habitats. For some groups of hydrobiids of similar morphology, small size and simple shells and anatomy, describing species boundaries is particularly challenging since most diagnostic characters are related to the morphometrics of soft parts. Recent papers have demonstrated that molecular data are useful to support the morphological delimitation of hydrobiid genera and species (Hershler et at. 2003, Szarowska et al. 2005, Arconada and Ramos 2006, Arconada et al. 2007) and that a combined approach using morphological and molecular data can help reveal intraspecific variability unveiling cryptic species within the genus *Pseudamnicola* (Szarowska et al. 2006, Delicado et al. 2012).

Two subgenera are currently recognized within the *Pseudamnicola: P.* (*Corrosella*), occurring in the Iberian Peninsula and one small area in the South of France; and *P.* (*Pseudamnicola*), widely distributed in freshwater ecosystems of the Mediterranean basin. The diversity of the subgenus *Corrosella* is much lower than that of *Pseudamnicola* and only 11 nominal species (described by: Dupuy 1851, Fagot 1907, Boeters 1970, 1984, 1986, 1988, 1999, Girardi 2009 and Delicado et al. 2012) have been ascribed to this subgenus in a more restricted distribution area.

One of these 11 species is *P.* (*C.*) *astieri* (Dupuy, 1851), originally described from the surroundings of Grasse in the department of Alpes-Maritimes (France). Several other species were later cited from the neighbouring Var department (*Bythinella anteisensis* Berenguier 1882, *B. berenguieri* Bourguignat in Berenguier 1882, *B. doumeti* Bourguignat in Locard 1893, among others) and synonymised with *P.* (*C.*) *astieri* (see Falkner et al. 2002 and Girardi 2009 for a review). Then, when *Corrosella* Boeters, 1970 was introduced, *B. anteisensis* was included by the author and *B. berenguieri* considered a younger synonym. In 1981, Gasull recorded the presence of *Pseudamnicola* (*Corrosella*) *astieri* (Dupuy) in the Castellón province (Spain). However, Falkner et al. (2002) later claimed certain misunderstandings in the exchange of information between Boeters and Gasull (Boeters pers. com.) which had led to the report that *P.* (*C.*) *astieri* also inhabited several central and eastern Spanish provinces (see Gasull 1981, Vidal-Abarca and Suárez 1985). As a result of Falkner's review, the Catalogue of Continental Molluscs in France (Falkner et al. 2002) included this entity as an endemism of the Var department.

Our paper provides a wide conchological and anatomical description of a new species of *Pseudamnicola* (*Corrosella*) from eastern Spain (Iberian Peninsula), *P.* (*C.*) *hauffei* sp. n., and, through its re-description, compares it with the species *P.* (*C.*) *astieri* from Var (France) and with other *P.* (*Corrosella*) species with close-by distribution areas in the Iberian Peninsula. Morphological studies were combined with cytochrome *c* oxidase subunit I (COI) sequence analysis in the light of previously published molecular data (Delicado et al. 2012) to test divergence and phylogenetic relationships among *Pseudamnicola* (*Corrosella*) species. Overall, our results delimit the two species indicating that *P.* (*C.*) *astieri* is an endemic species of the Var department of France and add a new clade to the already known phylogeny of the subgenus *Corrosella*. These results would necessarily have to be considered to design the conservation strategies for these restricted species both in France and in Spain.

Material and methods

The study area comprised the Departments of Alpes-Maritimes and Var in southeastern France and the provinces of Castellón and Valencia in eastern Spain. Specimens were collected from several sites in this area (see Figure 1) and deposited in the Collection of Molluscs of the Museo Nacional de Ciencias Naturales (MNCN), Madrid, Spain.

Anatomical observations and morphometric measurements were made on specimens relaxed with menthol crystals and fixed in ethanol following the procedures described in Ramos et al. (2000) and Arconada and Ramos (2001). Morphological descriptions are based on the terminology of Hershler and Ponder (1998) except for characters not described or included in that paper for which we use the nomenclature of Delicado et al. (2012). Spire whorls were counted following the method of Ramos et al. (2000). The number of specimens undergoing morphometry, the localities, and sampling dates for each species are indicated in the corresponding section of the text and tables. The unequal sample size of measured specimens affects both standard deviation and coefficient of variation of the morphometric study. In order to correct these biases, we followed the methods of Holtzman (1950) and Biemann and Kearney (2010) respectively. All calculations have been done using the package MBESS (Kelley and Lai 2011) for the R statistical environment (R Development Core Team 2011). Student´s t-test was used to check statistical differences between shell dimensions in both species.

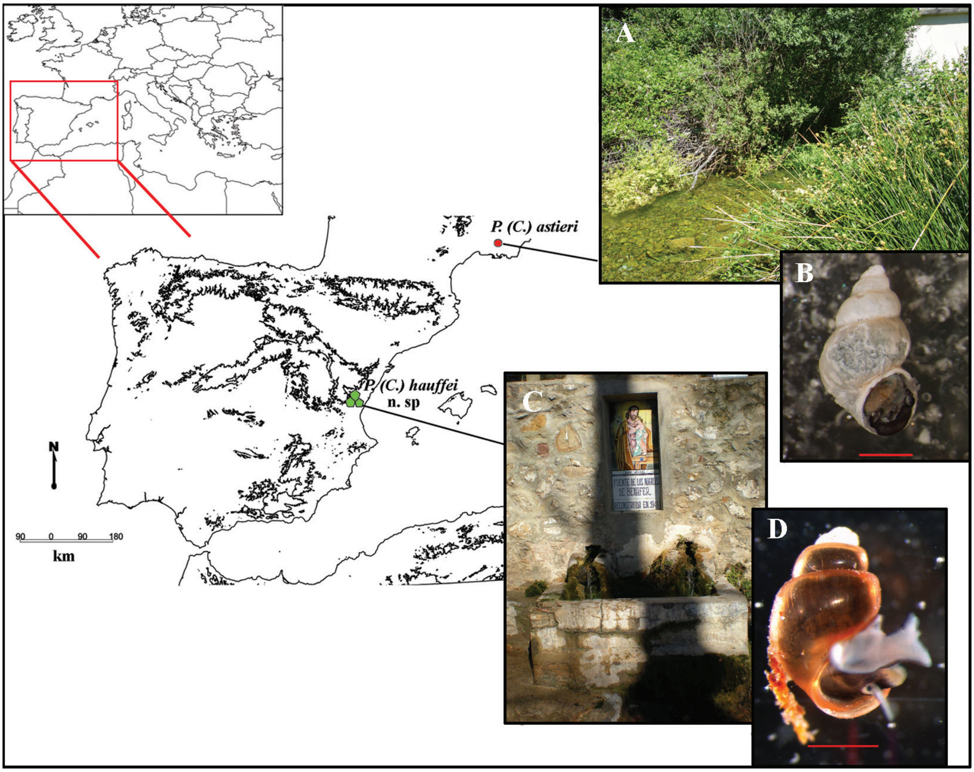


Figure 1. Map of localities of *Pseudamnicola (Corrosella) astieri* and *P. (C.) hauffei* sp. n. **A** Photograph of Source d'Argens, Brue-Aurillac, Var, France **B** Preserved specimen of *P. (C.) astieri* **C** Photograph of Nogales spring, Benafer, Castellón, Spain (type locality of *P. (C.) hauffei* sp. n.) **D** Alive specimen of *P. (C.) hauffei* sp. n. Scale bar in B and D represents 1 mm.

Specimens were dissected under a Leica MZ 16 A stereomicroscope and photographed using a Nikon ds fi1 camera. All measurements were made using Nis-Elements V. 2.2. software. Anatomical illustrations were prepared from camera lucida drawings. Environmental scanning electron microscope (ESEM) images of shells were captured using a Philips Quanta 200 in low-vacuum mode, after removal of the periostracum by immersion in 5% sodium hypochlorite and then cleaning by ultrasonication. The radula and operculum were cleaned by immersion in KOH solution (10g/l) at room temperature. Both structures were then rinsed in distilled water and air-dried before mounting on stubs and coating with a thin (10–20 nm) gold layer in an Emitech K550X sputter coating unit followed by observation in high-vacuum mode.

Total DNA was isolated from the foot tissue of the snails using the ChargeSwitch gDNA Micro Tissue (Invitrogen, Paisley, UK) extraction kit. Partial COI sequences were amplified by polymerase chain reaction (PCR) using LCO1490 (Folmer et al. 1994) and COR722 (Davis et al. 1998) as primers, following the protocol described in Delicado et al. 2012. We examined three specimens from the type locality of *P.*

(*C.*) *hauffei* and three specimens from the Source d'Argens of *P.* (*C.*) *astieri* and the sequences obtained were edited using the SEQUENCHER v.4.6 program (Gene Code Corporation, Ann Arbor, MI, USA). A molecular data set was created together with other published sequences for *Pseudamnicola* (see Table 1 for Genbank accession numbers). Uncorrected divergences were calculated in PAUP 4b10 and Bayesian analysis was performed with MRBAYES 3.1.2 (Huelsenbeck 2000; Huelsenbeck and Ronquist 2001) employing two parallel runs of 5 million of generations and sampling one every 1000 replicates. The 10% of sampled trees were discarded as burn-in (see details in Delicado et al. 2012).

Table 1. Species name, locality details, Genbank accession numbers and publication references for mt-COI sequences.

Species name	Locality	Genbank accession number	Reference
P. (C.) luisi	La Gitana spring, La Peza, Granada, Spain.	JF312220	Delicado et al. 2012
P. (C.) falkneri	La Armada spring, Orce, Granada, Spain.	JF312224	Delicado et al. 2012
P. (C.) manueli	La Garganta stream, Nava de San Pedro, Jaén, Spain.	JF312227 JF312228	Delicado et al. 2012
P. (C.) bareai	Spring in Ermita de las Santas, Granada, Spain.	JF312225 JF312226	Delicado et al. 2012
P. (C.) marisolae	Pilar del Mono spring, Dúrcal, Granada, Spain.	JF312218 JF312219	Delicado et al. 2012
P. (C.) iruritai	Don Pedro spring, Loja, Granada, Spain.	JF312221 JF312222	Delicado et al. 2012
P. (C.) andalusica	La Salud spring, Toscarejo, Jaén, Spain.	JF312223	Delicado et al. 2012
P. (P.) lucensis	Thermal spring in Bagni di Lucca, Tuscany, Italy.	AF367651	Wilke et al. 2001
P. (P.) macrostoma negropontina	Artificial pond in Marmaris, Evvoia island, Greece.	EF061915	Szarowska et al. 2006
Hydrobia acuta acuta	Lac de Tunis, Tunisia.	AF278804	Wilke et al. 2000
Pyrgula annulata	Lake Garda, Brescia, Italy.	AY341258	Szarowska et al. 2005

Abbreviations used in the text and tables

Shell and operculum characters: AH: aperture height; AL: aperture length; AW: aperture width; LBW: length of body whorl; NL: length of opercular nucleus; NW: width of opercular nucleus; NSW: number of spire whorls; OL: operculum length; OLWL: length of the last whorl of the operculum; OLWW: width of the last whorl of the operculum; OW: operculum width; SL: shell length; SW: shell width; WAW: width of the antepenultimate whorl; WBW: width of the body whorl; WPW: width of the penultimate whorl.

Anatomical characters: Ag: albumen gland; Bc: bursa copulatrix; CC: cerebral commissure; Cg: capsule gland; Ct: ctenidium; dBc: duct of the bursa copulatrix; LCG: left cerebral ganglion; LPG: left pleural ganglion; Os: osphradium; P: penis; Po: pallial oviduct; Pr: prostate gland; RCG: right cerebral ganglion; Ro: renal oviduct; RPG: right pleural ganglion; SR: seminal receptacle; Ss: style sac; St: stomach; SubC: suboesophageal connective; SubG: suboesophageal ganglion; SupC: supraoesophageal connective; SupG: supraoesophageal ganglion; L: length; W: width. The concentration of the nervous system was measured as the "RPG" ratio (Davis et al. 1976) and also characterised using the categories of Davis et al. (1984, 1986, 1992) as follows: dorsal nerve ring concentrated (≤ 0.29); moderately concentrated (0.30–0.49); elongated (0.50–0.67); extremely elongated (≥ 0.68).

Collections. BOE, Boeters, München, Bundesrepublik Deutschland; MHNG, Muséum d'histoire naturelle de la Ville de Genève, Switzerland; MNCN, Museo Nacional de Ciencias Naturales, Madrid, Spain.

Collectors. B.A., B. Arconada; C.N., C. Noreña; D.D., D. Delicado; D.M., D. Moreno; J.M.R, J.M. Remón; R.A., R. Araujo.

Results

Systematic descriptions

***Pseudamnicola* (*Corrosella*) *astieri* (Dupuy, 1851)**

http://species-id.net/wiki/Pseudamnicola_astieri
Figs 2–4

Hydrobia astierii Dupuy, 1851: 556–557, pl. XXVII, fig. 12, Paris (Type loc. surroundings of Grasse, Alpes-Maritimes, France [shell description]).

Paludinella astieri (Dupuy): Frauenfeld 1865: 575.

Bythinella astieri (Dupuy): Locard 1882: 227; Bérenguier 1882: 83; Locard 1893: 79, fig. 81 (shell description); Berenguier 1902: 378, pl. 16 fig. 6 (1990).

Bythinella anteisensis Bérenguier, 1882: 83, 89–90 (Type loc. Foux de Draguignant, Var, France [shell description]); Bérenguier 1902: 378–379 (shell description) pl. 16 fig. 7 (1990). (Synonymy: Girardi 2009: 56).

Bythinella berenguieri Bourguignat in Bérenguier, 1882: 83, 99–100 (Type loc. Foux de Draguignant, Var, France [shell]); Bérenguier 1902: 379–380 (shell description) pl. 16 fig. 8 (1990) (Synonymy: Girardi 2009: 56).

Bythinella doumeti Bourguignat in Locard, 1893: 91. (Type loc. surroundings of Nimes, Gard, France [shell description]) (Synonymy: Falkner et al. 2002: 81, after revision of two syntypes in the Bourguignat collection, MHNG).

Corrosella anteisensis (Bérenguier): Boeters, 1970: 64, figs. 2, 4, 7, 9 [(shell, operculum, male and female genital systems of topotypes; Boeters could not find the syntypes)], (=*Bythinella berenguieri* Bourguignat in Bérenguier). (Synonymy: Girardi 2009: 56).

Pseudamnicola (*Corrosella*) *astierii* (Dupuy): Falkner et al. 2002: 29, 80-81; Girardi 2009: 56–61, figs. 1–3 (Var, France: Source d'Argens, Source du Pavillon, Source de la Foux à Draguignan [shell and anatomy]).

Type locality. Surroundings of Grasse, France (Dupuy, 1851).

Type material. Boeters (1970) reported the existence of one specimen with the label "*Paludinella astieri*, typus ex Dupuy" in Paladilhe's collection at the Faculté des Sciences, Montpellier, France. We tried in vain to confirm the existence of such material at the university mentioned. Consequently, we should consider that the type specimen is presently inaccessible for study. However, some topotypes of *Corrosella anteisensis* (Bérenguier) from Foux à Draguignan, Var exist: BOE 261, 285 a-c, 291b Boeters (1970) and Girardi (2009). This author also reported *P.* (*C.*) *astieri* from Source d'Argens, Brue-Aurillac à Seillons, Var and the Source du Pavillon, Ruisseau Fauvery à Pontevès, Var (Girardi 2009).

Material examined. A few specimens collected from Source d'Argens , Brue-Aurillac, Var, France after finding the type area and other localities in Alpes-Maritimes and Var practically destroyed by severe storms. A total of two females and four males have been examined for anatomical descriptions.

Localities. Source d'Argens, Brue-Aurillac, Var, France, 43°30.24'N, 5°54.43'E, D.D., 21 June 2010, MNCN 15.05/60025 (70° ethanol, Figures 2–4) and MNCN/ADN 54949–54951 (absolute ethanol). For more localities, see Girardi 2009.

New diagnosis. Shell yellowish or whitish with a body whorl occupying 2/3 shell length and a deep suture between whorls; protoconch microsculpture granulated; central radular tooth formula 7-C-7; style sac surrounded by a black pigmented intestine; elongate bursa copulatrix U-shaped; elongate seminal receptacle without duct; penis slender with a black patch of pigmentation and some folds in its middle region; nervous system brown pigmented with supraoesophageal connective about two times longer than suboesophageal.

Description. Shell ovate-conic with 4–4.75 spire whorls, height 2.5–3.5 mm (Figure 2A–C; Table 2); periostracum yellowish or whitish; protoconch approximately 370 µm wide with 1.5 whorls and a nucleus around 150 µm long (Figure 2D,E); protoconch microsculpture granulated, more intense on apex (Figure 2E); body whorl about 2/3 total length; teleoconch whorls convex with a deep suture; peristome orthocline; aperture complete, oval, with an inner lip thicker than outer lip; peristome margin simple, straight (Figure 2B).

Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral with nucleus submarginal (Figure 3A,B; Table 3); muscle attachment area oval, located near the nucleus.

Radula intermediate length (20% total shell length) bearing some 50 rows of teeth (Figure 3C, Table 4); central tooth has a tongue-shaped median cusp and seven blunt lateral cusps (Figure 3D,E); lateral teeth with three tapered cusps on each side of a long central tongue-shaped cusp; inner marginal teeth have 18 sharp cusps, shortening towards the tooth base; outer marginal teeth with 19 sharp cusps (Figure 3D,F).

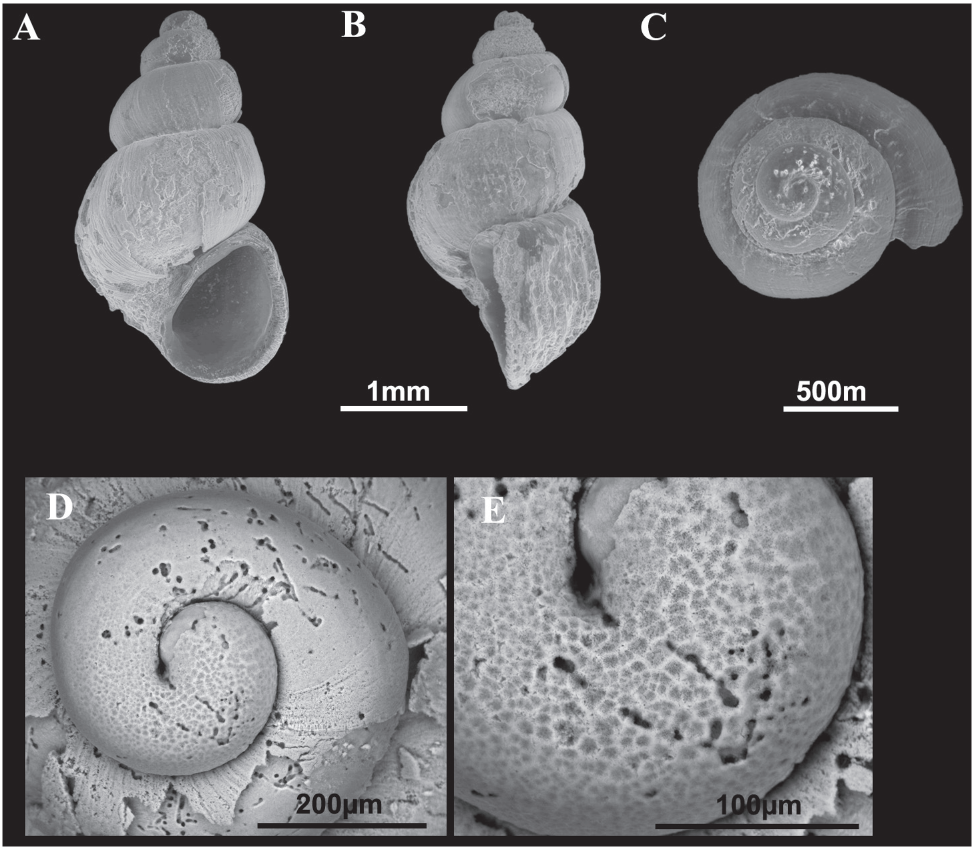


Figure 2. Shells of *Pseudamnicola (C.) astieri*. **A–E** Shells from Source d'Argens, Brue-Aurillac, Var, France **D–E** Protoconch and detail of the microsculpture from the protoconch.

Pigmentation and anatomy: Head dark brown pigmented from snout to neck (Figure 4D); pigmentation clearer on neck; tentacles also brown pigmented except for a narrow band on these and on ocular lobes; snout long as wide, with medial lobation; foot intermediate length and pigmented in dorsal region. Ctenidium in middle region of pallial cavity filling ca. 70% of its length with 17–18 gill filaments; osphradium intermediate width under central gill filaments (Figure 4C, Table 5). Stomach slightly longer than wide with a small posterior caecum; style sac shorter than stomach and surrounded by intestine black pigmented (Figure 4F, Table 5).

Female genitalia with a slender pallial oviduct (Figure 4G; Table 6); capsule gland longer than albumen gland and consisting of two regions, the posterior one being more transparent; elongate bursa copulatrix, long, folded and U-shaped with a duct about 70% of bursa length; renal oviduct straight and less pigmented from the insertion point of the bursal duct to where it begins to fold and black pigmented making one or two loops; elongate seminal receptacle without duct (Figure 4H) joining renal oviduct just before the point where the bursal duct joins the renal oviduct.

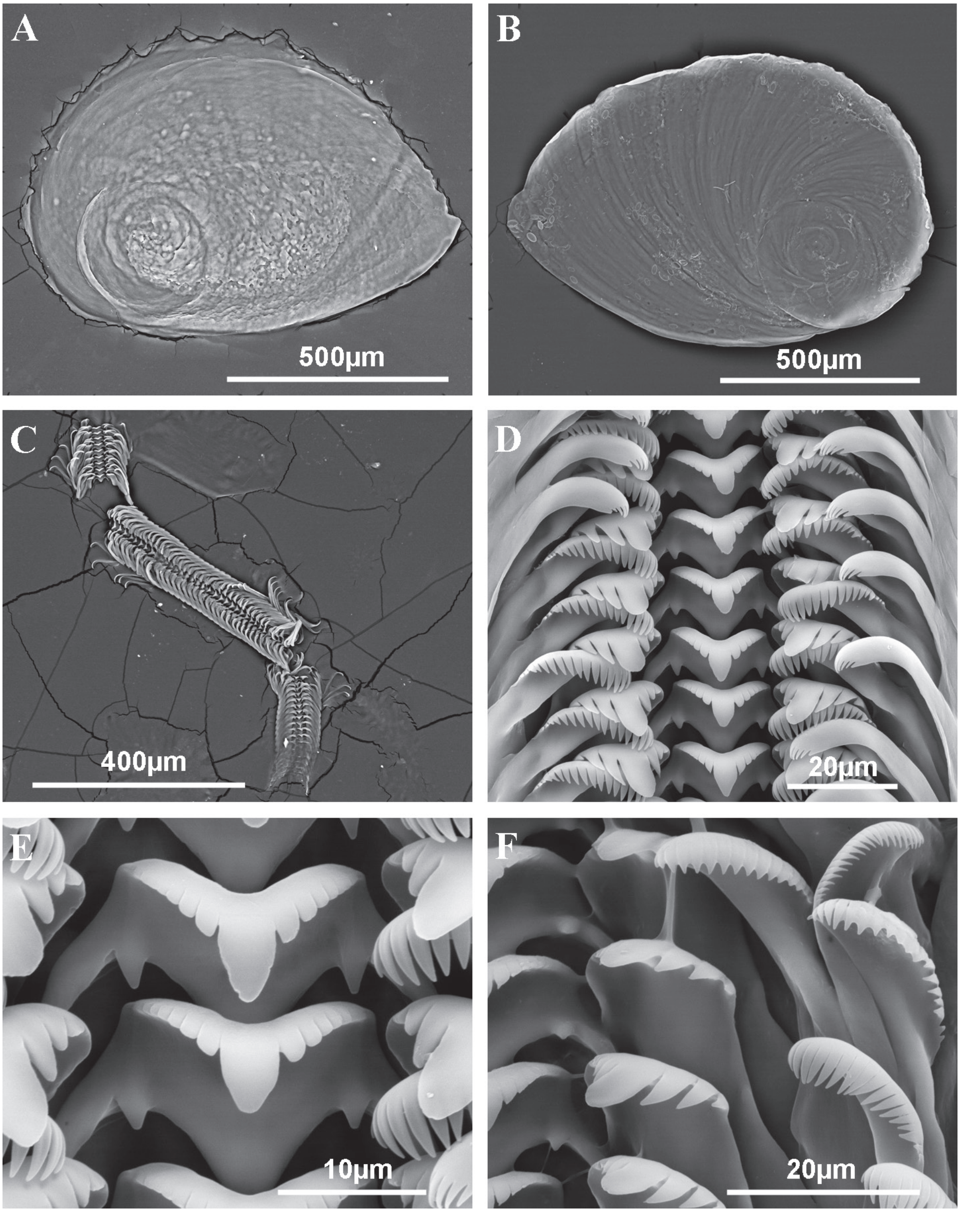


Figure 3. Operculum and radula of *Pseudamnicola (C.) astieri* from Source d'Argens, Brue-Aurillac, Var, France. **A** Internal side of the operculum **B** External side of the operculum **C** Radula **D** Rows of teeth of the radula **E** Central tooth **F** Lateral, internal and external marginal teeth.

Male genitalia bear a bean-shaped prostate gland about three times longer than wide (Figure 4E, Table 6); penis long, slender, with a black patch of pigmentation and some folds in its middle region; attachment area behind right eye (Figure 4D); penial duct scarcely visible running straight close to the outer penis margin.

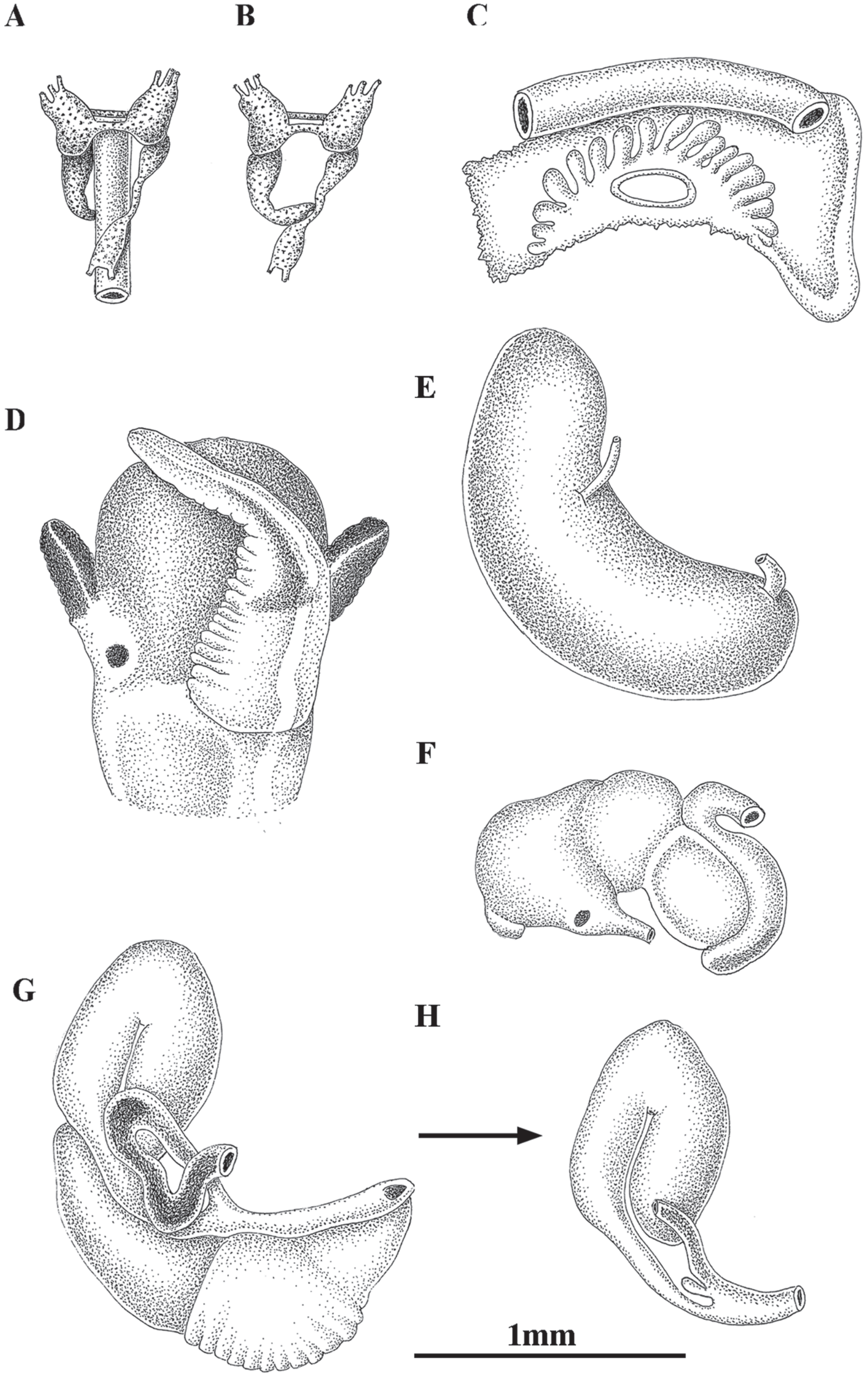


Figure 4. Anatomy of *Pseudamnicola (C.) astieri* from Source d'Argens, Brue-Aurillac, Var, France. **A, B** Partial nervous system **C** Ctenidium and osphradium **D** Head of a male and penis **E** Prostate gland **F** Stomach **G** Female genitalia **H** Bursa copulatrix and seminal receptacle.

Table 2. Shell measurements (in mm) of *P. (C.) astieri* from d'Argens spring, Seillons, France and *P. (C.) hauffei* sp. n. from Los Nogales spring, Benafer, Castellón, Spain. Probability *p* values of *T*-test are provided for each variable, n.s. = no significant.

	P. (C.) astieri **(n=11)**			***P. (C.) hauffei* sp. n. (n=18)**			**T-test (*p* values)**
	Mean (Max-Min)	**SD_N**	**CV**	**Mean (Max-Min)**	**SD_N**	**CV**	
SL	3.11 (3.56–2.58)	0.25	0.08	2.50 (2.85–2.17)	0.18	0.07	$p < 0.001$
SW	1.92 (2.28–1.74)	0.15	0.08	1.59 (1.78–1.44)	0.10	0.06	$p < 0.001$
SL/SW	1.62 (1.76–1.45)	0.10	0.06	1.57 (1.73–1.46)	0.07	0.04	n.s.
AH	1.37 (1.78–1.26)	0.14	0.10	1.20 (1.36–1.04)	0.08	0.07	$p < 0.001$
SL–LBW	1.10 (1.39–0.76)	0.16	0.15	0.69 (1.00–0.49)	0.13	0.19	$p < 0.001$
WBW	1.76 (1.89–1.59)	0.09	0.05	1.42 (1.57–1.29)	0.07	0.05	$p < 0.001$
AL	1.39 (1.72–1.28)	0.12	0.08	1.26 (1.41–1.11)	0.09	0.07	$p < 0.01$
AW	1.01 (1.33–0.85)	0.14	0.14	0.89 (1.11–0.78)	0.08	0.09	$p < 0.01$
WPW	1.23 (1.33–1.11)	0.08	0.07	0.95 (1.12–0.80)	0.08	0.08	$p < 0.001$
WAW	0.19 (0.32–0.15)	0.06	0.32	0.28 (0.37–0.19)	0.04	0.14	$p < 0.001$
NSW	4.30 (4.75–4.00)	0.31	0.07	4.18 (4.50–4.00)	0.21	0.05	n.s.

SD_N, Unbiased estimate for Standard Deviation, CV, Coefficient of Variation.

Nervous system brown pigmented, consisting of disperse points of pigmentation; cerebral ganglia equal in size; supraoesophageal connective more than two times longer than suboesophageal (Figure 4A,B; Table 7). Mean RPG ratio 0.42 (moderately concentrated).

Remarks. The only available information on the anatomy of this species in the literature corresponded to populations from Foux à Draguignan (figure 2, 4, 7, 9 in Boeters 1970 and figure 2 by M. Bodon in Girardi 2009) and Source du Fauvery in Pontevès (figure 1 by M. Bodon in Girardi 2009). The specimens examined from Source d'Argens (Brue-Aurillac) are similar in shell and gastric complex shapes to specimens from Source du Fauvery though they more resemble specimens from the Foux à Draguignan in terms of pallial oviduct shape and number of gill filaments. However, other important diagnostic characters such as the shape of the penis and bursa copulatrix as well as seminal receptacle shape and its position on the renal oviduct are similar in the three populations. Based on these comparisons we conclude that specimens of the three localities belong to the same taxonomic unit with some inter-population variability shown.

Table 3. Operculum measurements (in mm) of *P.* (*C.*) *astieri* from d'Argens spring, Seillons, France and *P.* (*C.*) *hauffei* sp. n. from Los Nogales spring, Benafer, Castellón, Spain.

	P.* (*C.*) *astieri **(n=5)**			***P.* (*C.*) *hauffei* sp. n.** **(n=7)**		
	Mean (Max-Min)	**SD_N**	**CV**	**Mean (Max-Min)**	**SD_N**	**CV**
OL	1.10 (1.18–0.96)	0.05	0.05	1.08 (1.18–0.96)	0.08	0.08
OW	0.79 (0.84–0.73)	0.04	0.05	0.74 (0.84–0.66)	0.06	0.08
OLWL	0.48 (0.56–0.40)	0.05	0.11	0.49 (0.63–0.36)	0.08	0.17
OLWW	0.34 (0.40–0.25)	0.05	0.16	0.33 (0.38–0.29)	0.03	0.09
NL	0.47 (0.52–0.39)	0.04	0.09	0.36 (0.44–0.26)	0.06	0.17
NW	0.36 (0.42–0.31)	0.04	0.12	0.29 (0.45–0.21)	0.06	0.22

SD_N, Unbiased estimate for Standard Deviation, CV, Coefficient of Variation.

Table 4. Radula formulae and measurements (in mm) of three radulae of *P. (C.) astieri* from d'Argens spring, Seillons, France and three of *P. (C.) hauffei* sp. n. from Los Nogales spring, Benafer, Castellón, Spain.

	P.* (*C.*) *astieri	***P.* (*C.*) *hauffei* sp. n.**
Central teeth	7+C+7/1–1	5+C+5/1–1
Central teeth width	~ 20 μm	~ 15 μm
Lateral teeth	3–C–3	3–C–3
Inner marginal teeth	≥ 18 cusps	≥ 15 cusps
Outer marginal teeth	≥ 19 cusps	≥ 19 cusps
Radula length	~ 700 μm	~ 600 μm
Radula width	~ 90 μm	~ 95 μm
Number of rows	~ 55	~ 50

Comparing shell sizes among the *Pseudamnicola* (*Corrosella*) species from the northern half of Iberian Peninsula, the shells of *P.* (*C.*) *astieri* are larger (2.5–3.5 mm) than those of *P.* (*C.*) *hauffei* sp. n. (2.20–2.90 mm) (see statistically significant differences in shell measurements in Table 2) and *P.* (*C.*) *hinzi* Boeters, 1986 (2.2–2.7 mm, Boeters 1986) yet similar in size to those of *P.* (*C.*) *navasiana* (Fagot, 1907) (3.0–3.5 mm, Boeters 1988). The only two shell variables resulting no significant between *P.* (*C.*) *astieri* and *P.* (*C.*) *hauffei* sp. n. were the rate SL/SW and NSW. That means that both species share the same ovate-conic shape and around 4 spire whorls, which are common characteristics among all *Pseudamnicola* (*Corrosella*) species. Anatomically, *P.* (*C.*) *astieri* bears a similar or higher number of gill filaments (about 17–18) than *P.*

(*C.*) *hinzi* (16–17, Boeters 1986), *P.* (*C.*) *navasiana* (15–16, Boeters 1988) and *P.* (*C.*) *hauffei* sp. n. (about 15). The penis in *P.* (*C.*) *astieri* is narrower and more slender than in *P.* (*C.*) *hauffei* sp. n. and *P.* (*C.*) *navasiana*, although it is wider and longer than in *P.* (*C.*) *hinzi*. The copulatory organ is pigmented in its distal region in all four species, but the pigmentation patch is larger in *P.* (*C.*) *astieri*. Although the bursa copulatrix is usually elongate or pyriform shaped among (*P.*) *Corrosella* species, it is U-shaped and folded in *P.* (*C.*) *astieri* whereas it is J-shaped in *P.* (*C.*) *hauffei* sp. n., *P.* (*C.*) *hinzi* and *P.* (*C.*) *navasiana*. A small seminal receptacle (around 0.15 mm) is a character common to all four species.

***Pseudamnicola* (*Corrosella*) *hauffei* sp. n.**

urn:lsid:zoobank.org:act:4DC6D03C-09B8-4E52-924C-39D6B01BBF42
http://species-id.net/wiki/Pseudamnicola_hauffei

Type locality. Los Nogales spring, Benafer, Castellón, Spain, 30°55.80'N, 0°34.34' W.

Type material. Holotype MNCN 15.05/60026a (SEM preparation, Figure 5A) and paratypes (Figures 5D–G, 6, 7) MNCN 15.05/60026b (SEM preparation, Figures 5D–G, 6, and 70° ethanol, Figure 7) and MNCN/ADN 54952–54969 (frozen material and 70° ethanol), D.D. & C.N., 19 March 2009; MNCN 15.05/60027 (70° ethanol), 26 May 1998, B.A.

Material examined. Four males and four females from type locality were examined for anatomical study. In addition, some populations from provinces of Castellón and Valencia (Spain) were also found and studied, dissecting likewise two males and two females from each for their identification.

Localities. Los Nogales spring, Benafer, Castellón, Spain (type locality), 30° 55.80'N, 0°34.34'W, B.A., 26 May 1998, MNCN 15.05/60027 (70° ethanol); D.D. & C.N., 19 March 2009, MNCN 15.05/60026 (70° ethanol and ESEM preparation, Figures 5A, D–G) and MNCN/ADN 54952–54969 (frozen material); Agadín spring, Benafer, Castellón, Spain, 39°56.38'N, 0°34.54'W, D.D. & C.N., 19 March 2009, MNCN 15.05/60028 (70° ethanol and ESEM preparation, Figure 5C) and MNCN/ADN 54970–54974 (frozen material); irrigation ditch in Navajas, Castellón, Spain, 39°52.09'N, 0°30.37'W, R.A., D.M. & J.M.R 7 March 1990, MNCN 15.05/60029 (70° ethanol); Curso spring, Navajas, Castellón, Spain, 39°52.43'N, 0°30'W, B.A., 25 May 1998, MNCN 15.05/60030 (70° ethanol) and MNCN/ADN 54975–54989 (frozen material); La Peña spring, Navajas, Castellón, Spain, 39°52.77'N, 0°30.03'W, R.A., D.M. & J.M.R, 7 March 1990, MNCN 15.05/60031 (70° ethanol); La Esperanza spring, Navajas, Castellón, Spain, 39°52.19'N, 0°30.43'W, R.A., D.M. & J.M.R, 7 March 1990, MNCN 15.05/60032 (70° ethanol); Del Prado spring, Viver, Castellón, Spain, 39°56.23'N, 0°36.81'W, D.D. & C.N., 19 March 2009, MNCN 15.05/60033 (70° ethanol) and MNCN/ADN 54990–54992 (frozen material); San Miguel spring, Viver, Castellón, Spain, 39°55.68'N, 0°36.64'W, B.A., 25 May 1998, MNCN 15.05/60034 (70° ethanol); D.D. & C.N., 19 March 2009, MNCN 15.05/60035 (70° ethanol and

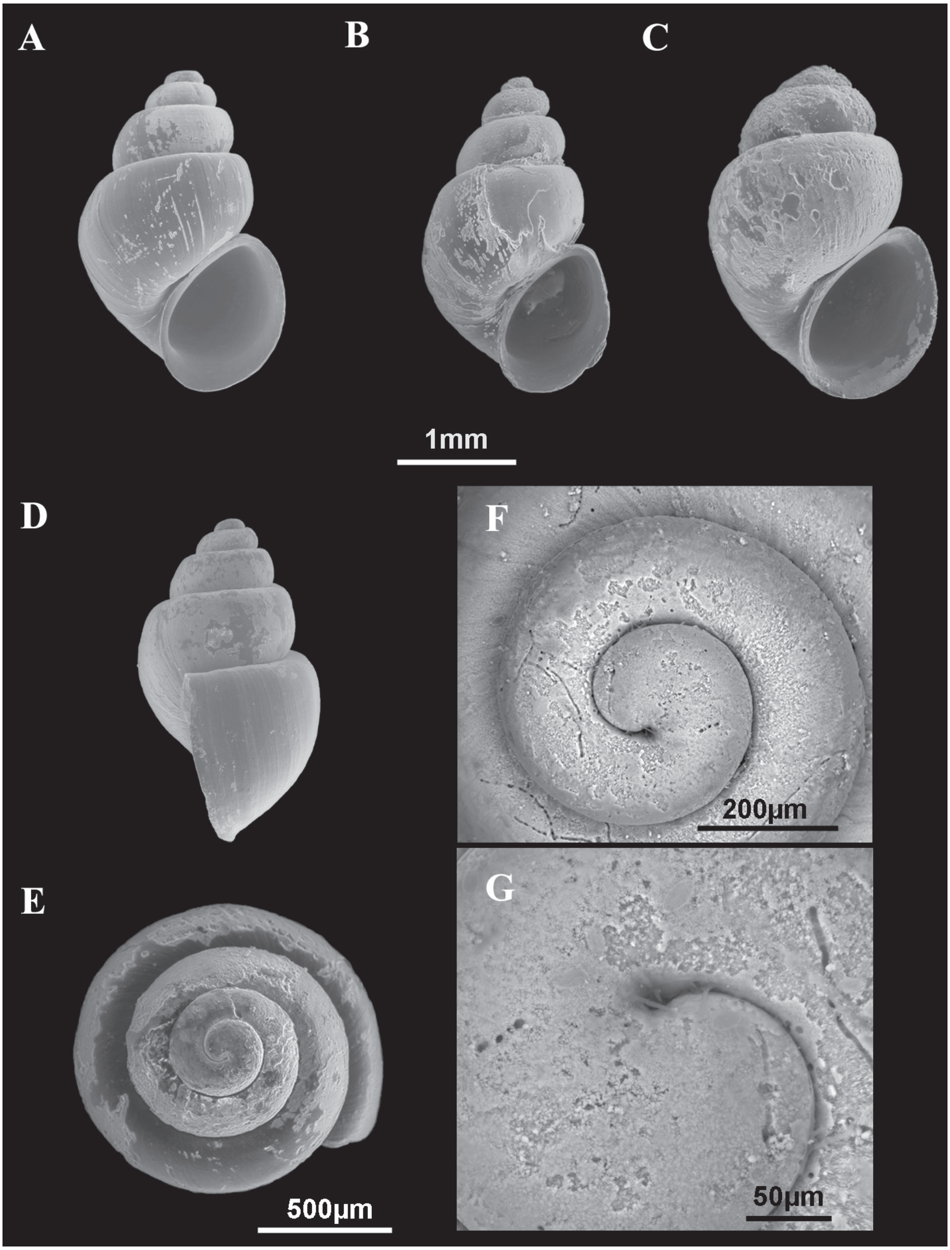


Figure 5. Shells of *Pseudamnicola (C.) hauffei* sp. n. **A, D–G** Shells from Nogales spring, Benafer, Castellón, Spain **B** Shell from San Miguel spring, Viver, Castellón, Spain **C** Shell from Agadín spring, Benafer, Castellón, Spain **E–G** Protoconch and microsculpture.

ESEM preparation, Figure 5B) and MNCN/ADN 54997–54999 (70° ethanol); San Miguel ditch, Viver, Castellón, Spain, 39°55.68'N, 0°36.64'W, D.D. & C.N., 19 March 2009, MNCN 15.05/60036 (70° ethanol) and MNCN/ADN 54993–54996

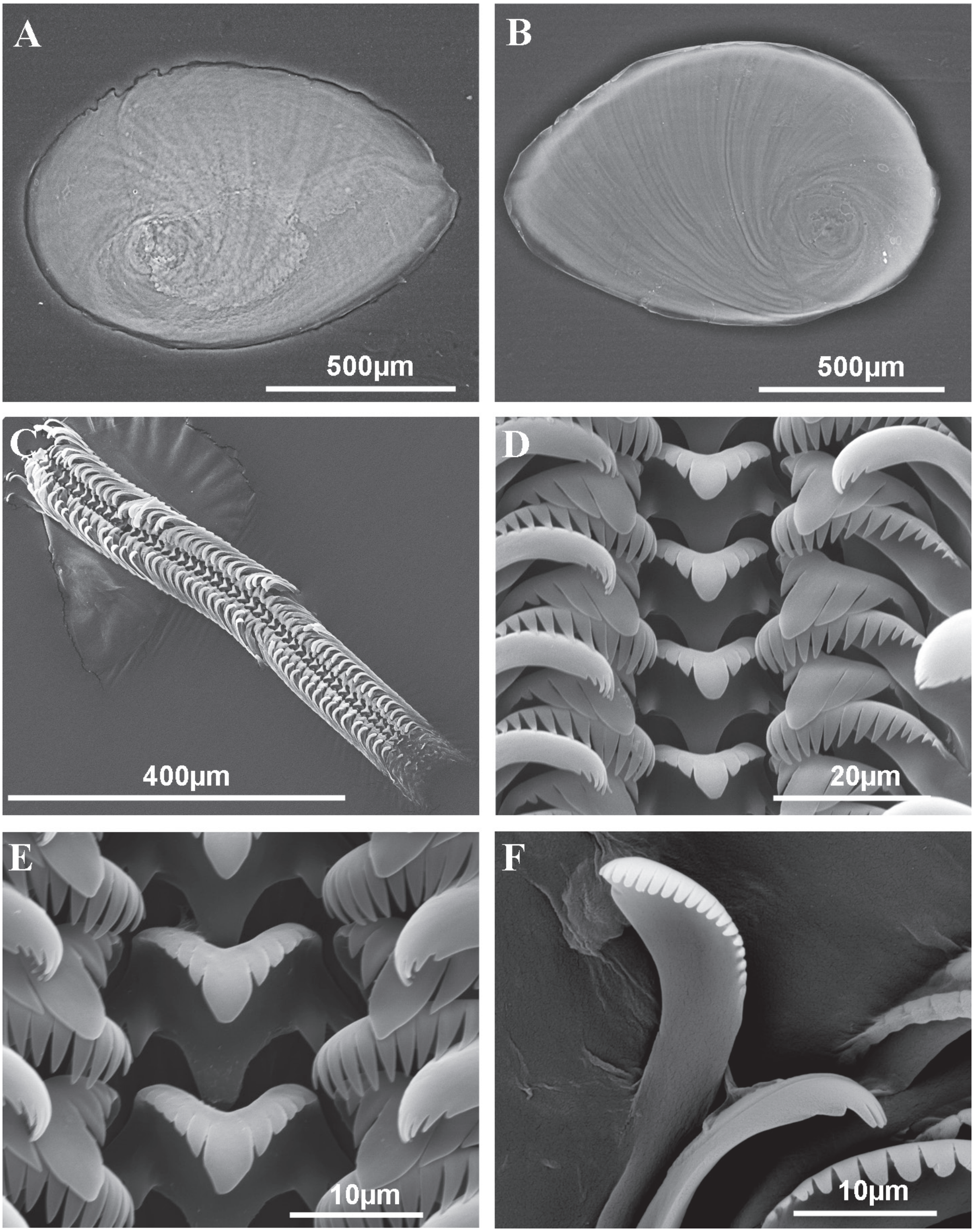


Figure 6. Operculum and radula of *Pseudamnicola (C.) hauffei* sp. n. from Nogales spring, Benafer, Castellón, Spain **A** Internal side of the operculum **B** External side of the operculum **C** Radula **D** Rows of teeth of the radula **E** Central tooth **F** External marginal teeth.

(frozen material); Font Nova, Benifaió, Valencia, Spain, 39°0.55'N, 0°5.87'W, B.A., 26 May 1998, MNCN 15.05/60037 (70° ethanol); Cortés de Pallás, Valencia, Spain, 39°14.61'N, 0°26.01'W, B.A., 26 May 1998, MNCN 15.05/60038 (70° ethanol).

Material examined for morphometry. Shell, anatomical, operculum and radular measurements (Tables 2–7) were made on specimens from the type locality, Los Nogales spring in Benafer, Castellón.

Etymology. Dedicated to the malacologist and ecologist Torsten Hauffe, for his help and support during the stay of the first author in Germany.

Diagnosis. Shell yellowish with body whorl occupying 2/3 shell length; umbilicus slightly visible; protoconch microsculpture grooved; central radular tooth formula 5-C-5; style sac protruding below non-pigmented intestine; elongate bursa copulatrix J-shaped; renal oviduct pigmented until seminal receptacle, which has a pigmented short duct; penis triangular with a wide base attached to central area of head; nervous system brown pigmented with supraoesophageal connective about three times longer than suboesophageal.

Description. Shell ovate-conic (Figure 5A–C), yellowish periostracum with 4–4.5 spire whorls, height around 2.0–3.0 mm (Table 2); protoconch approximately 450 μm wide with 1.5 whorls and a nucleus around 200 μm long (Figure 5E,F); protoconch microsculpture grooved (Figure 5G); body whorl about 2/3 total length; whorls convex with deep suture; peristome frontal, complete, oval, with thick inner lip partly hiding umbilicus; outer peristome simple, straight (Figure 5D).

Operculum corneous, yellowish, thin, pliable, ellipsoidal, paucispiral, with nucleus submarginal (Figure 6A,B; Table 3); oval muscle attachment near nucleus.

Radula with around 50 rows of teeth, medium in size (25% total shell length) (Figure 6C, Table 4); central tooth with a tongue-shaped median cusp and five lateral cusps, slightly sharpening towards central one (Figure 6D,E); lateral teeth with a long tongue-shaped median cusp and three tapered laterals; inner and outer marginal teeth bear 15 and 19 sharp cusps respectively (Figures 6D,F).

Pigmentation and anatomy: Head intensely brown pigmented from snout to neck (Figure 7D); pigment on neck clearer than on head; brown band of pigment also on tentacles, but not on ocular lobes; snout as long as wide, with medial lobation; foot intermediate length, pigmented on dorsal region. Ctenidium in the anterior region of pallial cavity with about 15 gill filaments; osphradium ellipsoidal under central gill filaments (Figure 7C, Table 5). Stomach slightly longer than wide (Figure 7F); style sac barely shorter than stomach, protruding below intestine (Table 5).

Female genitalia with a pallial oviduct about four times longer than wide (Figure 7G; Table 6); capsule gland slightly longer than albumen gland and denser in posterior region; genital aperture in the anterior extreme of pallial oviduct; elongate bursa copulatrix, J-shaped folded with a duct less than 50% bursa length; renal oviduct scarcely pigmented from the insertion point of bursal duct to where it begins to fold and black pigmented, making two or three loops; elongate seminal receptacle with pigmented short duct (Figure 7H) joining renal oviduct slightly above the point where the bursal duct joins the renal oviduct.

Male genitalia bearing a bean-like prostate gland about three times longer than wide (Figure 7E, Table 6); penis triangular with a wide base attached to central area of head

Table 5. Ctenidium, osphradium and digestive system measurements (in mm) of *P. (C.) astieri* from d'Argens spring, Seillons, France and *P. (C.) hauffei* sp. n. from Los Nogales spring, Benafer, Castellón, Spain.

	P. (C.) astieri **(n=5)**			***P. (C.) hauffei*** **sp. n. (n=7)**		
	Mean (Max-Min)	**SD_N**	**CV**	**Mean (Max-Min)**	**SD_N**	**CV**
Ct L	1.06 (1.25–0.90)	0.15	0.14	1.11 (1.27–0.92)	0.16	0.14
Os L	0.32 (0.43–0.20)	0.09	0.27	0.33 (0.45–0.24)	0.07	0.22
Os W	0.13 (0.15–0.11)	0.02	0.16	0.21 (0.25–0.15)	0.03	0.15
Ss L	0.59 (0.67–0.50)	0.06	0.11	0.62 (0.66–0.58)	0.03	0.05
Ss W	0.35 (0.37–0.31)	0.02	0.06	0.37 (0.41–0.33)	0.03	0.08
St L	0.71 (0.74–0.66)	0.04	0.06	0.73 (0.84–0.66)	0.06	0.09
St W	0.61 (0.67–0.56)	0.04	0.07	0.69 (0.78–0.61)	0.06	0.09

SD_N, Unbiased estimate for Standard Deviation, CV, Coefficient of Variation.

with some folds in middle section and a narrow patch of black pigment on distal surface (Figure 7D); vas deferens uncoiled in penis running straight close to the external margin.

Nervous system brown pigmented, but ganglia darker than connectives and commissures; cerebral ganglia equal in size; supraoesophageal and suboesophageal ganglia similar in shape and size; supraoesophageal connective around three times longer than suboesophageal (Figure 7A,B; Table 7). Mean RPG ratio 0.51 (elongated).

Remarks. Some of the localities where this species was found were cited by Gasull (1981) incorrectly as inhabited by *P.* (*C.*) *astieri*. Both species show marked differences such as: 1) *P.* (*C.*) *astieri* has a longer shell, longer spire (SL-LBW) (Table 2) and the protoconch microsculpture is more granulated than in *P.* (*C.*) *hauffei* sp. n. Moreover, the inner lip of the shell aperture in *P.* (*C.*) *hauffei* sp. n. is thicker than in *P.* (*C.*) *astieri* and partly hides the umbilicus; 2) central radular tooth with seven lateral cusps in *P.* (*C.*) *astieri,* five in *P.* (*C.*) *hauffei* sp. n. (Table 4); 3) style sac surrounded by black pigmented intestine in *P.* (*C.*) *astieri* yet lacks pigment and protrudes under the intestine in *P.* (*C.*) *hauffei* sp. n. (Figures 4F and 7F); 4) bursa copulatrix J-shaped and seminal receptacle with a short duct in *P.* (*C.*) *hauffei* sp. n., while bursa copulatrix is U-shaped and seminal receptacle is shorter and lacks a duct in *P.* (*C.*) *astieri* (Figures 4H and 7H)*;* 5) penis triangular with a wide base in *P.* (*C.*) *hauffei* sp. n. and slender in *P.* (*C.*)

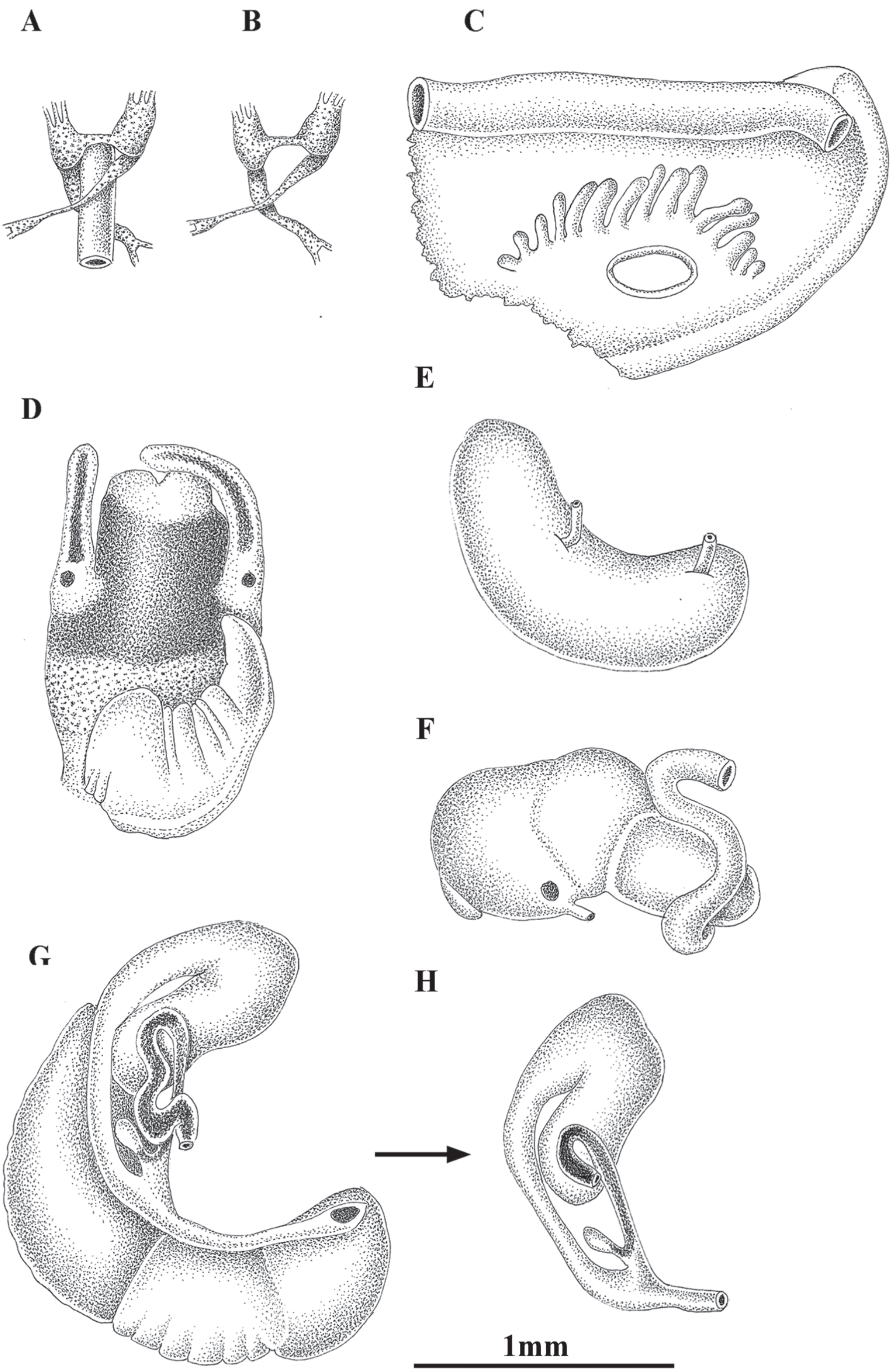


Figure 7. Anatomy of *Pseudamnicola (C.) hauffei* sp. n. from Nogales spring, Benafer, Castellón, Spain. **A, B** Partial nervous system **C** Ctenidium and osphradium **D** Head of a male and penis **E** Prostate gland **F** Stomach **G** Female genitalia **H** Bursa copulatrix and seminal receptacle.

Table 6. Female and male genitalia measurements (in mm) of two females and four males of *P. (C.) astieri* from d'Argens spring, Seillons, France and four females and four males of *P. (C.) hauffei* sp. n. from Los Nogales spring, Benafer, Castellón, Spain.

	P. (C.) astieri			***P. (C.) hauffei* sp. n.**		
	Mean (Max-Min)	**SD_N**	**CV**	**Mean (Max-Min)**	**SD_N**	**CV**
Po L	2.22 (2.35–2.08)	0.24	0.11	2.04 (2.32–1.75)	0.26	0.13
Po W	0.58 (0.59–0.56)	0.03	0.04	0.45 (0.49–0.41)	0.04	0.10
Ag. L	0.95 (1.04–0.84)	0.18	0.18	0.81 (1.00–0.71)	0.14	0.17
Cg. L	1.01 (1.11–0.91)	0.18	0.17	1.00 (1.11–0.93)	0.09	0.09
SR1 L	0.14 (0.15–0.14)	0.01	0.09	0.18 (0.22–0.15)	0.03	0.18
BC L	1.24 (1.32–1.15)	0.15	0.12	1.37 (1.56–0.95)	0.30	0.22
BC W	0.30 (0.35–0.25)	0.09	0.29	0.31 (0.36–0.25)	0.05	0.18
dBC L	0.60 (0.75–0.65)	0.09	0.15	0.62 (0.72–0.46)	0.13	0.21
Pr L	1.67 (1.86–1.55)	0.14	0.08	1.37 (1.46–1.26)	0.09	0.06
Pr W	0.58 (0.69–0.52)	0.09	0.15	0.45 (0.51–0.41)	0.05	0.12
P L	1.26 (1.35–1.15)	0.09	0.07	1.28 (1.60–1.12)	0.24	0.19
P W	0.37 (0.40–0.33)	0.03	0.09	0.66 (0.75–0.50)	0.12	0.18
PL/Head length	1.02 (1.16–0.86)	0.16	0.16	0.91 (1.07–0.82)	0.15	0.17

SD_N, Unbiased estimate for Standard Deviation, CV, Coefficient of Variation.

Table 7. Nervous system measurements (in mm) of *P. (C.) astieri* from d'Argens spring, Seillons, France and *P. (C.) hauffei* sp. n. from Los Nogales spring, Benafer, Castellón, Spain.

	***P. (C.) astieri* (n=5)**			***P. (C.) hauffei* sp. n. (n=5)**		
	Mean (Max-Min)	**SD_N**	**CV**	**Mean (Max-Min)**	**SD_N**	**CV**
LRCG	0.22 (0.24–0.20)	0.02	0.10	0.21 (0.23–0.18)	0.02	0.10
LLCG	0.22 (0.25–0.18)	0.03	0.15	0.22 (0.24–0.19)	0.02	0.10
LCC	0.11 (0.12–0.07)	0.02	0.19	0.15 (0.19–0.12)	0.02	0.14
LRPG	0.13 (0.18–0.10)	0.03	0.25	0.15 (0.16–0.12)	0.02	0.14

	P. (C.) astieri **(n=5)**			***P. (C.) hauffei*** **sp. n. (n=5)**		
	Mean (Max-Min)	**SD_N**	**CV**	**Mean (Max-Min)**	**SD_N**	**CV**
LLPG	0.17 (0.20–0.13)	0.03	0.19	0.23 (0.28–0.20)	0.03	0.14
LsupG	0.14 (0.17–0.11)	0.03	0.23	0.12 (0.15–0.10)	0.02	0.18
LsubG	0.11 (0.13–0.09)	0.01	0.10	0.12 (0.14–0.09)	0.02	0.18
LPsupC	0.18 (0.24–0.11)	0.05	0.30	0.29 (0.39–0.21)	0.07	0.26
LPsubC	0.07 (0.09–0.06)	0.01	0.15	0.10 (0.13–0.05)	0.03	0.32
RPG	0.40 (0.49–0.38)	0.05	0.13	0.51 (0.58–0.46)	0.05	0.10

SD_N, Unbiased estimate for Standard Deviation, CV, Coefficient of Variation.

astieri (Figures 4D and 7D); 6) nervous system elongated (RPG= 0.51) in *P.* (*C.*) *hauffei* sp. n. yet moderately concentrated (RPG= 0.42) in *P.* (*C.*) *astieri.*

Compared to the other *P.* (*Corrosella*) species living in nearby areas, *P.* (*C.*) *hinzi* and *P.* (*C.*) *navasiana, P.* (*C.*) *hauffei* sp. n. has a shorter and more ovate shell shape, a longer bursa copulatrix, bursa duct and seminal receptacle, and a more triangular wider-based penis.

Molecular analysis

The data set analysed included data for 11 *Pseudamnicola* species and 658 characters of the COI gene. New sequences for both species were deposited in Genbank under accession numbers JQ067672 – JQ067677, while the rest of the sequences were obtained from this same database (see Table 1). *Hydrobia acuta acuta* (Draparnaud, 1805) and *Pyrgula annulata* (Linnæus, 1758) were used as outgroups.

Pseudamnicola (*Corrosella*) *hauffei* sp. n. differed 7.44% with respect to *P.* (*C.*) *astieri* specimens and moreover, both were clustered as sister species (Table 8 and Figure 8). Through Bayesian analysis, the subgenus *Corrosella* was found to be well supported and divided into three clades, whose phylogenetic relationships are still unclear. The clades comprising *P.* (*C.*) *hauffei* sp. n. and *P.* (*C.*) *astieri*, or *P.* (*C.*) *manueli* and *P.* (*C.*) *bareai* were well supported (posterior probabilities over 0.90). However the clade including *P.* (*C.*) *marisolae*, *P.* (*C.*) *luisi*, *P.* (*C.*) *andalusica*, *P.* (*C.*) *iruritai* and *P.* (*C.*) *falkneri* was not well supported.

Table 8. Genetic divergence matrix for the species examined based on the COI gene sequence.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *P. (C.) astieri*	-												
2. *P. (C.) hauffei* sp. n.	7.44	-											
3. *P. (C.) marisolae*	10.26	10.41	-										
4. *P. (C.) luisi*	10.79	12.16	6.00	-									
5. *P. (C.) iruritai*	8.13	9.42	6.38	6.69	-								
6. *P. (C.) andalusica*	9.11	10.64	6.46	6.54	5.39	-							
7. *P. (C.) falkneri*	8.97	8.81	9.19	10.33	7.83	8.05	-						
8. *P. (C.) bareai*	7.86	7.65	9.14	9.59	7.87	8.50	8.42	-					
9. *P. (C.) manueli*	8.73	8.74	9.65	11.15	8.81	9.03	7.67	5.46	-				
10. *P. (P.) lucensis*	15.06	14.78	15.15	15.21	13.69	14.31	13.66	12.90	14.22	-			
11. *P. (P.) macrostoma negropontina*	14.45	14.47	14.85	14.89	14.16	14.32	13.67	14.42	14.54	7.68	-		
12. *H. acuta acuta*	17.91	19.49	17.60	17.26	15.78	16.35	16.62	17.12	18.29	18.65	18.97	-	
13. *Pyrgula annulata*	16.67	17.44	17.55	16.67	14.99	16.82	17.58	16.09	17.05	16.90	18.14	17.38	-

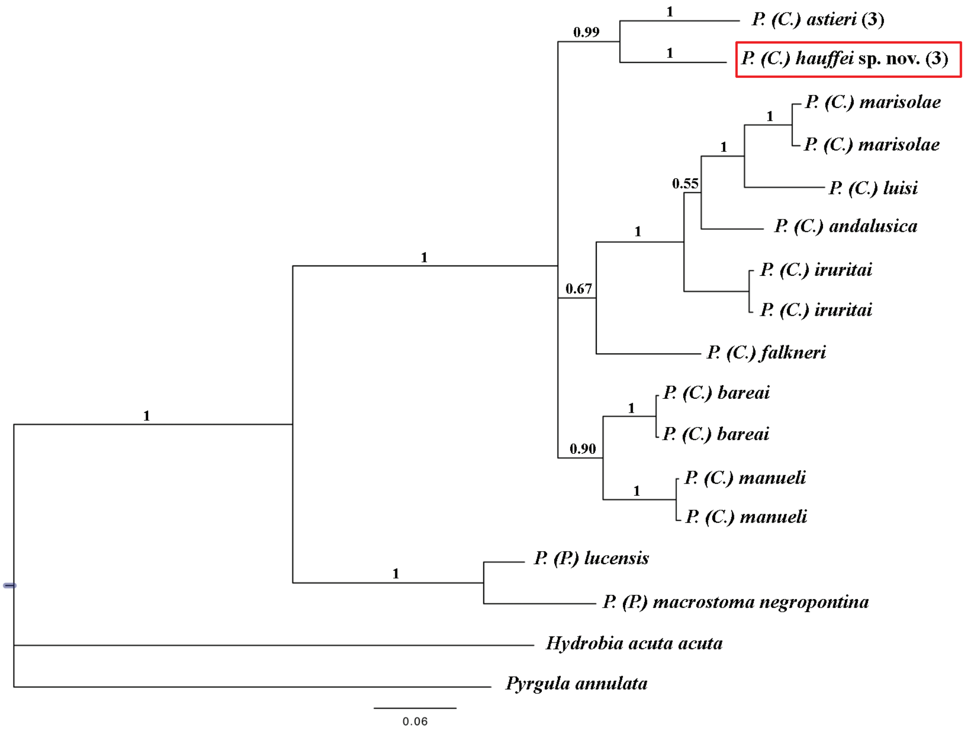


Figure 8. Bayesian 50% majority rule consensus tree inferred employing COI mitochondrial gene partial sequence. The numbers above branches represent Bayesian posterior probabilities. The numbers between brackets symbolize specimens with identical haplotypes. Scale bar: expected changed per site.

Conclusions

Based on this wide morphological study and our molecular data, we were able to delimit both species and clearly rule out the hypothesis of the presence of *P.* (*C.*) *astieri* in the Iberian Peninsula, identifying it as an endemism of the Alpes-Maritimes and Var departments of France, as proposed by Falkner in 2002. Consequently, the Iberian populations formerly cited as *P.* (*C.*) *astieri* in Castellón province (Gasull 1981, Vidal-Abarca and Suárez 1985) actually correspond to the new species *P.* (*C.*) *hauffei* sp. n. Morphologically, the most diagnostic characters are provided by shell habitus, central radular tooth, male and female genital systems and the RPG ratio. In effect, a considerable difference between the two species was detected in nervous system condensation (RPG ratio). In addition, *P.* (*C.*) *hauffei* sp. n. has more elongated connectives, which is considered a primitive state (Fretter and Graham 1962).

Through a phylogenetic approach based on partial sequence data for the COI gene provided in GenBank for other *P.* (*Corrosella*) species, we were able to estimate a mean genetic divergence of about 8% (5.39 to 11.15%) (Delicado et al. 2012). In comparison, our preliminary molecular data for the same gene sequence indicate a genetic divergence of 7.4% between *P.* (*C.*) *astieri* and *P.* (*C.*) *hauffei* sp. n., suggesting the two taxa are in fact different taxonomic entities.

Besides clarifying the taxonomic status of these two species and their phylogenetic relationship as sister species, our findings point to a greater diversity of *Pseudamnicola* (*Corrosella*) than previously thought, with implications for the protection of this poorly known group of molluscs. Indeed, their fragile ecosystems susceptible to the effects of human activities, altered water regimes, pollution, etc. means that most of these hydrobiid species are seriously threatened or even endangered (see Hydrobiidae spp. by Arconada et al. in Verdú and Galante 2006). Moreover, the fact that both taxonomic entities, *P.* (*C.*) *astieri* and *P.* (*C.*) *hauffei* sp. n. are endemisms inhabiting restricted areas of France and Spain respectively instead of belonging to a single species with a large distribution area, suggests that they should be assessed for inclusion in the Red Lists of both countries and conservation measures should be taken to protect their fragile habitats.

Acknowledgements

The authors thank Dr. M. Haase and Dr. E. Gittenberger for their constructive comments and suggestions. A.L. Tormo, M. Furio and A. Jorge from the MNCN assisted with the ESEM study and photomicrographs. Drawings were redone by I. Díaz Cortaberría. Dr. M.A. Alonso-Zarazaga provided advice on nomenclature. The English was reviewed by A. Burton. The manuscript was also improved by the comments and suggestions of Dr. R. Hershler. This work has been financed by the MICINN projects Fauna Ibérica IX (CGL2007-66786-C8-01) and FaIb X (CGL2010-22267-C07-01) and the support of a JAE Predoctoral fellowship (JAEPre047) to DD.

References

Arconada B, Ramos MA (2001) New data on Hydrobiidae systematics: two new genera from the Iberian Peninsula. Journal of Natural History 35: 949–984. doi: 10.1080/002229301300323884

Arconada B, Ramos MA (2003) The Ibero-Balearic region: one of the areas of highest Hydrobiidae (Gastropoda, Prosobranchia, Rissooidea) diversity in Europe. Graellsia 59(2–3): 91–104. doi: 10.3989/graellsia.2003.v59.i2-3.237

Arconada B, Ramos MA (2006) Revision of the genus *Islamia* Radoman, 1973 (Gastropoda, Caenogastropoda, Hydrobiidae), on the Iberian Peninsula and description of two new genera and three new species. Malacologia 48(1–2): 77–132.

Arconada B, Delicado D, Ramos MA (2007) A new genus and two new species of Hydrobiidae (Mollusca, Caenogastropoda) from the Iberian Peninsula. Journal of Natural History 41(29–32): 2007–2035. doi: 10.1080/00222930701529273

Bérenguier P (1882) *Essai sur la faune malacologique du departement de Var*. Société d'études scientifiques et archéologiques de Draguignan, France, 131 pp.

Bérenguier P (1902) Malacographie du Département du Var. C. et A. Latil, Draguignan, 540 pp.

Biemann T, Kearney E (2010) Size does matter: How varying group size in a sample affect the most common measures of group diversity. Organizational Research Methods 13: 582–599. doi: 10.1177/1094428109338875

Boeters HD (1970) *Corrosella* n. gen. (Prosobranchia: Hydrobiidae). Journal de Conchiliologie 108(3): 63–69.

Boeters HD (1976) Hydrobiidae Tunesiens. Archiv für Molluskenkunde 107(1/3): 89–105.

Boeters HD (1984) Unbekannte westeuropäische Prosobranchia, 6. Heldia 1(1): 9–11.

Boeters, HD (1986) Unbekannte westeuropäische Prosobranchia, 7. Heldia 1(4): 125–128.

Boeters HD (1988) Moitessieriidae und Hydrobiidae in Spanien und Portugal. Archiv für Molluskenkunde 118(4/6): 181–261.

Boeters HD (1999) *Alzionella navarrensis* n. sp., *Pseudamnicola* (*Corrosella*) *hydrobiopsis* n. sp. and the type especies of *Pseudamnicola* Paulucci, 1878 unknown West european Prosobranchia. Basteria 63: 77–81.

Davis GM, Kitikoon V, Temcharoen P (1976) Monograph on "*Lithoglyphosis" aperta*, the snail host of Mekong River Schistosomiasis. Malacologia 15: 241–287.

Davis GM, Chen CE, Wu C, Kuang TF, Xing G, Li L, Liu WJ, Yan YL (1992) The Pomatiopsidae of Hunan, China (Gastropoda: Rissoacea). Malacologia 34(1–2): 143–342.

Davis GM, Guo YH, Hoagland KE, Chen PL, Zheng LC, Yang HM, Chen DJ, Zhou YF (1986) Anatomy and systematics of Triculini (Prosobranchia: Pomatiopsidae: Triculinae), freshwater snails from Yunnan, China, with descriptions of new species. Proceedings of the Academy of Natural Sciences of Philadelphia 138: 466–575.

Davis GM, Kuo YH, Hoagland KE, Chen PL, Yang HM, Chen DJ (1984) *Kunmingia*, a new genus of Triculinae from China: phenetic and cladistic relationships. Proceedings of the Academy of Natural Sciences of Philadelphia 136: 165–193.

Davis GM, Wilke T, Spolsky C, Qiu C, Qiu D, Xia M, Zhang Y, Rosenberg G (1998) Cytochrome Oxidase I-Based Phylogenetic Relationships Among the Pomatiopsidae, Hydrobiidae, Rissoidae and Truncatellidae (Gastropoda: Caenogastropoda: Rissoacea). Malacologia 40(1–2): 251–266.

Delicado D, Machordom A, Ramos MA (2012) Underestimated diversity of Hydrobiid snails. The case of *Pseudamnicola* (*Corrosella*) (Mollusca: Caenogastropoda: Hydrobiidae). Journal of Natural History 46(1–2): 25–89. doi: 10.1080/00222933.2011.623358

Dupuy D (1851) Histoire naturelle des mollusques terrestres et d'eau douce. Chez Victor Masson, Paris, 956 pp.

Falkner G, Ripken ThEJ, Falkner M (2002) Mollusques continentaux de France. Liste de Reférénce annotée et bibliographie. Patrimoines Naturels 52, 350 pp.

Fauna Europaea (2011) Fauna Europaea database version 2.4, Mollusca: Gastropoda: Hydrobiidae. Available: http://www.faunaeur.org. Accessed: (04/11/2011).

Fagot P (1907) Contribution à la faune malacologique de la province d'Aragon. Boletín de la Sociedad Aragonesa de Ciencias Naturales 6: 136–160.

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.

Frauenfeld GR (1865) Verzeichniss der Namen der fossilen und lebenden Arten der Gattung *Paludina* (Lam.) nebst jenen der näckststehendeu und Eiureihung derselben in die verschiedenen neueren Gattungen. Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien XIV: 562–672.

Fretter V, Graham A (1962) British Prosobranch Molluscs. The Ray Society, London, 755 pp.

Gasull L (1981) Fauna malacológica terrestre y de agua dulce de la provincia de Castellón de la Plana. Bolletí de la Societat d'História Natural de les Balears 25: 55–102.

Ghamizi M, Vala JC, Bouka H (1997) Le genre *Pseudamnicola* au Maroc avec description de *Pseudamnicola pallaryi* n. sp. (Gastropoda: Hydrobiidae). Haliotis 26: 33–49.

Girardi H (2009) *Pseudamnicola* (*Corrosella*) *astierii* (Dupuy, 1851), dans les eaux de Var, France (Mollusca: Caenogastropoda: Hydrobiidae). Documents malacologiques 3: 56–61.

Glöer P, Pesič V (2009) New freshwater gastropod species of the Iran (Gastropoda: Stenothyridae, Bithyniidae, Hydrobiidae). Mollusca 27(1): 33–39.

Glöer P, Bouzid S, Boeters HD (2010) Revision of the genera *Pseudamnicola* Paulucci 1878 and *Mercuria* Boeters 1971 from Algeria with particular emphasis on museum collections. Archiv für Molluskenkunde 139(1): 1–22.

Hershler R, Ponder W (1998) A review of morphological characters of Hydrobioid snails. Smithsonian Contributions to Zoology 600: 1–55. doi: 10.5479/si.00810282.600

Hershler R, Liu HP, Thompson FG (2003) Phylogenetic relationships of North American nymphophiline gastropods based on mitochondrial DNA sequences. Zoologica Scripta 32: 357–366. doi: 10.1046/j.1463-6409.2003.00115.x

Holtzman WH (1950) The unbiased estimate of the population variance and standard deviation. American Journal of Psychology 63: 615–617. doi: 10.2307/1418879

Huelsenbeck JP (2000) MrBayes: Bayesian inference of phylogeny. University of Rochester, Department of Biology, NY.

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. Bioinformatics 17: 754–755. doi: 10.1093/bioinformatics/17.8.754

Kelley K, Lai K (2011) MBESS: MBESS. R package version 3.2.1. http://CRAN.R-project.org/package=MBESS

Locard A (1882) Catalogue general mollusques vivants de France: Mollusques terrestres, des eaux douces et des eaux salmastres. H. Georg, Lyon, 462 pp. doi: 10.5962/bhl.title.10669

Locard A. (1893) Les coquilles des eaux douces et saumâtres de France. B Baillière, Paris, 327 pp.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Ramos MA, Arconada B, Moreno D, Rolán E (2000) A new genus and a new species of Hydrobiid snail (Mollusca:Gastropoda:Hydrobiidae) from eastern Spain. Malacologia 42(1–2): 75–101.

Szarowska M, Falniowski A, Riedel F, Wilke T (2005) Phylogenetic relationships of the subfamily Pyrgulinae (Gastropoda: Caenogastropoda: Hydrobiidae) with emphasis on the genus *Dianella* Gude, 1913. Zootaxa 891: 1–32.

Szarowska M, Grzmil P, Falniowski A (2006) *Pseudamnicola* Paulucci, 1878 (Gastropoda: Hydrobiidae) in the Balkans. Folia Malacologica 14(4): 179–190.

Verdú JR, Galante E eds (2006) Lista Roja de los Invertebrados de España p 45–66. In Libro Rojo de los Invertebrados de España. Dirección General para la Biodiversidad, Ministerio de Medio Ambiente, Madrid.

Vidal-Abarca C, Suárez MA (1985) Lista faunística y bibliográfica de los moluscos (Gastrópoda & Bivalvia) de las aguas continentales de la Península Ibérica e Islas Baleares. Asociación Española de Limnología, Madrid, Spain, 193 pp.

Wilke T, Davis GM, Falniowski A, Giusti F, Bodon M, Szarowska M (2001) Molecular systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. Proceedings of the Academy of Natural Science of Philadelphia 151: 1–21. doi: 10.1635/0097-3157(2001)151[0001:MSOHMG]2.0.CO;2

Wilke T, Rolán E, Davis GM (2000) The mudsnail genus *Hydrobia* s.s. in the northern Atlantic and western Mediterranean: a phylogenetic hypothesis. Marine Biology 137: 827–833. doi: 10.1007/s002270000407

ZooKeys 190: 81–94 (2012)
doi: 10.3897/zookeys.190.2975
www.zookeys.org

RESEARCH ARTICLE

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

Acanthodasys paurocactus sp. n., a new species of Thaumastodermatidae (Gastrotricha, Macrodasyida) with multiple scale types from Capron Shoal, Florida

Sarah Atherton[1,†], Rick Hochberg[2,‡]

[1] *University of Massachusetts Lowell, One University Avenue, Lowell, MA , 01854, USA*

† *urn:lsid:zoobank.org:author:1F597997-CD78-4F36-A82B-977B14DCAA6C*
‡ *urn:lsid:zoobank.org:author:8C5BB2F6-35A3-4A4D-9F67-C2A21A5DADE1*

Corresponding author: *Sarah Atherton* (sarah_atherton@student.uml.ed), *Rick Hochberg* (rick_hochberg@uml.edu)

Academic editor: *M. Todaro* | Received 20 February 2012 | Accepted 25 April 2012 | Published 4 May 2012

urn:lsid:zoobank.org:pub:6A07609A-7FA3-4645-AC1C-6B84B274CA4A

Citation: Atherton S, Hochberg R (2012) *Acanthodasys paurocactus* sp. n., a new species of Thaumastodermatidae (Gastrotricha, Macrodasyida) with multiple scale types from Capron Shoal, Florida. ZooKeys 190: 81–94. doi: 10.3897/zookeys.190.2975

Abstract

A new species of *Acanthodasys* (Gastrotricha, Macrodasyida, Thaumastodermatidae) is described from sublittoral sediments off the Atlantic coast of Florida. *Acanthodasys paurocactus* **sp. n.** is a relatively small species (to 450 µm long) with a strap-shaped outline, a series of anterior, lateral, and ventrolateral adhesive tubes, paired caudal pedicles with posterior adhesive tubes, and a morphologically diverse cuticle. The cuticle contains both spined and unspined scales. Unspined scales are present in two general shapes: lanceolate and eye shaped, with some transitional shapes. All scales have a thickened rim and depressed central region; some scales of both shapes bear either one or more central bumps, a parallel ridge, or a perpendicular ridge that gives the appearance of a cross-shaped pattern under transmitted light. Spined scales are somewhat quadrangular in shape and bear uniancres to 15 µm long with a cross-shaped sectional profile. The new species is now one of five described species to possess both spined and spineless scales, and only one of two species to possess two types of spineless scales (the second species is an incompletely described specimen from Norway).

Keywords

Meiofauna, Caribbean, gastrotrich, taxonomy, Macrodasyida, cuticle

Introduction

Gastrotrichs are microscopic invertebrates found in all oceans, seas and inland water bodies. The phylum Gastrotricha is composed of two orders, Chaetonotida, which includes 322 freshwater species (Balsamo et al. 2009) and 133 marine or brackish water species (Hummon and Todaro 2010), and Macrodasyida, which includes 295 marine species and 2 freshwater species (Hummon and Todaro 2010). As permanent members of the meiobenthos, marine gastrotrichs are often numerous in fine to medium grain sediments, ranking second or third in abundance behind nematodes and copepods (Todaro et al. 1995; Hochberg 1999). However, their minuscule size combined with a lack of taxonomic expertise across the globe has hindered studies of gastrotrich biodiversity, particularly in tropical regions like the Caribbean where even general studies of meiofauna are sorely lacking (Miloslavich et al. 2010).

To date, little is known of gastrotrich biodiversity in the tropics and subtropics, particularly the Tropical Northwestern Atlantic (TNWA, aka wider Caribbean), which extends from South Florida to the French Guiana-Brazil border. Todaro (1994) and Todaro et al. (1995) conducted the first surveys of gastrotrich biodiversity in the Gulf of Mexico, one of the five ecoregions that defines the TNWA, uncovering 45 species from Texas to the Florida peninsula. Other ecoregions have received attention by researchers looking to catalog their marine biodiversity including: the Bahamanian ecoregion (Renaud-Debyser 1963), the Central Caribbean ecoregion (Hummon 1974, 2010; Hochberg 2008, 2010; Hochberg and Atherton 2011), the Lesser Antilles ecoregion (Kisielewski 1984; Hummon 2010; Kånneby et al. 2012; Todaro et al. 2012) and the South Florida ecoregion (Thane-Fenchel 1970; Schoepfer-Sterrer 1974; Decho et al. 1985; Evans and Hummon 1991; Evans 1992, 1994; Hummon 2010). Hummon's (2010) study remains the most extensive exploration to date, revealing species with distributions that span multiple ecoregions, thereby providing new insights into the biogeography of tropical and subtropical marine gastrotrichs.

In this study, we document a new species of *Acanthodasys* (Macrodasyida, Thaumastodermatidae) from sublittoral sediments off the Atlantic coast of Florida. This description forms part of a larger study that aims to classify the meiofauna from Capron Shoal, an offshore sandy shoal known to harbor diverse meiofauna (Winston and Håkansson 1986).

Methods

Gastrotrichs were collected from Capron Shoal (27°26'52"N, 80°13'81"W), a 3 m deep station approximately 7 km off the coast of Fort Pierce, Florida. Samples were collected via anchor dredge in March, 2005 and August 2011 and analyzed back at the Smithsonian Marine Station in Fort Pierce, Florida. Extraction of gastrotrichs followed a standard protocol: 1) approximately 100 cm^3 of sediment was combined with 900 cm^3 of 7% aqueous MgCl2 solution in a 1 L Erlenmeyer flask and allowed to rest for 10 min; 2) the flask was gently shaken and the supernatant was decanted over a 48 µm mesh; and

3) the mesh was gently washed with seawater into a Petri dish. Specimens were sorted under a Leica EZ4 stereomicroscope, transferred to a glass slide, and viewed with a compound microscope (Zeiss A1) equipped with DIC (differential interference contrast). Light micrographs and digital videos were captured with a Sony Handycam digital camera. Measurements of individual specimens were performed with an ocular micrometer. Lengths and positions of organ systems are described in terms of percentage body units, where total body length from anterior (U00) to posterior (U100) is 100 units.

Specimens were prepared for scanning electron microscopy with the following protocol: fixation in 3% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2) for 24h; rinsing four times (15 min each); postfixation in 1% OsO_4 in 0.1 M cacodylate buffer for 1 h; rinsing in 0.1M cacodylate buffer (4 × 15 m); dehydration in an ethanol series; transfering to BEEM capsules and dehydration in a critical point dryer. Specimens were then sputter coated with gold and examined on a JEOL 6400 SEM at 10 kV.

One specimen was prepared for museum archival using the following protocol, which is deemed more permanent than standard glycerin mounts: fixation in 2.5% glutaraldehyde in 0.1M phosphate buffer saline (PBS; pH 7.4) for 24 hr; rinsing with PBS for 1 hr; postfixation in 1% OsO4 in 0.1M PBS for 30 sec (to increase contrast); rinsing in PBS for 15 min; dehydration through an ethanol series; transfering to propylene oxide for 30 min; and embedding in epon resin on a glass microscope slide (coverslipped and placed in an oven at 60° C for 24 hr). Type specimen is deposited in the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Abbreviations: PIJ, pharyngeointestinal junction; TbA, anterior adhesive tubes below ventral mouth rim; TbL, lateral adhesive tubes; TbP, posterior adhesive tubes on caudal pedicles; TbVl, ventrolateral adhesive tubes.

Results

Order Macrodasyida Remane, 1925 [Rao and Clausen, 1970]
Family Thaumastodermatidae Remane, 1927
Subfamily Diplodasyinae Ruppert, 1978
Genus *Acanthodasys* Remane, 1927

Acanthodasys paurocactus sp. n.

urn:lsid:zoobank.org:act:16C6323A-A944-4C69-9FBE-3F060876360F
http://species-id.net/wiki/Acanthodasys_paurocactus

Type locality. Capron Shoal, Florida (27°26'52"N, 80°13'81"W), 3m depth, coarse sand. Sediments collected via anchor dredge by Hugh Reichardt and Woody Lee in March 2005; also in August 2011.

Materials examined. Florida: Five adult specimens observed with DIC optics on 4 August 2011; two specimens prepared during an earlier expedition (March 2005) for scanning electron miocroscopy.

Holotype. Adult specimen, ~ 375 µm long, curled, lateral orientation. Epidermal glands are artifically swollen. Cat no. USNM 1179053. Also, digital video of same specimen, live, deposited at the Smithsonian.

Diagnosis. *Acanthodasys* with body length 300–450 µm (mature specimens at ~ 325 µm length). Body mostly strap-shaped with a distinct pair of caudal pedicles curled under body. Maximum body width at mouth/PIJ/midpoint of body is 35/42/67 µm. Pharynx to 136 µm long with pharyngeal pores near base. Area around mouth naked (no scales or spines) and up to 12 µm long, bearing numerous sensory cilia to 10 µm long. Scales cover entire body with oblique and transverse orientations; scales of two shapes, elongate lanceolate and short eye shaped, each with a centrally depressed region. Some scales have a small bump(s) or ridge at the center. Spined scales of dorsal and lateral cuticle bear uniancres 4–15 µm long; ventral uniancres 2–4 µm long scattered in ciliary fields and in median columns between locomotory cilia. Scales extend on to the caudal pedicles. Lateral sensory cilia to 15 µm long. Epidermal glands to 13 µm in diameter, 15–20 per side. Five TbA per side inserting directly on body surface at mouth rim. Up to 4 robust and elongate TbL per side, present only in trunk region. Up to 20 TbVl per side beginning posterior of PIJ, with the most posterior group of five TbVl becoming distinctly lateral in position close to the caudal pedicles. Caudal pedicles distinct with one lateral, two terminal, and one medial tube per lobe. Hermaphroditic, with paired testes and single glandular caudal organ. Rosette gland on dorsolateral left side of body; large egg present (~50 µm diameter); ovaries paired at caudal end.

Etymology. This species is named for its spiky appearance, reminiscent of cactus (*pauro*, Greek: little, small; *cactus*, Greek: a prickly plant).

Description. The description is based on specimens measured *in vivo*; most specimens were dorsoventrally curled (see Fig. 1B). Body strap-shaped and 300–450 µm long (subadults ~ 300 µm long, most specimens 350–400 µm long) (Fig. 1). Terminal mouth 30–35 µm wide; body width increasing slightly to 43 µm at PIJ and to 67 µm in adults with developing ova. The trunk gradually tapers and leads to a pair of distinct caudal pedicles (Fig 1. inset). The entire body is covered with scales and spined scales except for the hood-like region around the mouth (Fig 1, 4). Epidermal glands to 13 µm diameter, up to 15–20 per side (Figs. 1A, 2).

Cuticlular armature. Scales and spined scales present (Figs. 1A, 2–4). Scales often appear as interwoven fibers in brightfield optics, but DIC reveals numerous scales in between the spined scales (uniancres); several scales with various raised structures at their center (es, Fig. 3A). At high magnification with DIC (1000X) and SEM (> 1000X), at least two types of scales are observed: elongate, lanceolate-shaped scales (ls) and shorter, eye-shaped scales (es, Figs. 3, 4B); scales of intermediate size and shape are also present (Fig. 4B). All scales have a slightly thickened rim and central depression that extends along the longitudinal axis of the scale (Figs. 3B, 4B). Scales are arranged in several different orientations (longitudinal, transverse, oblique) across the dorsal and lateral body walls (Fig. 4C). SEM reveals that several scales, both lanceolate and eye shaped, have either a raised, oval bump at the center of the depression (white arrow, Fig. 3B) or a

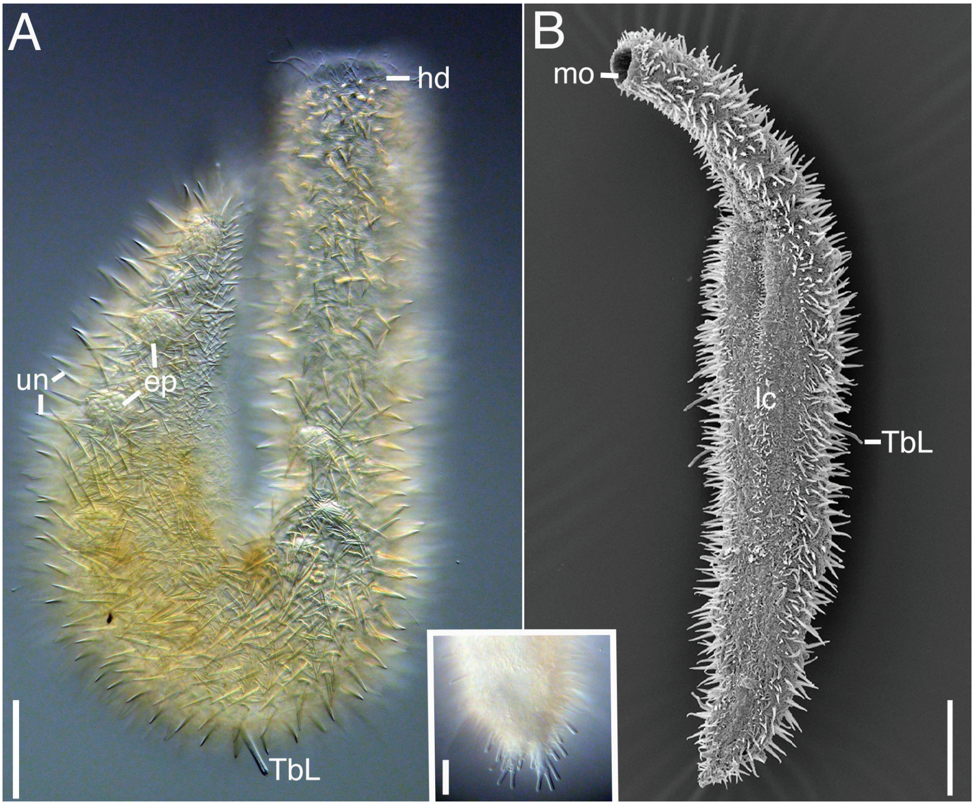


Figure 1. *Acanthodasys paurocactus* sp. n. **A** Adult specimen, dorsolaterally curled, DIC optics. Scale bar = 30 µm. **B** Adult specimen, ventral view, SEM. Note that the caudal pedicles are curled thereby obscuring the TbP. Scale bar = 50 µm. Inset Posterior end showing arrangement of TbVl and TbP. Scale bar = 14 µm. Abbreviatons: **ep** epidermal gland **oh** oral hood **lc** locomotory cilia **mo** mouth **TbL** lateral adhesive tube **un** uniancres.

raised, bar-shaped ridge that is parallel (es, Fig. 3A) or perpendicular (white arrow, Fig. 3B) to the long axis of the scale. Lanceolate scales measure to 7 µm long and eye-shaped scales to 4 µm long with a maximum width to 1.5 µm. Spined scales bearing uniancres are interspersed among spineless scales (Fig. 3A–C). Uniancres with a cross-shaped (cruciform) sectional profile (asterisk, Fig. 3B) arise from the center of thick-rimmed scales that also have a somewhat quadrangular shape (Figs. 3B, 4B). Dorsal and lateral uniancres close to the oral hood are 3–5 µm long and increase in length along the trunk and reach a maximum of 15 µm long. Several small uniancres (2–3 µm) extend onto the caudal pedicles. Uniancres are mostly straight and oriented perpendicular to the body surface or in a slightly posterior direction; some uniancres had a bent tip that might have been the result of dehydration during preparation for SEM. Openings to the epidermal glands were surrounded by a raised cuticular ridge. Ventrolaterally, the uniancres decrease in size to 4 µm long where they border the locomotory cilia (Fig. 3C). Several

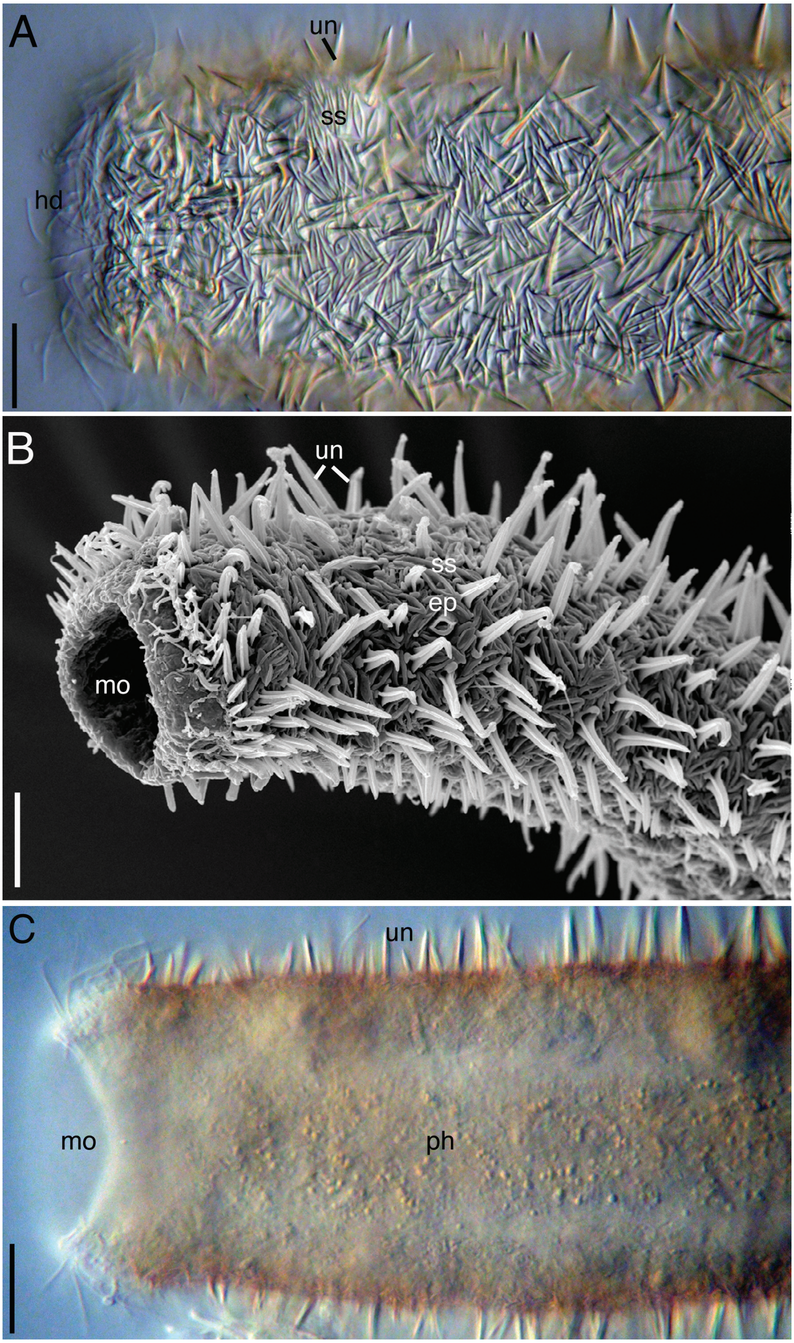


Figure 2. *Acanthodasys paurocactus* sp. n. **A** Closeup of anterior end of adult specimen, dorsal view, DIC optics. Scale bar = 15 μm. **B** Closeup of anterior end of adult specimen, lateral view, SEM. Scale bar = 15 μm. **C** Closeup of anterior end of adult specimen, ventral view, DIC optics. Scale bar = 12 μm. Abbreviations: **ep** opening of epidermal gland **hd** oral hood **mo** mouth **ph** pharynx **ss** spineless scales **un** uniancres.

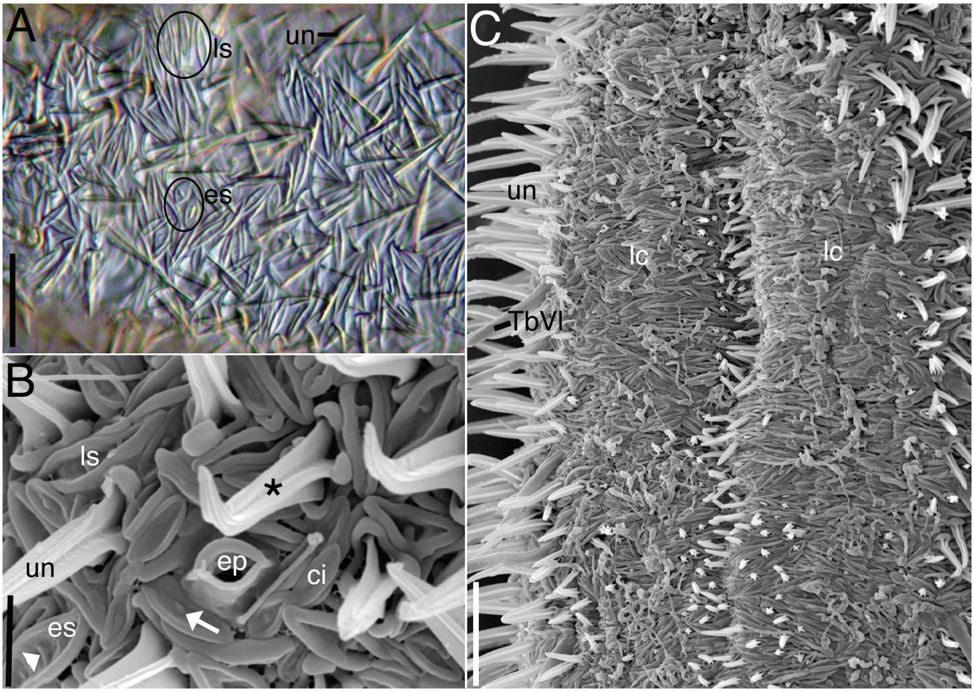


Figure 3. *Acanthodasys paurocactus* sp. n. **A** Closeup of dorsal cuticle of adult specimen, with focus on two types of spineless scales (circled) and uniancres (un), DIC optics. Scale bar = 12 µm. **B** Closeup of lateral cuticle of specimen showing lanceolate-shaped scales and eye-shaped scales, SEM. Some scales have perpendicular ridges (white arrowhead) or bumps (white arrow). Uniancres (*) arise from quadrangular-shaped scales. Scale bar = 4 µm. **C** Closeup of ventral trunk region of adult specimen showing location of ventral locomotory cilia (**lc**) and small ventral uniancres. Scale bar = 12 µm. Abbreviations: **ci** sensory cilium next to scales **ep** epidermal gland opening with raised cuticular ridge **es** eye-shaped scales **lc** locomotory cilia **ls** lanceolate scales **TbVl** ventrolateral adhesive tube **un** uniancre.

very small uniancres, 1–3 µm long, are scattered among the cilia on the ventral body wall (Fig. 3C). Several tiny (1–2 µm) and slightly larger (2–4 µm) uniancres are present in between the ciliary columns in the trunk region.

Cilia. Sensory cilia to 10 µm long extend across the oral hood and form a thin corona around the head (Figs. 1A, 2A). A thicker patch of sensory cilia on either side of the head extends to 15 µm length. Smaller cilia 5–8 µm long line the mouth rim on the ventral body wall. At least ten stiff, hair-like cilia to 12 µm long extend down the length of the body on either side. Sensory cilia were observed to project out between the scales under SEM (Fig. 3B). Ventral locomotory cilia cover most of the pharyngeal region, extending from approximately U05 to the PIJ (Figs. 3C, 4A). At the PIJ, the cilia continues as a series of continuous rows to the posterior end but with a narrow column of naked cuticle (and uniancres) in between (Fig. 3C).

Adhesive tubes. Five pairs of anterior adhesive tubes (TbA) up to 5 µm long are present at the mouth margin: one either side of the midline is a close-set pair of tubes

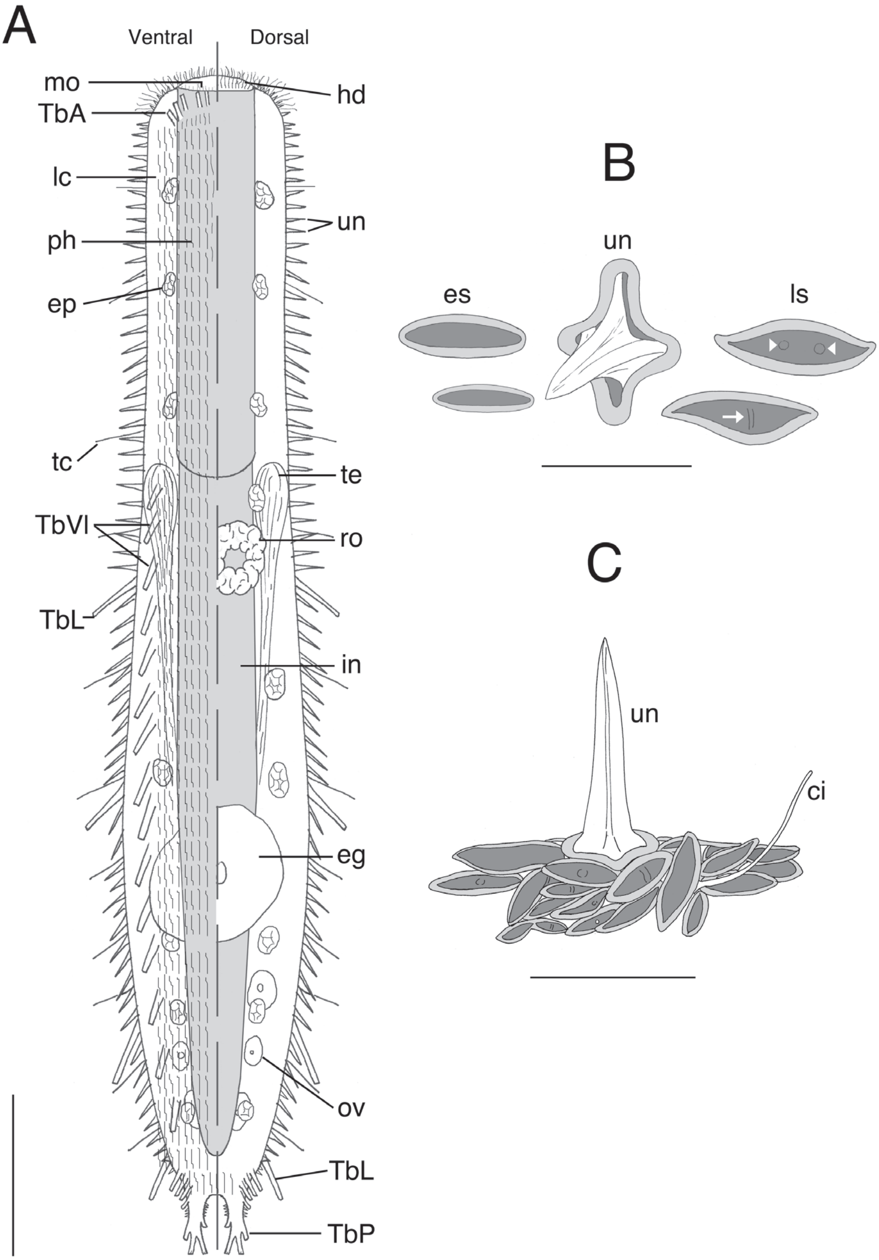


Figure 4. *Acanthodasys paurocactus* sp. n. **A** Composite sketch showing ventral (left) and dorsal (right) features. Scale bar = 40 μm. **B** Sketches of some scales based on SEM photographs of the dorsal cuticle. **C** Sketch of the general orientation of various spineless scales around a single spined scale based on SEM micrographs. Scale bar = 12 μm. Abbreviations: **ci** sensory cilium **eg** mature egg **ep** epidermal gland **es** eye-shaped scale **hd** oral hood **in** intestine **lc** locomotory cilia **ls** lanceolate-shaped scale **mo** mouth **ov** developing ova **ph** pharynx **ro** rosette organ **TbA** anterior adhesive tubes **TbL** lateral adhesive tubes **TbVl** ventrolateral adhesive tubes **TbP** posterior adhesive tubes **tc** lateral tactile cilia **te** testis **un** uniancres.

that is present medially and three tubes that form a group that is oriented diagnonally and closer to the lateral margin of the body (Fig. 4). Four pairs of TbL are present in the trunk region. Each tube is 21–25 μm long and robust in appearance. One specimen showed tubes at U45, U54, U70 and U80; three specimens were curled and difficult to measure. One specimen only had two TbL at positions U68 and U79. Up to twenty ventrolateral adhesive tubes (TbVl) to 12 μm long are inserted posterior to the PIJ. Most TbVl appear evenly spaced down the trunk; five TbVl become slightly more lateral in position and are clustered anterior to the caudal pedicles. The pedicles reach a maximum of 16 μm long including the posterior adhesive tubes (TbP) and bear a total of four TbP each: one lateral (6 μm), two terminal (4–5 μm), and one medial (4 μm) (Fig. 1 inset).

Digestive tract. Mouth terminal and circular to 35 um wide (Figs. 1B, 2B), surrounded by naked cuticle that forms a dorsal oral hood with a 12 μm rim (Fig. 2A, B); the naked cuticle around the ventral rim of the mouth is only 6 μm wide (hd, Figs. 2C). Pharynx to 136 μm long and 22 μm wide. Pharyngeal pores near base of pharynx (~ U34), not observable in all specimens. Intestine narrow and tapering at posterior; anus not observed.

Reproductive system. Hermaphroditic, with paired, bilateral testes beginning at the PIJ around U36 (Fig. 4A). Vasa deferentia extend posteriorly but could not be followed beyond mid-trunk region. Caudal organ observed in one specimen (body length: 400 μm), and pear-shaped, but the animal was too damaged for measurements. Rosette organ to 28 μm in diameter at U43–U46 in largest specimen (Fig. 4A). Paired ova were observed on either side of the posterior intestine in one specimem, with one large egg dorsal to intestine at approximately U65.

Taxonomic remarks

At present, there are sixteen species of *Acanthodasys* known from several oceans and inland seas including the Atlantic ocean (e.g., Forneris 1961; Kisielewski 1987, Evans 1992), Indian ocean (e.g., Gerlach 1961; Naidu and Rao 2004), Black and Mediterranean seas (reviewed in Todaro et al. 2004), and other localities worldwide (reviewed in Hummon 2009). However, only nine species have formally published descriptions that meet the criteria of Article 13 of the ICZN (1999) including: *A. aculeatus* Remane, 1927; *A. algarvensis* Hummon, 2008 (see Hummon and Todaro 2010); *A. arcassonensis* Kisielewski, 1987; *A. caribbeanensis* Hochberg & Atherton, 2010; *A. carolinensis* Hummon, 2008; *A. fibrosus* Clausen, 2004; *A. flabellicaudus* Hummon & Todaro, 2009; *A. lineatus* Clausen, 2000; and *A. silvulus* Evans, 1992. Five species named by Ruppert (1978) – *A. diplodasyoides*, 1978, *A. platydasyoides*, 1978, *A. tetranchyrodermatoides*, 1978, *A. thrinax*, 1978 and *A. vermiformis*, 1978 – are considered *nomina nuda* according to Article 13 of the ICZN because they lack formal descriptions. Two species described from Norway (Clausen 2000) are incompletely known (*Acanthodasys* sp. 1, sp. 2) but presumably represent undescribed taxa. Of the described species, *Acantho-*

dasys aculeatus Remane, 1927 has the most extensive geographic distribution (but see below), and is only one of two species reported from the Tropical Northwestern Atlantic (Hummon 2009), the other species being *A. caribbeanensis* Hochberg & Atherton, 2010 originally described from Panama in the Central Caribbean ecoregion.

In general, *A. paurocactus* sp. n. can be easily distinguished from its congeners by the structure of the cuticle, while most other characteristics overlap with those of previously described species. For example, the strap-shaped body outline is characteristic of most species in the genus, while the presence of a pair of distinct caudal pedicles (lobes) is known from *A. aculeatus*, *A. carolinensis*, *A. caribbeanensis*, *A. fibrosus, A. lineatus* and *Acanthodasys* sp. 1. Among these species, *A. paurocactus* sp. n. shows the most overall similarity with *A. aculeatus* sp. n. regarding body shape and general distribution of TbVl and TbP. Unfortunately, details about the number and distribution of adhesive tubes in *A. aculeatus* are questionable as the original description by Remane is incomplete: "Die Verteilung der Haftröhrchen konnte ich nur teilweise feststellen" (1927: 213). Furthermore, recent accounts of *A. aculeatus* from around the globe (e.g., Todaro et al. 1992; Fregni et al. 1999) indicate high variability in both body length and the number and position of adhesive tubes (e.g., Todaro et al. (1992) found TbD on specimens from the Tuscan archipelago that were not reported previously), calling into question the monophyletic status of *A. aculeatus*. Therefore, the taxonomic status of *A. aculeatus* must remain questionable until such time that further details, including genetic analyses, are provided on morphotypes from a wide range of geographic localities (e.g., see Kieneke et al. 2012 for genetic analyses of morphotypes of species of *Turbanella* across Europe).

Acanthodasys paurocactus sp. n. is now one of five species that is known to possess both spined scales (uniancres) and spineless scales. The other species are *A. aculeatus*, *A. arcassonensis*, *A. caribbeanensis* and *Acanthodasys* sp. 2. The uniancres of the new species are larger than those reported for *A. aculeatus* (variable: up to 9 µm, Forneris 1961), *A. arcassonensis* (11 µm), and *Acanthodasys* sp. 2 (6–10 µm), but much smaller than those of *A. caribbeanenesis* (up to 50 µm). Interestingly, the zone between spined scales appears to be made of a series of fine, interwoven cuticular fibers (based on transmitted light) similar to that of *A. fibrosus* Clausen, 2004, which lacks spineless scales. Closer inspection with differential interference contrast (DIC) at high magnification, however, revealed two important characteristics: all uniancres arise from a scale that makes the entire structure appear as an inverted t-shape (similar to that observed for *A. flabellicaudus*), and there are numerous spineless scales in between the uniancres. However, only when specimens were examined with scanning electron microscopy (SEM) could we unambiguously discern the structure of the spineless scales.

There were two general types of spineless scales revealed with SEM. One type of scale was lanceolate in shape, very thin and up to 7 µm long. Interspersed among these scales were eye-shaped scales that were somewhat wider and to 4 µm long. Scales of intermediate size and shape were also present. All scales had a central depressed region that extended the length of the longitudinal axis of the scale; some of these scales also had raised regions (e.g., bumps, a single parallel ridge, a single perpendicular ridge) in the depressed region. The rim of all scales, which appeared thicker than than the rest of the scale body, was always elevated above the central depression. We hypothesize that the raised ridge and depressed center of each scales alters their refractive index under transmitted light, thereby imparting the fiber-like appearance of the scales at low magnification. A similar case may also be found in *A. fibrosus* once that species is viewed with SEM. Interestingly, *A. paurocactus* sp. n. is now only the second known species to possess two types of spineless scales, the other species being an undescribed specimen (*Acanthodasys* sp. 2) from Norway (Clausen 2000). Unfortunately, many details on the undescribed species remain to be determined, but based on Clausen's measurements of the spines (6–10 µm) and body length (800 µm), his specimens are clearly different from those present in Florida.

As noted for *A. arcassonensis* (Kisielewski 1987) and other species of *Acanthodasys* (Rieger and Rieger 1977), the uniancres of *A. paurocactus* sp. n. have a cross-shaped or cruciform sectional profile under transmitted light. This profile is reminiscent of the patterns noted in various spineless scales of the new species and spineless scales of species of *Diplodasys* (Rieger and Rieger 1977). We hypothesize that this pattern may be due to the presence of a perpendicular ridge in the center of some scales (see Fig. 3B, white arrowhead), which at low magnifications and under transmitted light can make the scale appear to have a cross-like pattern. Rieger and Rieger (1977) made similar observations on species at the ultrastructural level, and hypothesized that the cross-shape pattern characteristic of these scales (and the uniancres) may be plesiomorphic within the subfamily Diplodasyinae. Further observations on other species with SEM are warranted before accepting the cross-shaped pattern as a useful taxonomic character.

Acknowledgements

The authors acknowledge the helpful comments of Dr. M. Antonio Todaro, Dr. Alexander Kieneke, and one anonymous reviewer for improving this manuscript. The authors would also like to thank Hugh Reichardt, Woody Lee, Julie Piraino and Dr. Michael Boyle at the Smithsonian Marine Station in Fort Pierce, FL for their assistance in field collections and SEM work. This is Smithsonian Marine Station at Fort Pierce contribution number 845. This material is based upon work supported by the National Science Foundation under Grant No. DEB 0918499.

References

Balsamo M, d'Hondt JL, Pierboni L, Grilli P (2009) Taxonomic and nomenclatural notes on freshwater Gastrotricha. Zootaxa 2158: 1–19.

Clausen C (2000) Gastrotricha Macrodasyida from the Tromso region, northern Norway. Sarsia 85: 357–384.

Clausen C (2004) A new species of *Acanthodasys* (Gastrotricha: Thaumastodermatidae) from the west coast of Norway. Sarsia 89: 137–141. doi: 10.1080/00364820410004972

Decho AW, Hummon WD, Fleeger JW (1985) Meiofauna – Sediment interactions around tropical seagrass sediments using factor analysis. Journal of Marine Research 43: 237–255. doi: 10.1357/002224085788437389

Fregni E, Faienza MG, Grimaldi SDZ, Tongiorgi P, Balsamo M (1999) Marine gastrotrichs from the Tremiti archipelago in the southern Adriatic Sea, with the description of two new species of *Urodasys*. Italian Journal of Zoology 66: 183–194. doi: 10.1080/11250009909356254

Evans WA (1992) Five new species of marine Gastrotricha from the Atlantic coast of Florida. Bulletin of Marine Science 51: 315–328.

Evans WA (1994) Morphological variability in warm-temperate and subtropical populations of *Macrodasys* (Gastrotricha: Macrodasyida: Macrodasyidae) with description of seven new species. Proceedings of the Biological Society of Washington 107: 239–255.

Evans WA, Hummon WD (1991) A new genus and species of Gastrotricha from the Atlantic coast of Florida, U.S.A. Transactions of the American Microscopical Society 110: 321–327. doi: 10.2307/3226768

Forneris L (1961) Beitraege zur Gastrotrichenfauna der Nord- und Ostsee. Kieler Meeresforschungen 17: 206–218.

Gerlach SA (1961) Über Gastrotrichen aus dem Meeressand der Malediven (Indischer Ozean). Zoologischer Anzeiger 167: 471–475.

Hochberg R (1999) Spatiotemporal size-class distribution of *Turbanella mustela* (Gastrotricha: Macrodasyida) on a northern California beach and its effect on tidal suspension. Pacific Science 53: 50–60.

Hochberg R (2008) Gastrotricha of Bocas del Toro, Panama: A preliminary report. Meiofauna Marina 16: 101–107.

Hochberg R (2010) New species of *Oregodasys* (Gastrotricha: Macrodasyida) from Carrie Bow Cay, Belize with an ultrastructural description of the epidermal glandular system. Zootaxa 2660: 1–17.

Hochberg R, Atherton S (2011) A new species of *Lepidodasys* (Gastrotricha, Macrodasyida) from Panama wioth a description of its peptidergic nervous system using CLSM, anti-FMRFamide and anti-SCPb. Zoologischer Anzeiger 250: 111–122. doi: 10.1016/j.jcz.2010.12.002

Hummon WD (1974) Intertidal marine Gastrotricha from Colombia. Bulletin of Marine Science 24: 396–408.

Hummon WD (2008) Gastrotricha of the North Atlantic Ocean: 1. Twenty four new and two redescribed species of Macrodasyida. Meiofauna Marina 16: 117–174.

Hummon WD (2009) Global database for marine Gastrotricha (Taxonomic, Geographic, Bibliographic and Video). Server: http://132.235.243.28 or http://hummon-nas.biosci.ohiou.edu

Hummon WD (2010) Marine Gastrotricha of the Caribbean Sea: A review and new descriptions. Bulletin of Marine Science 86: 661–708.

Hummon WD, Todaro MA (2009) Italian marine Gastrotricha: VI. Seven new species of Macrodasyida. Zootaxa 2278: 47–68.

Hummon WD, Todaro MA (2010) Analytic taxonomy and notes on marine, brackish-water and estuarine Gastrotricha. Zootaxa 2392: 1–32.

International Code of Zoological Nomenclature [ICZN] (1999) International code of zoological nomenclature [the Code]. Fourth edition. The International Trust for Zoological Nomenclature, c/o Natural History Museum, London. i–xxix, +306 pp. [online version at www.iczn.org].

Kånneby T, Todaro MA, Jondelius U (2012) A phylogenetic approach to species delimitation in freshwater Gastrotricha from Sweden. Hydrobiologia 683:185–202. doi: 10.1007/s10750-011-0956-1

Kieneke A, Martínez Arbisu PM, Fontaneto D (2012) Spatially structured populations with a low level of cryptic diversity in European marine Gastrotricha. Molecular Ecology 121: 1239–1254. doi: 10.1111/j.1365-294X.2011.05421.x

Kisielewski J (1984) *Xenotrichula guadelupense* n.sp., a new marine gastrotrich from Guadeloupe. Bulletin du Museum D'Histoire Naturelle, Paris 6A: 37–40.

Kisielewski J (1987) New records of marine Gastrotricha from the French coasts of Manche and Atlantic I. Macrodasyida, with descriptions of seven new species. Bulletin du Museum d'Histoire Naturelle, Paris 9: 837–877.

Miloslavich P, Díaz JM, Klein E, Alvarado JJ, Díaz C, Gobin J, Escobar-Brioes E, Cruz-Motta JJ, Wiel E, Cortés J, Bastidas AC, Robertson R, Zapata F, Martin A, Castillo J, Kazandjian A, Ortiz M (2010) Marine biodiversity in the Caribbean: regional estimates and distribution patterns. Public Library of Science One 5: 1–25.

Naidu KV, Rao GC (2004) The Fauna of India and the Adjacent Countries–Gastrotricha. Director, Zoological Survey of India, Kolkata, 169 pp.

Rao GC, Clausen C (1970) *Planodasys marginalis* gen et sp. n. and Planodasyidae fam. nov. (Gastrotricha Macrodasyoidea). Sarsia 42: 73–82.

Remane A (1925) Organisation und systematische Stellung der aberranten Gastrotrichen. Verhandlungen der deutschen zoologischen Gesellschaft 30: 121–128.

Remane A (1927) Neue Gastrotricha Macrodasyoidea. Zoologische Jahrbuecher Abteilung für Systematik und Morphologie 54: 203–242.

Renaud-Debyser J (1963) Recherches écologiques sur la faune interstitielle des sables: Bassin d'Arcachon et île de Bimini, Bahamas. Vie Milieu (Supplement) 15: 1–157.

Rieger GE, Rieger RM (1977) Comparative fine structure study of the gastrotrich cuticle and aspects of cuticle evolution within the Aschelminthes. Zeitschrift für Zoologische Systematik und Evolutionsforschung 15: 81–124. doi: 10.1111/j.1439-0469.1977.tb00533.x

Ruppert E (1978) The reproductive system of gastrotrichs. III. Genital organs of Thaumastodermatinae subfamm n. and Diplodasyinae subfam. n. with discussion of reproduction in Macrodasyida. Zoologica Scripta 7: 93–114. doi: 10.1111/j.1463-6409.1978.tb00592.x

Schöpfer-Sterrer C (1974) Five new species of *Urodasys* and remarks on the terminology of the genital organs in Macrodasyids (Gastrotricha). Cahiers de Biologie Marine 15: 229–259.

Thane-Fenchel A (1970) Interstitial gastrotrichs in some south Florida beaches. Ophelia 7: 113–137.

Todaro MA (1994) *Chaetonotus triacanthus* and *Heteroxenotrichula texana*, two new chaetonotid gastrotrichs from the Gulf of Mexico. Transactions of the American Microscopical Socety 113: 15–21. doi: 10.2307/3226575

Todaro MA, Balsamo M, Tongiorgi P (1992) Marine gastrotrichs from the Tuscan archipelago (Tyrrhenian Sea): I. Macrodasyida, with description of three new species. Italian Journal of Zoology 59: 471–485.

Todaro MA, Dal Zotto M, Jondelius U, Hochberg R., Hummon WD, Kånneby T, Rocha CEF (2012) Gastrotricha: A Marine Sister for a Freshwater Puzzle. PLoS ONE 7(2): e31740. doi: 10.1371/journal.pone.0031740

Todaro MA, Fleeger JW, Hummon WD (1995) Marine gastrotrichs from the sand beaches of the northern Gulf of Mexico: species list and distribution. Hydrobiologia 310: 107–117. doi: 10.1007/BF00015529

Todaro MA, Matinato L, Balsamo M, Tongiorgi P (2004) Faunistics and zoogeographical overview of the Mediterranean and Black Sea marine Gastrotricha. Biogeographia 24: 131–160.

Winston JE, Håkansson E (1986) The interstitial bryozoan fauna from Capron Shoal, Florida. American Museum Novitates 2865: 1–50.

ZooKeys 190: 95–109 (2012)
doi: 10.3897/zookeys.190.3011
www.zookeys.org

RESEARCH ARTICLE

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

Phylogenetic reconsideration of *Myrmekiaphila* systematics with a description of the new trapdoor spider species *Myrmekiaphila tigris* (Araneae, Mygalomorphae, Cyrtaucheniidae, Euctenizinae) from Auburn, Alabama

Jason E. Bond[1,†], Chris A. Hamilton[2,‡], Nicole L. Garrison[3,§], Charles H. Ray[3,|]

1 *Auburn University Museum of Natural History, Auburn University, Auburn, AL, 36849, USA*

† *urn:lsid:zoobank.org:author:F5B56990-8D68-4D59-A5C3-07E11FD31661*
‡ *urn:lsid:zoobank.org:author:A11AB4EE-74C3-455C-8819-213462B86F2A*
§ *urn:lsid:zoobank.org:author:1B165257-BF5C-4FEC-8157-0470DFBF6D2F*
| *urn:lsid:zoobank.org:author:7EBE305E-0B5B-4CE2-8784-A4F30C9D1F5A*

Corresponding author: *Jason E. Bond* (jbond@auburn.edu)

Academic editor: *M. Kuntner* | Received 25 February 2012 | Accepted 25 April 2012 | Published 4 May 2012

urn:lsid:zoobank.org:pub:F6500AE5-B3F1-4DF3-9A11-B9B74D386B06

Citation: Bond JE, Hamilton CA, Garrison NL, Ray CH (2012) Phylogenetic reconsideration of *Myrmekiaphila* systematics with a description of the new trapdoor spider species *Myrmekiaphila tigris* (Araneae, Mygalomorphae, Cyrtaucheniidae, Euctenizinae) from Auburn, Alabama. ZooKeys 190: 95–109. doi: 10.3897/zookeys.190.3011

Abstract

The trapdoor spider genus *Myrmekiaphila* currently comprises 11 nominal species. A recent molecular phylogenetic evaluation of the group identified a number of problems with respect to how species and species groups were delineated by Bond and Platnick in their 2007 taxonomic revision of the genus. We report herein the discovery of a new species, *Myrmekiaphila tigris* **sp. n.** The phylogenetic position of the species is evaluated using a molecular phylogenetic approach based on a set of mtDNA markers. Our preferred phylogenetic hypothesis supports the recognition of a new species and further highlights the need to more carefully investigate species boundaries within the genus. These results further indicate that palpal bulb morphology is rapidly evolving and has likely been a contributing factor in rendering a number of species paraphyletic with respect to the molecular data.

Keywords

New species, Integrative taxonomy, Molecular taxonomy, Southeastern United States, *Myrmekiaphila*, Cyrtaucheniidae, Euctenizinae

Introduction

The genus *Myrmekiaphila* Atkinson, 1886 is a moderately diverse assemblage of trapdoor spiders distributed predominantly throughout the southeastern United States. Revised recently by Bond and Platnick (2007), it presently comprises 11 nominal species that range from Virginia southward to the Gulf Coast and extending as far west as central Texas. All known *Myrmekiaphila* species construct a silk-lined burrow, covered by a silken-soil trapdoor, from which they forage as sit and wait predators; many burrows are uniquely modified to include a side chamber that can be closed from the main chamber by a second trapdoor. As discussed by Bond and Platnick (2007) the familial placement of the genus has been somewhat problematic, however, molecular studies of mygalomorph phylogeny (Bond and Hedin 2006, Hedin and Bond 2006, and Bond et al. in review) place the genus in what is now recognized as the North American cyrtaucheniid subfamily Euctenizinae, to be elevated to family rank (Bond et al. in review).

Myrmekiaphila species delimitation and diagnosis relies heavily on modifications of the male first walking leg – often termed the mating clasper – and the morphology of the palpal bulb. Mating clasper morphology is typically discriminated on the basis of unique spination patterns whereas palpal bulb modifications are often quite dramatic comprising relatively pronounced branched and serrated structures (Fig. 1). Such major differences in palpal bulb morphology stand in relatively stark contrast to the paucity of differentiation observed for palpal bulb structure in other related mygalomorph taxa (see Bond 2004, Stockman and Bond 2008). Despite the disparities in male morphology, female specimens are relatively homogenous with only subtle differences in spermathecae morphology rendering them nearly impossible to identify without the aid of molecular markers. Consequently, Bond and Platnick (2007) provided a morphological key only for male specimens.

On the basis of palpal differences, presence or absence of a second prong and serration patterns (Fig. 1 but also see Bailey et al. 2010, Figure 1), Bond and Platnick (2007) divided the genus into three species groups; they acknowledged at the time that these groupings were without phylogenetic support. A subsequent molecular based study of *Myrmekiaphila* species relationships by Bailey et al. (2010) indeed demonstrated these species groups were not monophyletic. Most notably the results of their analysis indicated that *Myrmekiaphila torreya* Gertsch & Wallace, 1936, a species with a dual pronged bulb, is paraphyletic with respect to *M. coreyi* Bond & Platnick, 2007, a species with a single prong and diminutive serration. Consequently, the species "tree" (i.e., prior hypothesis of species boundaries) was in conflict with the gene tree. Bailey et al. (2010) hypothesized that palpal bulb evolution appeared to be quickly outpacing the evolutionary patterns conveyed by the mitochondrial and nuclear markers (incomplete lineage sorting) used to infer phylogeny and that it was unlikely that the putative *M. torreya* lineages harbored cryptic diversity because of a general lack of geographical concordance among its lineages. This paper documents the discovery of a new *Myrmekiaphila* species that brings into question the hypothesis that incomplete lineage is the cause of the observed gene-tree species-tree incongruence. Rather, the study system may suffer from

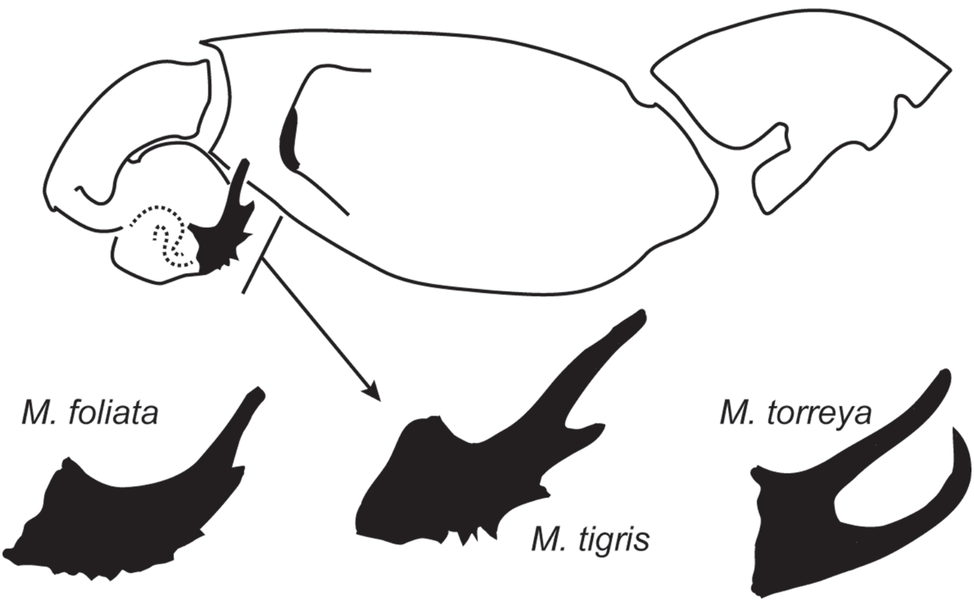


Figure 1. Line drawing of *Myrmekiaphila tigris* male pedipalp; distal aspect of palpal bulb shown for *M. foliata, M. tigris*, and *M. torreya* for comparative purposes.

"bad taxonomy" (see Funk and Omland 2003). That is, that Bond and Platnick (2007) may have overlooked some of the morphological diversity contained within the group.

The focus of this paper is the description of a new species, *Myrmekiaphila tigris* Bond & Ray. This species was brought to the attention of the first author by C. Ray who collected numerous specimens during the winter months (Dec-Jan) of 2011/2012 from sidewalks and swimming pools located in the town of Auburn, Alabama in a moderately populated housing subdivision. Bond and Platnick (2007) had examined specimens from the vicinity of the type locality reported herein, but seemingly misidentified them as *Myrmekiaphila foliata* Atkinson, 1886. Examination of the newly acquired specimens and reexamination of the earlier collected material found these specimens to bear some likeness to *M. foliata* but upon close inspection, notable differences in the palpal structure (discussed in the diagnosis of the species below) came to light. That said, given the putative widespread distribution of *M. foliata* it would not be unreasonable to infer that the variation noted in these specimens simply represents a geographic variant of the species and thus recognition of new species is not necessarily warranted (i.e., if we ignore the fact that mygalomorph taxa are prone to species crypsis; see Bond and Stockman 2008). As such, it seemed prudent to consider an independent assessment of the hypothesized new taxon using molecular data before proposing a new name. The study reported here provides molecular evidence that warrants recognition of a new species, further documents the somewhat complicated relationships of species contained within the genus, and formally describes this newly recognized lineage as a nominal species.

Materials and methods

Unique voucher numbers were assigned to all specimens (AUMS) and corresponding labels added to each vial. Collecting locality latitude/longitude data was georeferenced as described in Bond and Platnick (2007); georeferenced specimens from older museum labels given in brackets [ ] in the material examined section of the species description. All measurements were taken with a Leica MZ16.5 stereomicroscope equipped with a 10X ocular and ocular micrometer scale. Measurements were taken from the left appendage, usually in retrolateral view, using the highest magnification possible. Leg article measurements were taken as described in Bond and Platnick (2007). Illustrations were prepared using a Visionary Digital Imaging System (Ashland, VA). Photographs were recorded in multiple focal planes and assembled using the Zerene Stacker software package (Zerene Systems LLC, Richland, WA). Carapace and leg coloration are described using Munsell Soil Color Charts (Windsor, NY) and are given using the color name and hue value/chroma notation.

Species descriptions are formatted similarly to Bond (2004) and Bond and Platnick (2007) for consistency and comparative purposes. *Institutional abbreviations*: AMNH – American Museum of Natural History; CAU and AUMNH – Auburn University Museum of Natural History; CDF – personal collection of D. Folkerts, deposited in AUMNH; FMNH – Field Museum of Natural History. *Morphological abbreviations*: Cl/w (carapace length/width), STRl/w (sternum length/width), LBl/w (labium length/width), A/PER (anterior/posterior eye row), A/PME (anterior/posterior median eye), TSp/r and TSrd (male tibia I spines prolateral/retrolateral and retrolateral distal), PTw/l (male palpal tibia width/length), Bl (palpal bulb length), PT/TB3s (female patella/tibia III spines). Leg I article measurements are in the following order: femur, tibia, metatarsus, and tarsus; leg IV measurements are femur and tarsus only.

Protocols for sample, tissue, DNA extraction, polymerase chain reaction (PCR), and sequencing follow those outlined in Hendrixson and Bond (2007) and Bailey et al. (2010). Using those procedures we amplified for five exemplar specimens the 12S/16S mitochondrial DNA region using the PCR primers LR-J-12887 and SR-N-14612 (Simon et al. 1994). This mtDNA region was used by Bailey et al. (2010) in combination with a nuclear coding protein gene that showed a largely congruent phylogenetic pattern. Paratype specimens AUMS077, 089, 119 comprised a male and two female specimens collected from the type locality; AUMS095 is from a locality in the region, Tuskegee State Park. Specimen AUMS078 is a putative *M. torreya* Gertsch & Wallace, 1936 juvenile specimen from the proximity of the *M. tigris* type locality (outskirts of Auburn, AL, Lee County). The resulting fragment was sequenced using the PCR amplification primers plus an additional internal primer, SR-N-13xxxa (Bond and Stockman 2008). The fragments were assembled and edited using the computer program Geneious Pro v5.5.4 (Auckland, New Zealand). The final edited sequences (Genbank accession numbers JQ708211- JQ708215) were added to the existing *Myrmekiaphila* 12S/16S data matrix (Treebase accession S10740) and aligned to the existing alignment using the pairwise alignment tool in Mesquite (Maddison and Maddison 2009). Data partitions and model choice for Bayesian phylogenetic inference is outlined in Bailey et al. (2010).

Tree searches on the partitioned data set using Maximum Likelihood and Bayesian inference were conducted using RAxML ver. 7.2.8 (Stamatakis 2006) and MrBayes ver 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), respectively. RAxML analyses comprised 1,000 random sequence addition replicates (RAS) using the commands "-# 1000" and "–m GTRGAMMA". Bootstrap support values were calculated using the same search parameters with 1,000 replicates. Results from the bootstrap analysis were then applied to the best tree recovered from the RAS search. The MrBayes tree search comprised two independent runs of four simultaneous Markov Chain Monte Carlo (MCMC) chains. The Bayesian analyses were run for 20,000,000 generations with trees sampled every 1,000 generations; the first 25% were discarded as *burn-in*. Likelihood values for all post-analysis trees and parameters were evaluated for convergence and *burn-in* using the "sump" command in MrBayes and the computer program Tracer ver. 1.5 (Rambaut and Drummond; http://evolve.zoo.ox.ac.uk/software.html?id=tracer). Trees remaining after *burn-in* were used to calculate posterior probabilities using the "sumt" command.

Data resources

Phylogenetic data sets (NEXUS and PHYLIP format) for all specimens evaluated in this study; locality data for *Myrmekiaphila tigris*.

The data underpinning the analysis reported in this paper were deposited on 25 April 2012 in the Dryad Data Repository at doi: 10.5061/dryad.pk24v (Bond et al. 2012) and at GBIF, the Global Biodiversity Information Facility, http://ipt.pensoft.net/ipt/resource.do?r=myr_dataset.

Results and discussion

The –ln likelihood value for the best scoring tree recovered from the RAxML analysis was -11,248.44. The harmonic and arithmetic means of the log likelihood values for all post *burn-in* topologies following MrBayes analyses were -11,307.70 and -11,364.56, respectively. Figure 2 summarizes the results of the phylogenetic analysis. The trees inferred from the both the Bayesian and likelihood analyses were largely congruent with respect to tree topology; bootstrap support values were less than posterior probabilities at some nodes.

The inferred phylogeny clearly indicates the hypothesized new species is not closely related to *M. foliata* and is placed within the somewhat enigmatic *torreya* species group (sensu Bailey et al. 2010). Although its exact placement within the group as sister to a clade that includes both taxa with unbranched (*M. coreyi*) and branched (*M. torreya*, sensu lato) emboli lacks strong nodal support, its placement among taxa with divergent morphologies is strongly supported and unequivocal with respect to these data. Given the distinctiveness of the *M. tigris* palpal bulb (Fig. 1), it would be impossible to confuse the new species with other members of the *torreya* clade. And, these specimens clearly do not share common ancestry with *M. foliata*, thus do not represent a distinct

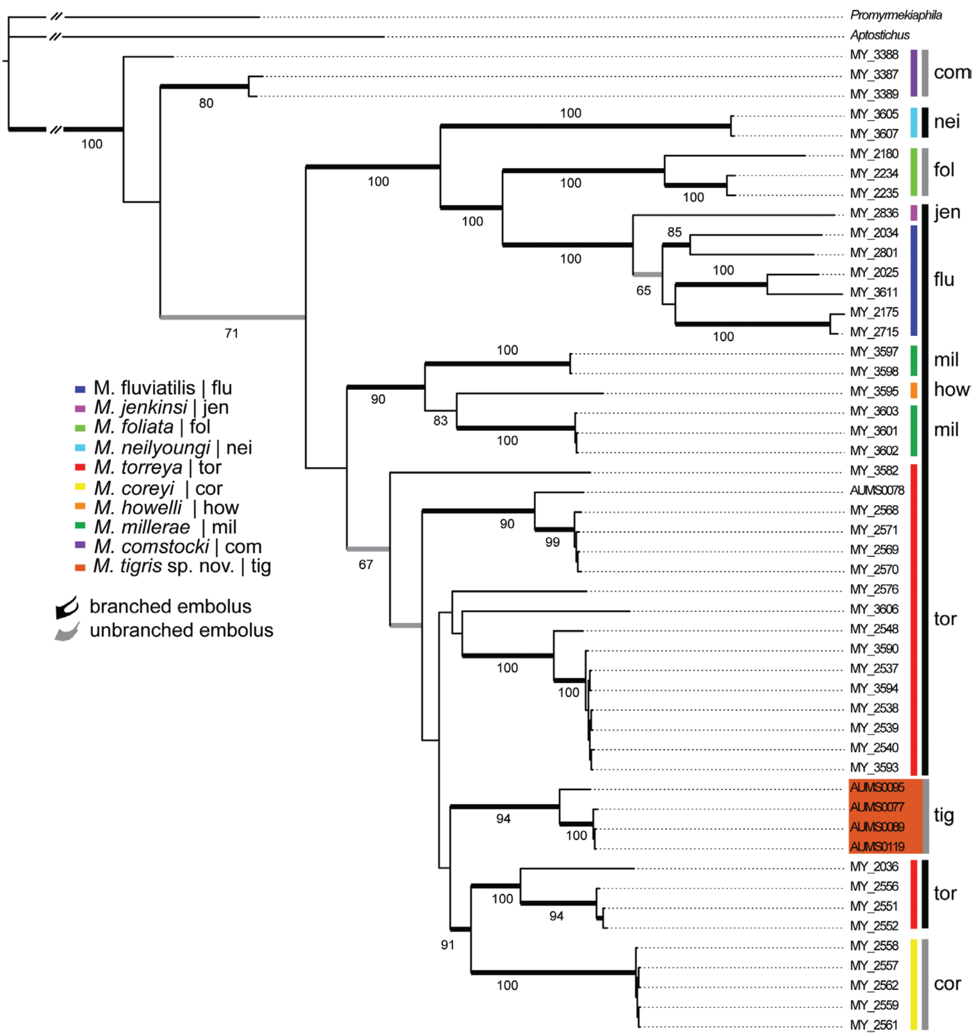


Figure 2. Preferred tree topology based on Bayesian analysis of the 12S/16S mitochondrial DNA data set. Color key and three-letter identifiers (inset) refer to species defined by Bond and Platnick 2007 along with the new species, *M. tigris*, described in this study and palpal bulb condition (one-pronged vs. two-pronged). Thickened black branches indicated posterior probabilities > 0.95; gray branches denote values of 0.90–0.95. Numbers at nodes are bootstrap percentages from the maximum likelihood analysis conducted in RAxML.

geographic variant of that species. Based on these molecular data coupled with the distinctive, easily diagnosable morphology (Fig. 1), the recognition of a new species is clearly warranted. As discussed in the Taxonomy section below, specimens incorrectly identified as *M. foliata* by Bond and Platnick (2007) are in need of reexamination.

The discovery of a second species with an unbranched embolus phylogenetically “embedded” within the *torreya* species group further complicates the systematics of the genus and generates more questions regarding species delimitation and the nature of the evolution of these somewhat enigmatic palpal bulb modifications. As discussed by Bailey et al. (2010), *M. torreya* remains a paraphyletic species with respect to *M. coreyi*, and now *M. tigris*. Solutions to resolving the conflict between the gene tree and the taxonomy of this species group include recognizing all "basal" lineages as species (speciation by remote control sensu Templeton 1998) or collapsing the entire clade into a single species. Given the degree of morphological divergence contained within the lineage (sensu lato), considering the entire clade as one species is not sufficient and would overlook significant diversity. Alternatively, recognizing all of the lineages within the *torreya* group as species seems premature. First, as hypothesized by Bailey et al. (2010) *M. torreya* paraphyly may simply reflect the rate at which genitalic change has occurred within the group as a consequence of sexual selection by female choice or sexual conflict thereby resulting in incomplete lineage sorting in the mtDNA data. The phylogeographic pattern reported here also fits with the hypothesis discussed by Bailey et al. (2010) that branched and unbranched palpal bulb conditions always occur where congeners are sympatric. Indeed, the phylogenetic position of specimen AUMS0078 (Auburn, AL, putative *M. torreya* specimen) seems to suggest that *M. torreya* and *M. tigris* may be sympatric. However, the full extent and potential overlap of both species has not yet been thoroughly investigated. Nevertheless it does seem plausible that genitalic evolution by selection and/or as a consequence of classical character displacement could be outpacing the rate of molecular divergence. However, that hypothesis may continue to breakdown as specimens are more closely examined in light of the molecular data. The discovery of a new species coupled with the phylogenetic hypothesis further magnifies the complexities contained with the genus and may indicate that the lineages contained within the torreya clade be considered as five species. As such it seems clear that considerable work remains in terms of sampling more extensively (i.e. geographical, morphological, and molecular data); we simply lack sufficient data to properly delineate all of the species at this time. Consequently, considerable work remains if we are to gain an understanding of species boundaries and genitalic evolution within this interesting group of trapdoor spiders.

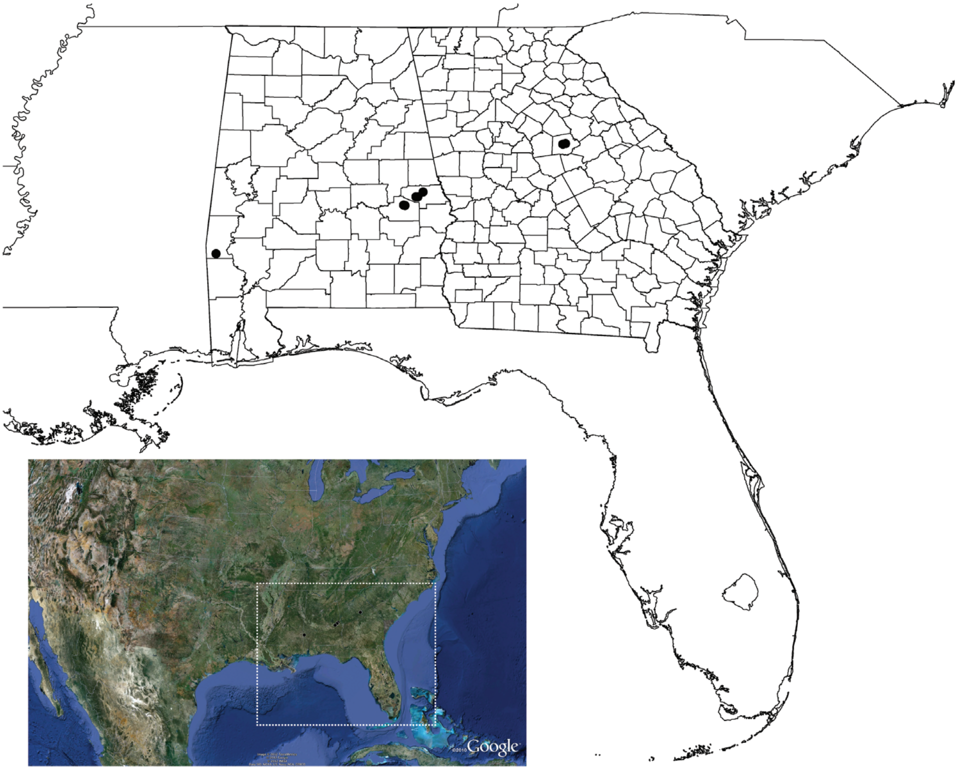
Taxonomy

Myrmekiaphila tigris Bond & Ray, sp. n.

urn:lsid:zoobank.org:act:1879EB33-3133-4A1A-96E5-5C2176A1AFB7
http://species-id.net/wiki/Myrmekiaphila_tigris
Common name: The Auburn Tiger Trapdoor Spider
Map 1; Figs 3–11

Myrmekiaphila foliata Atkinson, 1886 (misidentification): Bond and Platnick, 2007: 9–10.

Type material. Male holotype (AUMS090), female paratype (AUMS089, from Alabama, Lee County, Auburn, along Grove Hill Road, forested area across from intersec-



Map 1. Geographic distribution of *Myrmekiaphila tigris*. County outlines for Alabama and Georgia are shown. Lower color inset, dotted line, shows extent of distribution map.

tion with High Point Drive, 32.5786, -85.4543, 180m, coll. J. Bond 25.i.2012; additional male paratypes (AUMS077, 081–084) from same vicinity, coll. C. Ray i.2012. Male holotype and female paratype deposited in AUMNH; additional male paratypes deposited in AUMNH, AMNH and FMNH.

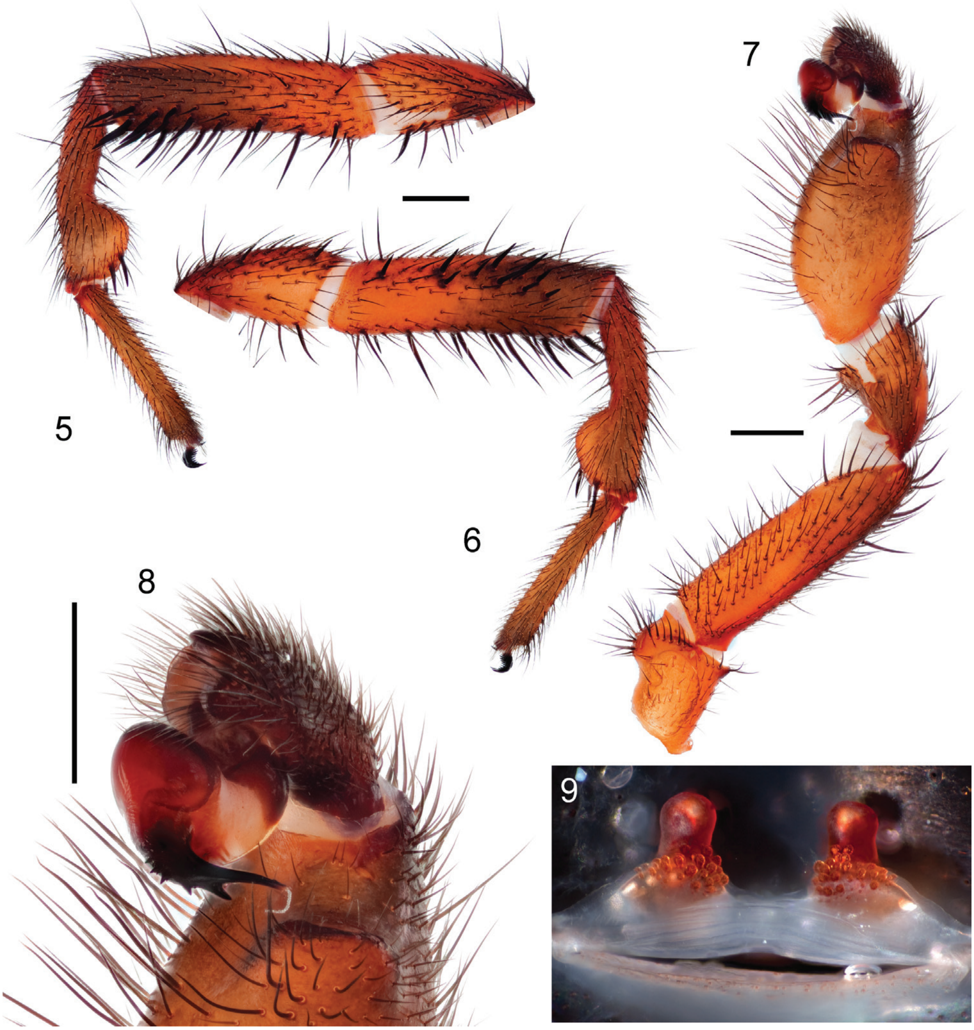
Additional material examined. ALABAMA: Choctaw Co.: Silas [31.7654, -88.3290, MYR013], 19.ii.1912 (H. Smith, CUC), 1♂. **Lee Co.:** Opelika [32.6454. -85.3783, MYR133], 1.i.1985 (D. Folkerts, CDF), 1♂; 3.2km S Auburn along Wire Road [32.5776, -85.5246, MYR135], 24.ii.1974 (R. Skinner, CAU), 1♂; Auburn [32.6099, -85.4808, MYR124], (AMNH), 2♀, 1 juv., [MYR127], (N. Banks, C. Baker, MCZ), 1♀, [MYR132], 10.iv.1941 (AMNH), 3♀, [MYR137], 3.iii.1968 (W. Ivey, CAU), 1♂, [MYR139], 10.v.1975 (B. Muse, CAU), 1♀, [MYR288], 1.vi.1986 (G. Mullen, CAU), 1♀; Auburn, Grove Hill subdivision, 32.5786, -85.4543 (AUMS086–088, 091, 096–115, 117–119, 121, 123–127, 130–138), 180m, xii.2011-i.2012 (C. Ray, D. Held, J. Bond, N. Garrison, AUMNH), 41♂ 2♀, 5 juv. **Macon Co.:** Tuskegee National Forest, Wire Road, S Interstate 85 [32.4577, –85.6576, MYR138], 12.xii.1975 (Weatherby, Brooks, CAU), 1♀; Tuskegee National Forest, 32.4522, -85.6378 [AUMS094–095], 30.i.2012 (C. Hamilton, AUMNH),

Figures 3, 4. *Myrmekiaphila tigris* sp. n. male holotype specimen in life. **3** oblique view **4** dorsal view. Scale bar = 5mm.

2 juv. **Montgomery Co.:** McGus Station [MYR140], 24.x.1915 (H. Smith, CUC), 1♂. **GEORGIA: Putnam Co.:** no specific locality [33.3335, -83.3499, MYR040], 23.iv.1974 (W. Merrill, FSCA) 1♂; Eatonton [33.3268, -83.3885, MYR131], 18.iv.1974 (W. Merrill, FSCA), 1♂.

Etymology. The specific epithet, the Latin name for tiger, is a noun taken in apposition and refers to the mascot of Auburn University.

Diagnosis. Male palpal bulb morphology (Figs 7, 8) is similar to *M. foliata* (Fig. 1, inset) but *M. tigris* specimens have a longer sinuous embolus with an elongate sub distal tooth. Potentially sympatric *M. torreya* males have two-pronged bulb whereas *M.*



Figures 5–9. *Myrmekiaphila tigris* sp. n. male holotype and female paratype. **5, 6** 1st walking leg of male, left side retrolateral and prolateral view **7** pedipalp, retrolateral view **8** palpal bulb **9** cleared spermathecae. Scale bars = 1mm.

tigris males have only a single prong. Males also appear to have a more robust palpal tibia with a larger retro-distal lateral ledge than in other species (Fig. 7). Females are much more difficult to definitively recognize from other species on the basis of morphological differences, however, the spermathecal base is considerably less wide than noted for *M. torreya* (Bond and Platnick 2007) and central bulb has a more elongate stalk (Fig. 9). Also, male and female *M. tigris* specimens tend to be larger in size than recorded for closely related *M. coreyi*: *M. tigris* Cl male > 6.50, female 7.36; *M. coreyi* Cl male < 4.50, female < 6.00. Specimens are phylogenetically distinct as a monophyl-

Figures 10, 11. *Myrmekiaphila tigris* sp. n. female paratype specimen in life. **10** oblique view **11** dorsal view. Scale bar = 5mm.

etic lineage exclusive of *M. torreya, M. coreyi*, and *M. foliata* (Fig. 2). Known only from central Alabama and Georgia.

Description of male holotype. *Specimen preparation and condition*. Specimen collected live from burrow, preserved in 80%. Pedipalp, leg I left side removed, stored in vial with specimen. *General coloration*. Carapace dark red 2.5YR 3/6; legs, chelicerae darker in color, dusky red 10R 3/4. Abdomen dark brown 7.5YR 3/2 with broad faint dusky stripes posteriorly dorsal (Figs 3, 4), ventrum spinnerets pale yellow. *Cephalothorax*. Carapace 6.56 long, 5.63 wide, hirsute with thin black short setae, stout black bristles along fringe; surface smooth, pars cephalica elevated. Fringe, posterior margin with black bristles. Foveal groove deep, straight. Eyes only slight elevated. AER slightly procurved, PER slightly recurved. PME slightly larger in diameter than AME. Sternum moderately setose, STRl 3.72, STRw 3.35. Posterior sternal sigilla large, irregularly shaped, nearly contiguous, anterior sigilla pairs small, oval, marginal. Chelicerae with distinct anterior tooth row comprising 11 teeth, posterior margin with single row small denticles. Palpal endites with patch of small cuspules on proximal, inner margin, labium lacks cuspules, LBw 1.19, LBl 0.70. Rastellum consists of 4 stout spines on distinct mound. *Abdomen*. Setose, heavy black setae intermingled with fine black setae. *Legs*. Leg I: 5.85, 2.88, 4.25, 3.41, 2.84; leg IV: 6.00, 3.25. Light scopulae on tarsi, metatarsi legs I, II. Tarsus I with single, slightly staggered row of 9 trichobothria. Leg I spination pattern illustrated in Figures 4, 5; TSp 12, TSr 10, TSrd 2. *Pedipalp*. Articles stout, lacking distinct spines (figs 6, 7). PTw 1.50, PTl 3.00, Bl 1.24. Distinct, elongate ledge on distal-retrolateral aspect tibia. Embolus stout, tapering sharply towards tip, with serrations, elongate distal tooth (Fig. 8).

Variation (8). Cl 6.81–10.30, 7.56±0.41; Cw 5.40–8.08, 6.08±0.30; STRl 3.80–5.25, 4.14±0.17; STRw 3.38–4.85, 3.71±0.17; LBw 1.02–1.57, 1.21±0.07; LBl 0.56–0.70, 0.64±0.02; leg I: 5.35–8.32, 6.29±0.31; 4.60–5.75, 4.73±0.16; 3.25–4.85, 3.75±0.18; 2.88–4.20, 3.14±0.16; leg IV: 5.75–8.88, 6.52±0.35; 3.09–4.45, 3.48±0.16; PTl 3.00–4.32, 3.27±0.15; PTw 1.56–2.13, 1.68±0.07; Bl 1.20–1.60, 1.30±0.04; TSp 12–21, 15.25±1.01; TSr 10–14, 11.75±0.41; TSrd 1–2, 1.88±0.13.

Description of female paratype. *Specimen preparation and condition*. Female collected live from burrow, preserved in same manner as male holotype. Genital plate removed, cleared in trypsin, stored in microvial with specimen. *Color*. Carapace dark reddish gray 2.5YR 3/1; legs, chelicerae, dark reddish brown 2.5YR 3/4. Abdomen reddish black dorsally 2.5YR 2.5/1 faint dusky bands dorsally; ventrum, spinnerets pale yellow (figs 10, 11). *Cephalothorax*. Carapace 7.36 long, 6.38 wide, generally glabrous, few thin setae, pars cephalica elevated. Fringe lacks setae. Foveal groove deep, slightly procurved. Eye group slightly elevated on very low mound. AER slightly procurved, PER slightly recurved. PME-AME subequal in diameter. Sternum widest at coxae II/III, moderately setose, STRl 4.60, STRw 4.08. Three pairs of sternal sigilla anterior pairs moderate size, oval, positioned marginally, posterior pair larger, irregularly shaped, nearly contiguous. Chelicerae anterior tooth row armed with 10 teeth with single posterior margin denticle row. Palpal endites with 47 cuspules concentrated at the inner promargin posterior heel; labium with 5 cuspules, LBw 1.46, LBl 0.97. Rastellum consist of 10 very stout spines positioned on distinct mound. *Abdomen*. Moderately setose, posterior median spinnerets reduced in size. *Walking legs*. Anterior two pairs noticeably more slender than posterior pairs. Leg I 16.39 long. Tarsus I with single staggered row of 10 trichobothria. Legs I, II with moderately heavy scopulae on tarsi, metatarsi. PT3s 14, TB3s 9. Rudimentary preening comb on retrolateral distal surface, tarsus - metatarsus joint metatarsus III, IV. *Spermathecae*. Two simple spermathecal bulbs, moderately elongate neck, arranged on low subtriangular base (fig. 9).

Variation. Females known only from three specimens.

Distribution and natural history. Known from central Alabama, counties of Choctaw, Lee, Macon, and Montgomery, and the piedmont region of Georgia, Putnam County (Map 1). The type locality comprises primarily young second growth mixed deciduous forest located at the transition from the Piedmont to Coastal Plan physiographic region. The population known from the additional material in western Alabama is located in Coastal Plain Province. Specimens were found to be presumably syntopic with *Cyclocosmia, Antrodiaetus, Ummidia*, and possibly *M. torreya* (collected from the region). Male specimens were collected from swimming pools and wandering on warm, damp mornings during the months of December and January. Females were collected from 6–8 cm deep burrow, some with below-ground side chambers with a trapdoor.

Genbank accession numbers. JQ708212–JQ708215

Acknowledgements

This project was supported by NSF grant DEB 0315160 and Auburn University. We are grateful to Dr. David Held for access to his property and specimens from his swimming pool. Rebecca Godwin assisted in taking measurements. Les Goertzen suggested the specific epithet tigris.

References

Atkinson GF (1886) Descriptions of some new trap-door spiders; their nests and food habits. Entomologica Americana 2: 109–137. doi: 10.1371/journal.pone.0012744

Bailey AL, Brewer MS, Hendrixson BE, Bond JE (2010) Phylogeny and classification of the trapdoor spider genus Myrmekiaphila: an integrative approach to evaluating taxonomic hypotheses. PLoS ONE 5(9): 1–15.

Bond JE (2004) Systematics of the Californian euctenizine spider genus Apomastus (Araneae: Mygalomorphae: Cyrtaucheniidae): the relationship between molecular and morphological taxonomy. Invertebrate Systematics 18: 361–376. doi: 10.1071/IS04008

Bond JE, Platnick NI (2007) A taxonomic review of the trapdoor spider genus *Myrmekiaphila* (Araneae, Mygalomorphae, Cyrtaucheniidae). American Museum Novitates 3596: 1–30. doi: 10.1206/0003-0082(2007)3596[1:ATROTT]2.0.CO;2

Bond JE, Hedin MC (2006) A total evidence assessment of the phylogeny of the diverse North American trapdoor spider subfamily Euctenizinae (Araneae, Mygalomorphae, Cyrtaucheniidae). Molecular Phylogenetics and Evolution 41: 70–85. doi: 10.1016/j.ympev.2006.04.026

Bond JE, Stockman AK (2008) An Integrative Method for Delimiting Cohesion Species: Finding the Population-Species Interface in a Group of Californian Trapdoor Spiders with Extreme Genetic Divergence and Geographic Structuring. Systematic Biology 57: 628–646. doi: 10.1080/10635150802302443

Funk D, Omland K (2003) Species-level paraphyly and polyphyly: Frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology, Evolution and Systematics 34: 397–423. doi: 10.1146/annurev.ecolsys.34.011802.132421

Gertsch WJ, Wallace HK (1936) Notes on new and rare American mygalomorph spiders. American Museum Novitates 884: 1–25.

Hedin M, Bond JE (2006) Molecular phylogenetics of the spider infraorder Mygalomorphae using nuclear rRNA genes (18S and 28S): Conflict and agreement with the current system of classification. Molecular Phylogenetics and Evolution 41: 454–471. doi: 10.1016/j.ympev.2006.05.017

Hendrixson BE, Bond JE (2007) Molecular phylogeny and biogeography of an ancient Holarctic lineage of mygalomorph spiders (Araneae: Antrodiaetidae: Antrodiaetus). Molecular Phylogenetics and Evolution 42: 738–755. doi: 10.1016/j.ympev.2006.09.010

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. doi: 10.1093/bioinformatics/17.8.754

Maddison WP, Maddison DR (2009) Mesquite: a modular system for evolutionary analysis. Version 2.7 ed. doi: 10.1093/bioinformatics/btg180

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Paul F (1994) Evolution, Weighting, and Phylogenetic Utility of Mitochondrial Gene-Sequences and a Compilation of Conserved Polymerase Chain-Reaction Primers. Annals of the Entomological Society of America 87: 651–701.

Stamatakis A (2006) RAxML-VI-HPC: Maximum Likelihood-based Phylogenetic Analyses with Thousands of Taxa and Mixed Models. Bioinformatics 22(21): 2688–2690. doi: 10.1093/bioinformatics/btl446

Stockman AK, Bond JE (2008) A taxonomic review of the trapdoor spider genus Promyrmekiaphila Schenkel (Araneae, Mygalomorphae, Cyrtaucheniidae, Euctenizinae). Zootaxa 1823: 25–41.

Templeton AR (1998) Species and speciation: geography, population structure, ecology, and gene trees. In: Howard DJ, Berlocher SH (Eds) Endless Forms: Species and Speciation. Oxford University Press, New York, 32–43.

Appendix I

Locality data for *Myrmekiaphila tigris*.

Explanation note: Locality data (CSV format) for *Myrmekiaphila tigris* specimens (doi: 10.3897/zookeys.190.3011.app1). File format: Microsoft Excel comma delimited.

Citation: Bond JE, Hamilton CA, Garrison NL, Ray CH (2012) Phylogenetic reconsideration of *Myrmekiaphila* systematics with a description of the new trapdoor spider species *Myrmekiaphila tigris* (Araneae, Mygalomorphae, Cyrtaucheniidae, Euctenizinae) from Auburn, Alabama. ZooKeys 190: 95–109. doi: 10.3897/zookeys.190.3011.app1