RESEARCH ARTICLE

The family Plectopylidae (Gastropoda, Pulmonata) in Laos with the description of two new genera and a new species

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Abstract

Previously only a single plectopylid species, *Helix laomontana* L. Pfeiffer, 1862 was reported from Laos. Here we erect *Naggsia* Páll-Gergely & Muratov, **gen. n.** for *H. laomontana* based on the description of its reproductive anatomy and radula. Another species, *Hunyadiscus saurini* Páll-Gergely, **gen. & sp. n.** is described from Northern Laos based on conchological data. *Helix* (*Plectopylis*) *andersoni* Blanford, 1869, which is known from the Burmese-Chinese border region, is also classified within *Hunyadiscus* Páll-Gergely, **gen. n.** A third species, *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* Páll-Gergely & Hunyadi, 2015 is reported from Laos for the first time. The new localities represent the westernmost sites of the genus *Gudeodiscus*. The reproductive anatomy of the latter species is described.

Keywords

Taxonomy, anatomy, radula, protoconch, new genus, new species

Introduction

Land snails of the family Plectopylidae are widely distributed in East Asia, from Nepal to southern Japan (Gude 1899b, Páll-Gergely and Hunyadi 2013, Páll-Gergely et al. 2015b). The taxonomic system of the Plectopylidae was established by Gude (1899a),

who recognized seven sections (*Endothyra* Gude, 1899a = *Endothyrella* Zilch, 1960, *Chersaecia* Gude, 1899a, *Endoplon* Gude, 1899a, *Plectopylis* Benson, 1860, *Sinicola* Gude, 1899a, *Enteroplax* Gude, 1899a and *Sykesia* Gude, 1897b). Two of these (*Enteroplax* and *Sykesia* = *Ruthvenia* Gude, 1911) are now classified in different families (Páll-Gergely and Hunyadi 2013). Recent investigation resulted in the redefinition of Gude's (1899a) genera as well as the description of three new genera (*Gudeodiscus* Páll-Gergely, 2013, *Halongella* Páll-Gergely, 2015 and *Sicradiscus* Páll-Gergely, 2013). With these, plectopylids are now classified in eight genera (Páll-Gergely and Hunyadi 2013, Páll-Gergely et al. 2015a, 2015b).

The single species reported from Laos to date was originally described as *Helix laomontana* L. Pfeiffer, 1862 (type locality: "Lao Mountains, Camboja") and was classified in *Chersaecia* by Gude (1899a). Revision of *Endothyrella* showed that *Chersaecia* had been treated as a catchall category and that it contained species with very diverse conchological characters (Páll-Gergely et al. 2015b). We transferred five species from *Chersaecia* to *Endothyrella*, mainly based on the ribbed protoconch and the absence of an apertural fold. In *Chersaecia* we retained only species that were similar to the type species, *Plectopylis leiophis* Benson, 1860, in terms of the presence of an apertural fold and the finely granulated (not ribbed) embryonic whorls. *Helix laomontana*, has also been excluded from *Chersaecia*, but could not be included into any other existing genus. Live collected specimens allowed the clarification of the taxonomic position of *Helix laomontana*. Another species, namely *Helix* (*Plectopylis*) *andersoni* W. Blanford, 1869 was also excluded from *Chersaecia* (see Páll-Gergely et al. 2015b). Although no ethanol-preserved specimens were available for anatomical study, its shell characters provide sufficient information to clarify its systematic status. Additionally, we report two other species from Laos, one being new to science.

Material and methods

Shell whorls were counted according to Kerney and Cameron (1979: 13) (precision 0.25).

For the nomenclature of lamellae (vertical parietal folds) and plicae (horizontal parietal folds and palatal folds) see Páll-Gergely et al. 2015b. Whenever possible, the internal lamellae and plicae were exposed by removing the shell wall at the appropriate part of the shells (inner view). If damaging the shells was not an option (too few shells available), the plicae were figured on the basis of their visibility through the shell wall (outer view). "Anterior" refers to the part or side of the armature closer to the aperture, "posterior" refers to the other side of the armature.

Ethanol-preserved specimens were dissected under a Leica stereomicroscope, equipped with a photographic camera. In the descriptions of the reproductive system, we used the terms "proximal" and "distal" in relation to the apical portion of the reproductive tract *i.e.* the ovotestis.

Individual buccal masses were removed and soaked in 2 M KOH solution for 5 hours before extracting the radula, which was later preserved in 70% ethanol. Radulae and shells were directly observed without coating under a low vacuum SEM (Miniscope TM-1000, Hitachi High-Technologies, Tokyo).

Abbreviations

HNHM   Hungarian Natural History Museum (Budapest, Hungary)
MNHN   Muséum National d'Histoire Naturelle (Paris, France)
NHM   Natural History Museum (London, UK)
NHMUK   when citing registered specimens from the Natural History Museum (London, UK)
NHMW   Naturhistorisches Museum (Vienna, Austria)
OK   Collection Kenji Ohara, Nishinomiya Shell Museum (Nishinomiya, Japan)
PGB   Collection Barna Páll-Gergely (Mosonmagyaróvár, Hungary)
RBINS   Royal Belgian Institute of Natural Sciences (Brussels, Belgium)
RE   Collection Reischütz (Horn, Austria)
SMF   Senckenberg Forschungsinstitut und Naturmuseum (Frankfurt am Main, Germany)
WM   Collection Wim J. M. Maassen (Echt, The Netherlands)
ZMH   Zoological Museum, Hamburg (Germany)

Taxonomic descriptions

Family Plectopylidae Möllendorff, 1898

Gudeodiscus Páll-Gergely, 2013

2013 *Gudeodiscus* Páll-Gergely, In: Páll-Gergely and Hunyadi, Archiv für Molluskenkunde 142(1): 4, 8.

Type species. *Plectopylis phlyaria* Mabille, 1887, by original designation.
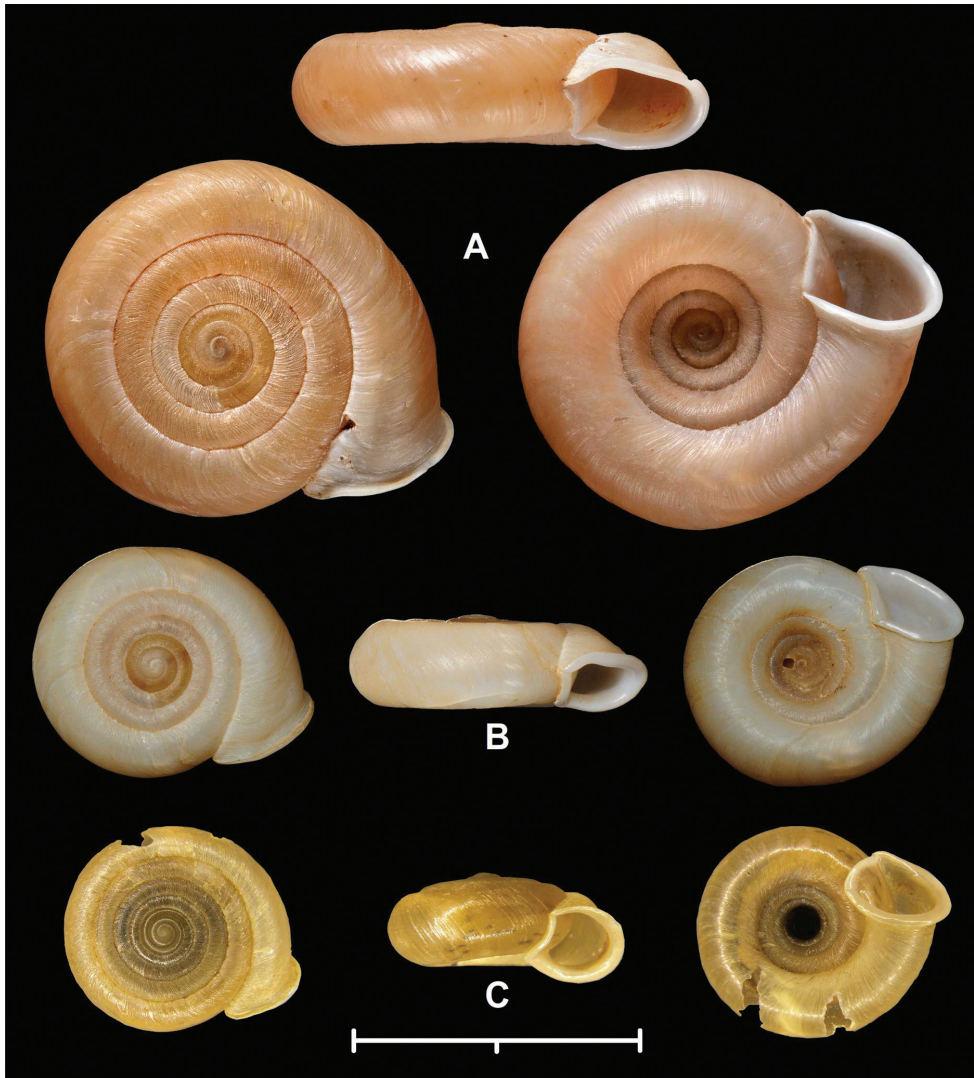
Subgenus _Gudeodiscus_

2015 *Gudeodiscus* (*Gudeodiscus*), — Páll-Gergely et al., ZooKeys 473: 13.

Gudeodiscus (_Gudeodiscus_) _messageri raheemi_ Páll-Gergely & Hunyadi, 2015
Figures 1C, 2B, 5G–H, 6, 9D–G

2015a *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi*, — Páll-Gergely et al., ZooKeys 473: 38–40, Figures 5D, 5E, 10A, 12R–V, 20, 28E, 29F–G, 31B, 35D–F.

Material examined. 11L06 Laos, Luang Prabang Province, ca. 18 km SE of Muang Xiang Ngeun, on the left side of Nam Khan, limestone, black soil in limestone pockets, clay, under rocks and logs in old forest, 455 m a.s.l., 19°40.931'N, 102°19.743'E, leg. A.

Figure 1. Shells of Plectopylidae species. **A** *Naggsia laomontana* (L. Pfeiffer, 1862) (syntype, NHMUK 20130004) **B** *N. laomontana* (Laos, Luang Prabang Province, Ban Pak Ou, Nam Wu (opposite side of Ban Pak Ou), 364 m 20°03.48276'N, 102°12.79912'E, leg. Ohara, K. 13.10.2006., coll. PGB) **C** *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* Páll-Gergely & Hunyadi, 2015, locality code: 11L06. Photos: T. Deli (**B**), B. Páll-Gergely (**C**) and H. Taylor (**A**). Scale represents 20 mm.

Abdou & I.V. Muratov, 30.10.2006, MNHN 2012-27054/38 shells (some of them are broken/juvenile) + anatomically examined specimen (Figs 1C, 2B, 5G–H, 6, 9D–G); **12L06** Laos, Luang Prabang Province, ca. 17 km SE of Muang Xiang Ngeun, on the left side of Nam Khan, limestone, black soil in limestone pockets, clay, under rocks and logs in old forest, 385 m a.s.l., 19°41.201'N, 102°19.197'E, leg. A. Abdou & I.V. Muratov, 30.10.2006, MNHN 2012-27055/21 shells (some of them are broken/juvenile); **34L06** Laos, Luang Prabang Province, ca. 6 km N of Phou Khoun, limestone, clay,
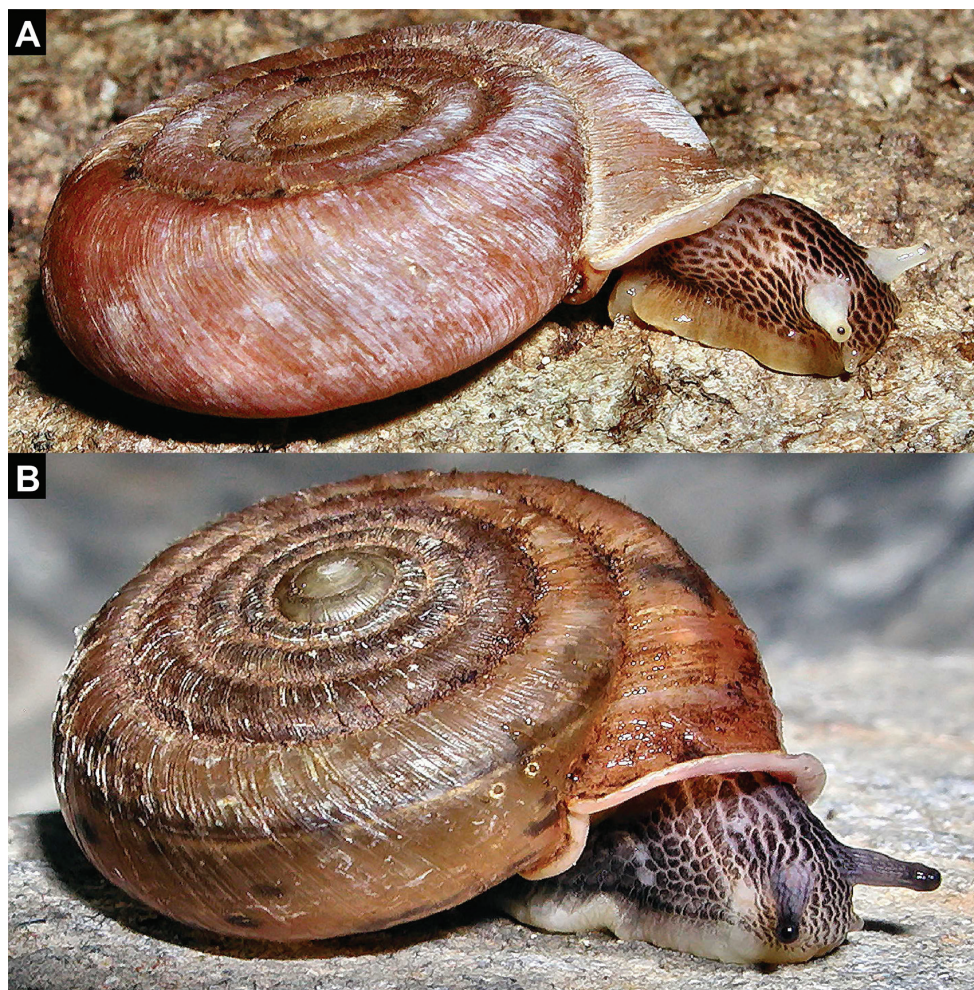
Figure 2. Living animals of Laotian Plectopylidae (anatomically examined specimens). A: *Naggsia laomontana* (L. Pfeiffer, 1862) **B** *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* Páll-Gergely & Hunyadi, 2015. Photos: Igor Muratov.

under rocks in dry secondary forest under and above cliff, 1244 m a.s.l., 19°29.653'N, 102°24.470'E, leg. A. Abdou & I.V. Muratov, 16.11.2006, MNHN 2012-27056/11 shells (some of them are broken/juvenile); Laos, Vientiane Province, Tam Chang, Vang Vieng, leg. Pongrat Dumrongrojwattana, 11.06.2009., PGB/1, WM/1.

Reproductive anatomy. A single living specimen collected in Laos, was killed by drowning and was stored in 70 % ethanol. A part of the body was extracted from the shell and was examined anatomically. The inner parts of the genitalia, such as the gametolytic sac, the diverticulum and the spermoviduct could not be extracted.

The reproductive anatomy of *Gudeodiscus messageri raheemi* was figured in the original description (Páll-Gergely et al. 2015a). The two anatomically examined Vietnamese specimens differed from each other mainly in the length of the penial

caecum. The Laotian specimen possesses a short caecum, similar to one of the Vietnamese specimens. The length of the caecum probably has minor taxonomic value because it varies considerably within species (see also *G. phlyarius* in Páll-Gergely et al. 2015a). The inner wall of the epiphallus of the Vietnamese specimens had three, rather low, longitudinal folds. In contrast, the Laotian specimen had a single thickened fold internally.

Remarks. *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* was described from Vietnam, where it inhabits the provinces Ninh Bình, Thanh Hóa, Sơn La, Hòa Bình and Nghệ An. The Laotian specimens agreed in shell morphology with the Vietnamese specimens. The new Laotian localities of *Gudeodiscus messageri raheemi* represent the westernmost record of the genus (Figure 14).

Ecology. This taxon inhabits primary or old secondary broad-leaved forests, in the humid microenvironments under leaves, logs, limestone rocks and in black soil accumulated inside limestone pockets. The live collected specimen co-occured with *Garnieria mouhoti* (L. Pfeiffer, 1862), a well-known species that is also associated with the moderate humidity of broad-leaved forests (Figure 12).

Hunyadiscus Páll-Gergely, gen. n.
http://zoobank.org/AD8518E3-C82B-4E66-A13D-BBB7F90EFC59

Type species. *Hunyadiscus saurini* sp. n.

Content. *andersoni* Blanford, 1869, *saurini* Páll-Gergely, sp. n.

Diagnosis. Shell dextral, body whorl keeled or angulated; protoconch with spiral and radial lines, with the spirals being dominant; palatal plicae slightly sinuate; parietal wall with a single lamella and some additional plicae/denticles anteriorly and/or posteriorly. Internal anatomy unknown. See also Remarks.

Differential diagnosis. *Hunyadiscus* gen. n. differs from all other plectopylid genera by the protoconch sculpture, which is characterized by both spiral and radial lines, the spirals being dominant. Moreover, all species of *Gudeodiscus*, *Halongella* and *Naggsia* gen. n. have a rounded body whorl, which is keeled in *Hunyadiscus* gen. n. The most similar genus to *Hunyadiscus* gen. n. is *Sinicola*, which also possess a keeled (shouldered) body whorl, and usually lacks the apertural fold. See also Table 1.

Etymology. The genus is dedicated to András Hunyadi, Hungarian malacologist and shell collector, who first called the attention of the first author on the necessity of revising the family Plectopylidae. The name *Hunyadiscus* is the combination of the family name Hunyadi and discus (Latin: *disc*), which refers to the shape of the shells.

Distribution. One species (*H. saurini* sp. n.) inhabits southern part of Northern Laos (exact locality unknown), the other species (*H. andersoni*) lives in southern Kachin state (Myanmar), at the bordering Chinese region (Figure 15).

Remarks. Many plectopylid species belonging to the genera *Endoplon*, *Endothyrella*, *Gudeodiscus*, *Halongella*, *Plectopylis*, *Sicradiscus* possess two parietal lamellae (anterior and posterior). Other species, however, possess only a single one. In most cases it is

Table 1. Key characters of the shell and genitalia of plectopylid genera possessing ribbed embryonic whorls. One star: see Páll-Gergely et al. 2015b; two stars: see Páll-Gergely et al. 2015a.

Genus	Coiling direction	Apertural fold	Body whorl	Epiphallus	Penial pockets	Protoconch
Endothyrella	sinistral or dextral	absent	rounded or keeled	present	whole penial wall	ribbed*
Sinicola	dextral	absent (rarely present)	keeled	present	whole penial wall	ribbed
Gudeodiscus	dextral	absent or present	rounded	present	apical part	ribbed
Halongella	dextral	present	rounded	present	whole penial wall	ribbed**
Hunyadiscus gen. n.	dextral	absent	keeled	unknown	unknown	spirally striated and ribbed
Naggsia gen. n.	dextral	absent	rounded	absent	absent	ribbed, ribs are wavy, with extremely fine spiral striation
eastern *Sicradiscus*	dextral	absent	keeled	present	whole penial wall	ribbed
western *Sicradiscus*	dextral	present	rounded	present	apical part	ribbed

possible to decide that the single lamella is homologous with either the anterior or the posterior lamella, because there are "remains" of the other lamella. For example, in some *Gudeodiscus* species (e.g. *G. multispira* [Möllendorff, 1883]), there are some small denticles in position of the anterior lamella, anterior to the well-developed, curved lamella. This indicates that the curved lamella is homologous with the posterior lamella. In contrast, in the genera *Sicradiscus* and *Endothyrella*, many species have small denticles on the posterior side of the single lamella. This suggests, that the single, well developed lamella is homologous with the anterior lamella (Páll-Gergely and Asami 2016). *Hunyadiscus andersoni* also has two small denticles on the posterior side of the lamella, one above, one below. This suggests that the single lamella of *H. andersoni* is homologous with the anterior lamella. The single lamella of *Hunyadiscus saurini* sp. n. is, on the other hand, probably homologous with the posterior lamella, because it has a strongly curved shape, and has a lower plica positioned anteriorly, which is, when present, situated under the anterior lamella in *Gudeodiscus* species. These hypotheses suggest that the two species of *Hunyadiscus* have remarkably different parietal plication (Fig. 11).

Hunyadiscus andersoni (W. Blanford, 1869)
Figures 3B–C, 4A–B, 5C–D, 11F

1869 *Helix* (*Plectopylis*) *andersoni* Blanford, Proceedings of the Zoological Society of London: 448 [Bhamo in regno Avæ et Hoetone in Yunan].

1874 *Helix andersoni*, — Hanley and Theobald, Conchologia Indica…: 46, plate 112, figs 8–9 [Bhamo, and Hoetone in Yunan] (1870–1876).

1875 *Helix* (*Plectopylis*) *andersoni*, — Godwin-Austen, Proceedings of the Zoological Society of London: 612, Plate 74, fig. 9.

1885 *Plectopylis Andersoni*, — Möllendorff, Jahrbücher der Deutschen Malakozoologischen Gesellschaft, 12: 389 ["bei Bhamo in Oberbirma", "»Hoitone« in der chinesischen Provinz Yünnan"].

1886 *Plectopylis Andersoni*, — Möllendorff, Jahrbücher der Deutschen Malakozoologischen Gesellschaft, 13: 188.

1887 *Helix andersoni*, — Tryon, Manual of Conchology. 2 (3): 161, Plate 34, fig. 71; Plate 35, figs 74–75 [Bhamo, in Ava; Hoetone, In Yunan].

1889 *Helix* (*Plectopylis*) *Andersoni*, — Tapparone Canefri, Annali del Museo Civico di Storia Naturale di Genova, 2a (7): 47 (=323). ["Catcin di Pun-Can, Cimfó, Monti Est di Bhamó", "Bhamó, Hoetone"]

1896 *Plectopylis andersoni*, — Gude, Science Gossip, 3: 154, figs 17a–c [Near Bhamo and Ava, in Upper Burma and on the Yunnan-frontier].

1899a *Plectopylis* (*Chersaecia*) *andersoni*, — Gude, Science Gossip, 6: 148.

1899b *Plectopylis* (*Chersaecia*) *andersoni*, — Gude, Science Gossip, 6: 175.

1914 *Plectopylis* (*Chersaecia*) *andersoni*, — Gude, The Fauna of British India including Ceylon and Burma. Mollusca II. (Trochomorphidae – Janellidae), 2: 73, 114, figs 55a–c.

2013 *Chersaecia andersoni*, — Páll-Gergely and Hunyadi, Archiv für Molluskenkunde 142 (1): 7, figs 14–15.

Types. According to Dance (1986) the collection of Blanford is deposited in the British Museum (now: Natural History Museum, London). In the type collection of the NHM we did not find syntypes, but there is a sample (NHMUK 1906.02.02.364) which is labelled as being collected from Bhamo, one of the type localities. This sample may represent the type lot.

Museum material examined. Yünan, Slg. Bosch, ex H. Rolle (1 juv.), SMF 172066; alte Schau-Slg/2, SMF 150117; no locality information (alte Schau-Slg.), SMF 150117/2; Upper Indwadi, NHMUK 1888.12.04.1561/2; Burma, coll. A. S. Kennard, ex Gude, NHMUK/2; Bhamo, Upper Burma, NHMUK 1906.02.02.364/5; Khakhyan Hills, Burmah, coll. Godwin-Austen, NHMUK/3; Bhamo, NHMUK/1.

Diagnosis. A very large, discoid, angulated species with elevated, sharp callus and spirally striated protoconch.

Description. Shell flat, angulated, light brown or corneous; ventral side of the body whorl keeled around the moderately wide, very deep umbilicus; protoconch spirally striated, radial ribs are mostly visible on its first whorl only; teleoconch equally ornamented with fine ribbing and spiral striae, resulting in rough, irregular reticulated surface on the dorsal side; ventral side also reticulated, but much weaker than the dorsal surface; 7.5–8.5 slowly increasing whorls separated by shallow suture; near the sutures the riblets sometimes supported with fine folds of the periostracum; aperture rounded, with white, slightly expanded and thickened apertural rim; callus slightly elevated, sharp, slightly S-shaped and forms two canals upon junction with the lip.

Figure 3. Shells of Plectopylidae species. **A** holotype of *Hunyadiscus saurini* Páll-Gergely sp. n. **B** *Hunyadiscus andersoni* (W. Blanford, 1869) (NHMUK 20130003 **C** Burmah, RBINS 10591. Photos: T. Deli (**C**), J. Harl (**A**) and H. Taylor (**B**). Scales represent 10 mm; upper scale refers to Fig. **A**, lower scale refers to Figs **B** and **C**.

The parietal side was examined in one specimen (SMF 150117), whereas the palatal plicae is examined in specimens of the SMF and the NHM. Parietal wall with one curved horizontal lamella with occasionally an elongated upper plica, which is in con-
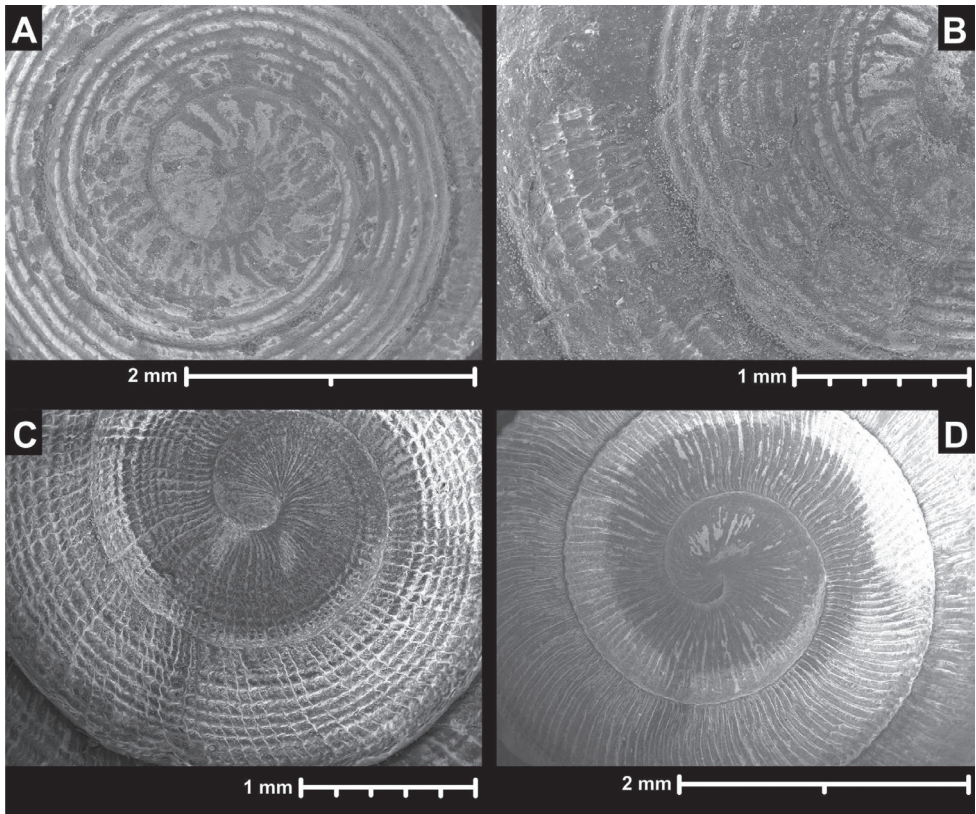
Figure 4. Embryonic whorls of Plectopylidae. **A–B** *Hunyadiscus andersoni* (W. Blanford 1869) (NHMUK 20130003, two different specimens) **C** *Hunyadiscus saurini* Páll-Gergely sp. n. **D** *Naggsia laomontana* (L. Pfeiffer, 1862), same specimen as Fig. 1B. All images by B. Páll-Gergely.

tact with the lamella; there are two small denticles on the posterior side of the lamella, these are in weak contact with the lamella; palatal side with eight horizontal plicae, first near the suture is small, the second is even smaller; the last also short and close to the lower suture and the penultimate resembles the second denticle; remaining four plicae between the first and last two are long and slim.

Measurements (in mm). D = 24.6–27.2, H = 10.9–11.4 (n = 2, RBINS I. G. 10591, Burmah).

Differential diagnosis. This species differs from large Chinese *Gudeodiscus* species (and *Naggsia laomontana*) by the keeled margin of the shells. It is much larger than all *Sinicola* species. The largest *Sinicola* species, *S. fimbriosa* (Martens, 1875) does not have a callus and has a stronger apertural margin.

Distribution. The species is known from Northern Burma and Western Yunnan. Hoetone (Hutung Village) and Bhamo are located in Kachin Provinces, whereas Ava is in Mandalay Province (all in Burma/Myanmar). The Kakhyen Hills are situated on the Chinese (Yunnan) and Burmese (Kachin) border.
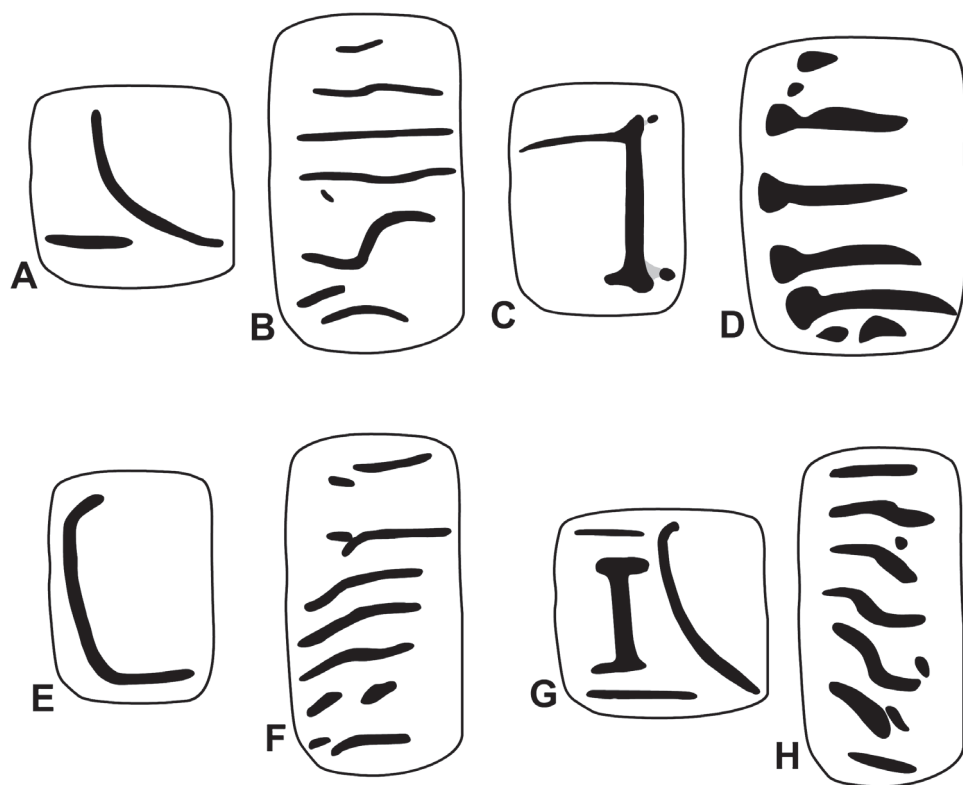
Figure 5. Parietal (**A, C, E, G**) and palatal (**B, D, F, G**) plication of Plectopylidae species. **A–B** *Hunyadiscus saurini* Páll-Gergely sp. n. **C–D** *Hunyadiscus andersoni* (W. Blanford 1869) (**C** SMF 150117 **D** after Gude 1896) **E–F** *Naggsia laomontana* (L. Pfeiffer, 1862), same sample as on Fig. 1B **G–H** *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* Páll-Gergely & Hunyadi, 2015, same specimen as on Fig. 1C. Figures not to scale. Inner view: **B, F, D**, Outer view: **H**.

Hunyadiscus saurini **Páll-Gergely, sp. n.**
http://zoobank.org/EF0B1D36-1FBB-49A5-972C-89E6BCA3FBCB
Figures 3A, 4C, 5A–B, 11A

1953 *Plectopylis laomontana*, Saurin, *Journal de Conchyliologie*, 93 (4), 113.

Type material. Laos, Pa Hia (Ancienne province Tran Ninh), Coll. Saurin, MNHN 24947 (holotype), MNHN 24948/7 paratypes + 5 juvenile shells (also paratypes), HNHM 97470/2 paratypes; Laos, Pah Xieng Tong, Pa Hia, Prov. Tran Ninh, Coll. Saurin, MNHN 249479/1 paratype + 1 juvenile shell (also paratype, protoconch figured: Fig. 4C); Laos, Pa Ka Tai, Prov. Tran Ninh, Coll. Saurin, MNHN 24950/5 paratypes, HNHM 97471/1 paratype; Laos, Pa Xieng Tong, Pa Hia, Prov. Tran Ninh, Coll. Saurin MNHN 24951/3 paratypes; Laos, Pa Hia (Tran Ninh), Coll. Saurin, MNHN 24952/1 juvenile shell (paratype).
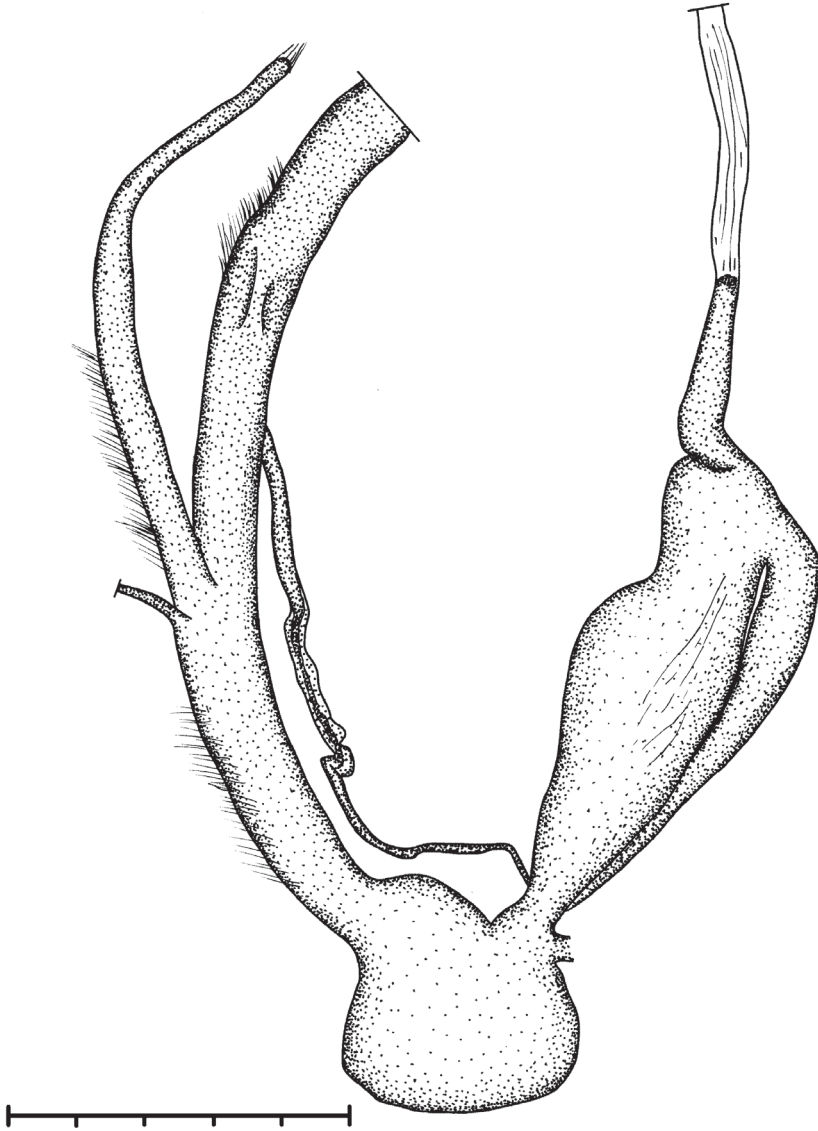
Figure 6. Reproductive anatomy of *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* Páll-Gergely & Hunyadi, 2015, same specimen as on Fig. 1C and Fig. 2B. Scale represents 5 mm.

Diagnosis. A dextral, medium-sized or large species with a relatively sharp upper keel and a blunt lower keel on the body whorl. On the parietal wall there is a single oblique lamella with a horizontal plica below it.

Description. The shell is yellowish or corneous (the type material consists mainly of weathered shells). The protoconch is very large, with regular riblets and spiral lines; the radial and spiral lines are approximately of the same strength. The 5.25–6 whorls

are separated by a shallow suture. The umbilicus is wide but moderately deep. The body whorl has a prominent upper keel and a less conspicuous lower keel. The apertural margin is slightly thickened. The parietal callus is blunt, not well developed and is only clearly apparent in older specimens.

Three shells were opened. On the parietal wall there is a single curved lamella that is oblique, its upper end situated much more anteriorly than the lower end. A short, but thick vertical plica is situated below and anteriorly of the lamella. On the palatal wall there are six more-or-less parallel plicae, with some additional short plicae. The most prominent additional plica is situated above the posterior end of the last plica. The fifth plica is usually S-shaped.

Measurements (in mm). D= 16.3–21.3, H= 6.7–8.6. (n=4, shells from different samples).

Differential diagnosis. *Hunyadiscus saurini* sp. n. is smaller than *H. andersoni*, its keel is situated higher (this results a more angular body whorl), has weaker parietal callus, a lower horizontal plica on the parietal wall which is absent in *H. andersoni*. *Naggsia laomontana* has a rounded body whorl and weaker spiral striation on its protoconch.

Etymology. The species is named in honour of the French geologist and malacologist Edmond Saurin (1904–1977) who collected it.

Type locality. Laos, Pa Hia (Ancienne province Tran Ninh).

Distribution. This species is known only from Southern Laos.

Remarks. This new species shows considerable diversity in terms of shell size. However, the other shell characters are stable within and between samples.

The village "Pa Hia" or "Pah Hia" is located 100 km south from Xieng-Khouang, the capital of Tran Ninh Province (see Saurin 1953: 113). However, the exact locality could not be determined (Nordsieck 2002, Páll-Gergely 2014, Páll-Gergely 2015c). A geological report (Marutani 2006), mentioned the name "Ban Namthong" in brackets after "Pa Hia". The two names are probably identical, but the origin of this information could not be traced. Marutani (2006) gave the following GPS coordinates for Ban Namthong: 19.05000°N, 103.28330°E. This location is situated approximately 75 km southwest from Xiangkhoang city. Google Earth placed "Ban Namthong" 7.6 km in southwest direction (18° 59'N, 103° 16'E), which agrees with the 1:50.000 map printed by the National Geographic Directorate, Vietnam, in 1965. We provisionally identify the village Pah Hia with Ban Namthong, because we could not locate the name Pah Hia on the maps available to us.

Naggsia **Páll-Gergely & Muratov, gen. n.**
http://zoobank.org/D8CDB123-6382-462E-A66E-ABFD7C838CA3

Type species. *Helix laomontana* L. Pfeiffer, 1862.

Content. *Naggsia laomontana* (L. Pfeiffer, 1862).

Diagnosis. Shell flat, widely umbilicated, body whorl rounded; protoconch with dense, regular, slightly waved ribs with extremely fine spiral striation. Epiphallus ab-

sent, diverticulum and gametolytic sac are both very short, but diverticulum is still shorter than the gametolytic sac. Cusp of central tooth missing, only basal plate of central tooth present. Marginals bicuspid.

Differential diagnosis. *Naggsia* gen. n. differs from the genera having ribbed embryonic whorls (*Endothyrella*, *Gudeodiscus*, *Halongella*, *Sicradiscus*, *Sinicola*) by the absence of an epiphallus and the presence of a very short diverticulum. Moreover, although the protoconch of *Naggsia* gen. n. is ribbed, the ribs (radial lines) are not straight, as in the other genera, but are somewhat wavy. *Naggsia* gen. n. differs from the genera without a ribbed protoconch (*Chersaecia*, *Endoplon*, *Plectopylis*) by the presence of regular, slightly waved ribs on the embryonic whorls. The latter genera are insufficiently known anatomically. *Plectopylis bensoni* Gude, 1914 (mentioned as *P. achatina* Pfeiffer, 1845) and *P. cyclaspis* Benson, 1859 have a well-developed epiphallus (Stoliczka 1871). Interestingly, these *Plectopylis* species have a relatively short and thickened diverticulum, which is somewhat similar to that of *Naggsia laomontana*. Similarly to *Naggsia laomontana*, *Chersaecia simplex* Solem, 1966 lacks the epiphallus (see Solem 1966), but it at has no diverticulum, which is well developed in *Naggsia*. See differential diagnosis under *Hunyadiscus* and Table 1.

Etymology. The new genus is dedicated to Fred Naggs (NHM) in acknowledgement of his help with our studies on the Plectopylidae.

Distribution. Northern Laos (Figure 15).

Remarks. The anatomy of *Naggsia laomontana* is rather similar to that of *Plectopylis* in the short diverticulum and to *Chersaecia* in the absence of epiphallus. The shell characters, namely the ribbed protoconch and the reduced parietal plication is similar to the genera *Endothyrella*, *Gudeodiscus*, *Halongella*, *Sicradiscus* and *Sinicola*. The radula of *Naggsia laomontana* shows similarities with those of *Gudeodiscus* (*Veludiscus*), *Halongella* and *Plectopylis* (see Stoliczka 1871, Páll-Gergely et al. 2015a) in terms of the small central tooth and the simple marginals. In contrast, the central tooth of *Endothyrella*, *Gudeodiscus* (*Gudeodiscus*), *Sicradiscus* and *Sinicola* species is large (as large as or larger than the ectocones of the first laterals), (Páll-Gergely et al. 2015a, 2015b), and the marginals are tricuspid or even quadricuspid. Before examining ethanol-preserved *Naggsia laomontana* specimens, we considered grouping the plectopylid genera into two tribes, namely one with a ribbed, and another with a smooth or granulated protoconch. The examination of *Naggsia laomontana* revealed that the character states considered to be primarily important do not allow the placement of *Naggsia* gen. n. in any of the two groups, and that the character states rather show a mosaic structure across genera.

Naggsia laomontana (L. Pfeiffer, 1862)
Figures 1A–B, 2A, 4D, 5E–F, 7–8, 9A–C, 10

1862 *Helix laomontana* L. Pfeiffer, Proceedings of the Zoological Society of London, 272, Plate 36, figs 9–10 [Lao Mountains, Camboja].
1863 *Helix laomontana*, — L. Pfeiffer, Novitates Conchologicae 2: 216, Plate 57, figs 7–9.

1868 *Helix laomontana*, — L. Pfeiffer, Monographia Heliceorum Viventium...: 394.

1875b *Helix* (*Plectopylis*) *laomontana*, — Godwin-Austen, Proceedings of the Zoological Society of London: 612.

1887 *Helix laomontana*, — Tryon, Manual of Conchology, 2 (3): 160, plate 34, figs 60–62.

1897a *Plectopylis laomontana*, — Gude, Science Gossip, 3: 245, figs 36a–c.

1899a *Plectopylis* (*Chersaecia*) *laomontana*, — Gude, Science Gossip, 6: 148.

1899b *Plectopylis* (*Chersaecia*) *laomontana*, — Gude, Science Gossip, 6: 175.

1914 *Plectopylis* (*Chersaecia*) *laomontana*, — Gude, The Fauna of British India including Ceylon and Burma. Mollusca.–II. (Trochomorphidae-Janellidae): 73.

1920 *Plectopylis laomontana*, — Gude, Proceedings of the Malacological Society of London, 14: 62, fig. 1.

2013 *Chersaecia laomontana*, — Páll-Gergely and Hunyadi, Archiv für Molluskenkunde 142 (1): 7–8.

Types examined. Cambodia, NHMUK 20130004 (3 syntypes).

Museum material examined. Laos, Luang Prabang (alte Schau-slg.), SMF 150121/2; Laos (Siam), Luang Prabang, ex Möllendorff, SMF 294866/3; Laos, Luang Prabang slg. Dosch ex H. Rolle, SMF 172067/1; Laos, Luang Prabang, SMF 150122/4; Cambodia, slg. Dosch ex H. Rolle ex Sowerby ex Fulton, SMF 172068/3; Laos, Luang Prabang, Französ. Hinterindien, C. Boettger 1904/43, SMF 102819/3; Cambodia, NHMW 342232/2; Laos, Lao Mountains, Altonaer Museum, coll. Semper, O. ex Cuming, ZMH 45901/2; Cambodge, coll. Achat Lallé, 1870, MNHN 2012-2506/1; Louang Prabang, MNHN 2012-2507/1; Cambodge, coll. Deshayes in coll. Crosse, MNHN 2012-2508/1; Laos, coll. Denis, MNHN 2012-2509/1; Louang Prabang, coll. Morgan, MNHN 2012-2510/2; Mts. Lao, Cambodja, MNHN 2012-2511/1; Louang Prabang, coll. Letellier 1949, MNHN 2012-2512/1; Louang Prabang (Laos), coll. Morlet-Fischer, MNHN 2012-2513/4; Louang Prabang (Laos), coll. Staadt 1969, MNHN 2012-2514/2; Louang Prabang (Laos), coll. Morlet-Fischer, MNHN 2012-2515/2; China, coll. Salisbury ex Beddome (also Canon Hoisley coll., 1918), NHMUK 20110363; Cambojia, Mr. Mouhot, Lao Mountains, NHMUK/3; Siam, Lao Mountains, coll. Godwin-Austen, NHMUK/2; India, NHMUK/2; Camboja, NHMUK/1; Laos, Luang Prabang, coll. Möllendorff, NHMW 7285/2; Tonkin, Prabang, coll. Gerstenbrandt, NHMW 8467/2; Laos. Luang Prabang, coll. Möllendorff, NHMW 40181/6; Cambodia, NHMW 34232/2;

New material examined. Laos, Luang Prabang Province, Ban Pak Ou, Nam Wu (opposite side of Ban Pak Ou), 364 m 20°03.48276'N, 102°12.79912'E, leg. Ohara, K. 13.10.2006., PGB/5; Laos, Tad Kuangsi Waterfall, about 20 km SW of Luang Prabang, 19°43'02.97"N 101°59'38.68"E., leg. Reischütz, A., February 2010., RE/3; Laos, Tad Kuangsi Waterfall, about 20 km SW of Luang Prabang, 19°43'02.97"N 101°59'38.68"E, leg. Theisl, T. April 2009., RE/3+2 juv.; Laos, Luang Prabang Province, Tad Kuangsi Xi (Waterfall), 466 m, 19°44.96071'N, 101°59.49286'E, leg. Ohara, K. 14.10.2006., PGB/1; **16L06** Laos, Luang Prabang Province, ca. 7 km S of
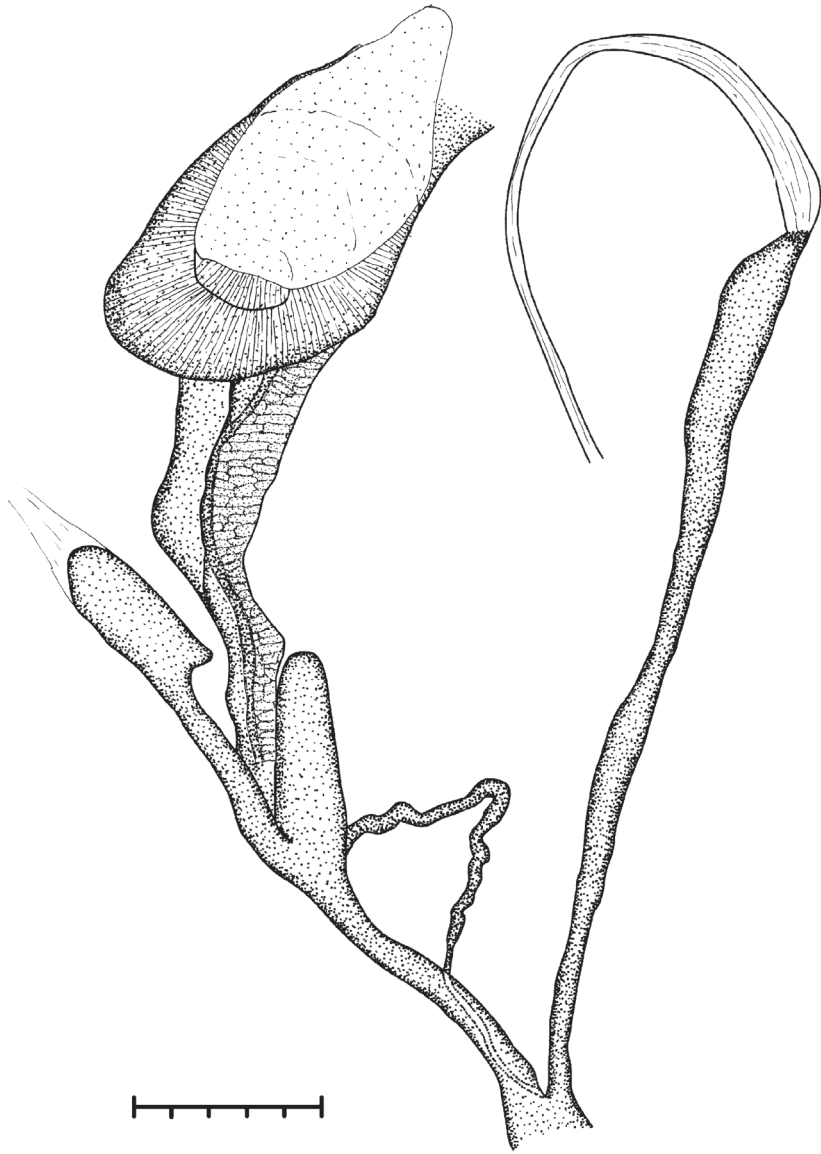
Figure 7. Reproductive anatomy of *Naggsia laomontana* (L. Pfeiffer, 1862), 16L06, spec.1. Scale represents 5 mm.

Luang Prabang, Near Tad Thong waterfall, 431 m a.s.l., 19°50.064'N, 102°07.755'E, leg. A. Abdou, I.V. Muratov, 3.11.2006., MNHN 2012-27057/45 shells + anatomically examined specimens (Figs 2A, 7–8, 9A–C, 10); **39L06** Laos, Luang Prabang Province, ca. 5 km SE of Luang Prabang, ca. 1.5 km NE of Ban Lak Sip, Phou Xuang mountain, 640 m a.s.l., 19°51.605'N, 101°11.081'E, leg. A. Abdou, I.V. Muratov, 24.11.2006., MNHN 2012-27057/20 shells (some of them broken/juvenile); **42L06**
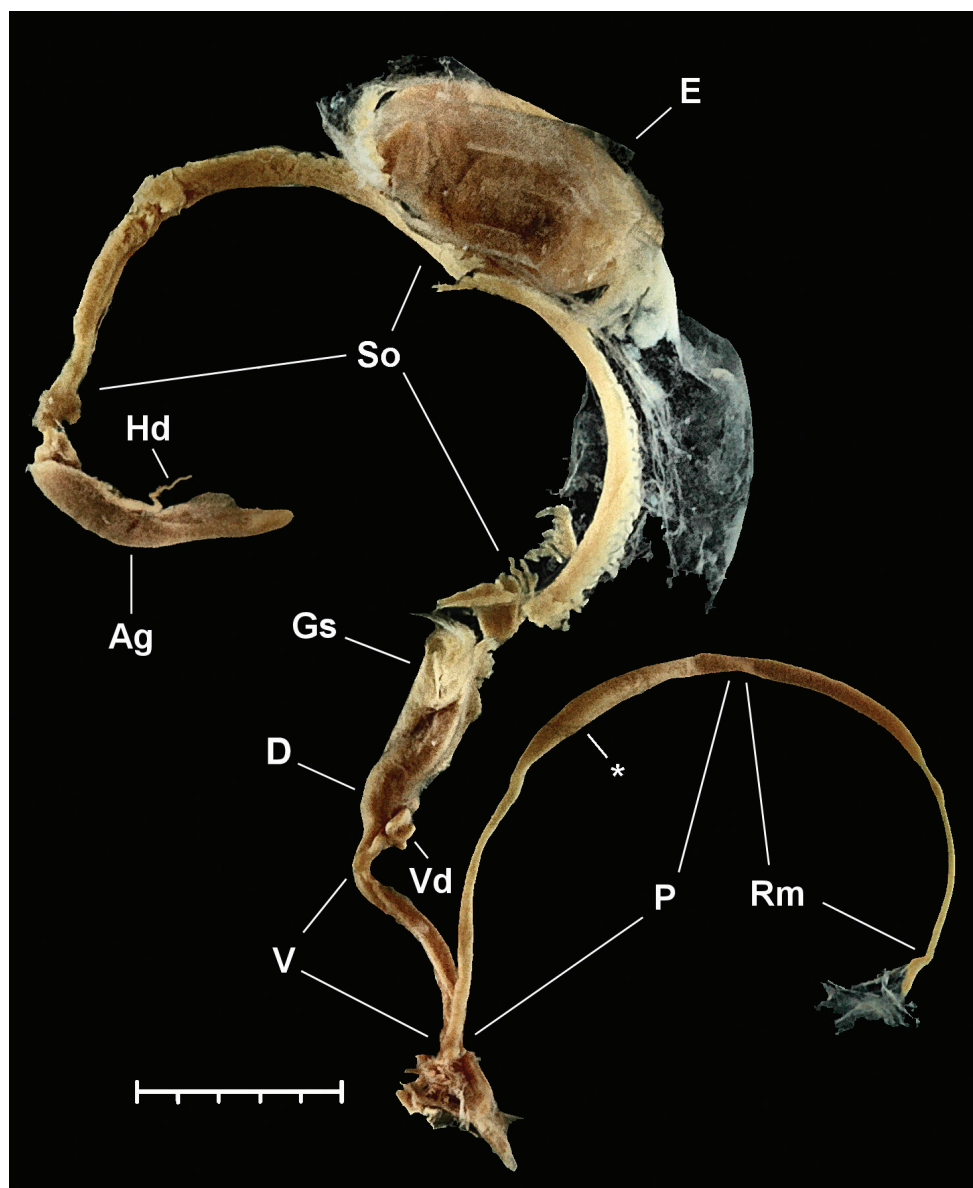
Figure 8. Photo of the reproductive anatomy of *Naggsia laomontana* (L. Pfeiffer, 1862), 16L06, spec.2. Abbreviations: Ag: albumin gland; D: diverticulum; E: embryo in the uterus (one out of five); Gs: gametolytic sac; Hd: hermaphroditic duct; P: penis; Rm: retractor muscle; So: spermoviduct; V: vagina with vas deferens alongside; Vd: coiled portion of vas deferens. Asterix indicates the place until where vas deferens could be traced back. Scale represents 5 mm.

Laos, Luang Prabang Province, ca. 22 km SW of Luang Prabang, Kuang Si waterfall, 482 m a.s.l., 19°44.966'N, 101°59.496'E, leg. A. Abdou, I.V. Muratov, 28.11.2006., MNHN 2012-27057/14 (some of them broken/juvenile).
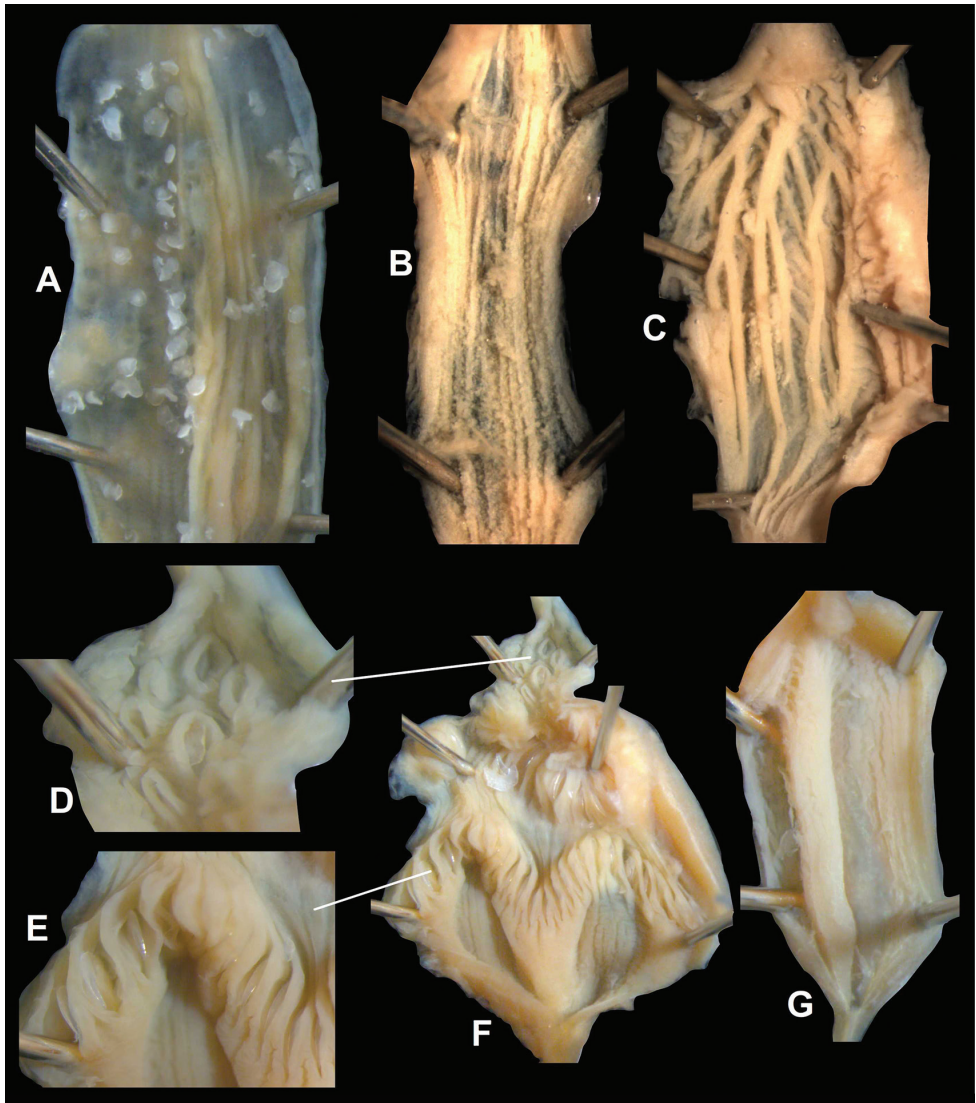
Figure 9. Inner walls of reproductive organs of Plectopylidae. **A–C** *Nagsia laomontana* (L. Pfeiffer, 1862) (same specimen as on Fig. 7); *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* Páll-Gergely & Hunyadi, 2015 (same specimen as on Fig. 1C). **A, E** penis **B, G** epiphallus **C** diverticulum **D** penial caecum **F** penis and penial caecum. Figures not to scale. All images: B. Páll-Gergely.

Diagnosis. A dextral, medium-sized or large species with a rounded body whorl, and no apertural fold. On the parietal wall there is a single curved lamella.

Description. The yellowish, sometimes pink or light brown shell is dextral, almost flat with the apex slightly elevated. The 5.5–6 whorls are separated by a moderately deep suture. The protoconch is very densely, regularly ribbed, with extremely fine spiral lines across the ribs. The teleoconch is irregularly ribbed; the space between the ribs
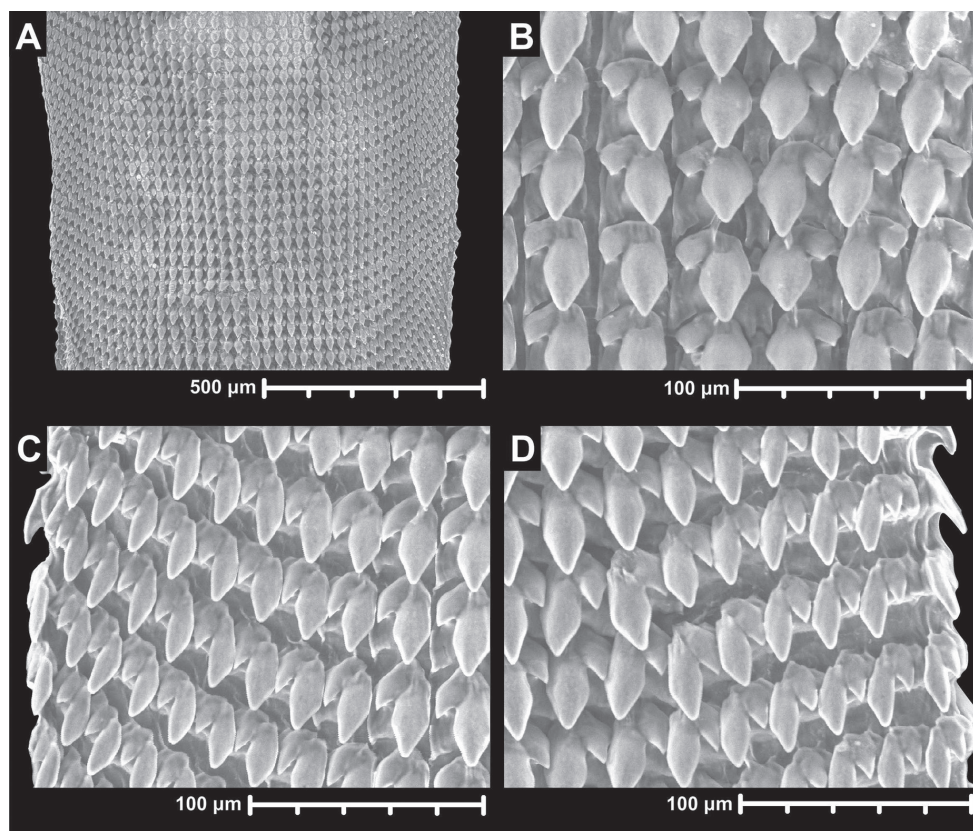
Figure 10. Radula of *Naggsia laomontana* (L. Pfeiffer, 1862). **A** anterior-posterior middle section of the Radula **B** central and first two lateral teeth **C–D** marginals. Sample: 16L06.

is greater than on the protoconch. The lip is only slightly thickened and reflexed. There is an elevated, but blunt parietal callus, which has two shallow channels at the meeting point with the parietal part of the lip.

Four specimens were opened. On the parietal wall there is a single curved lamella without additional plicae. On the palatal wall there are seven horizontal plicae. The first, (situated near the suture) is short, undivided, not inclined, sometimes having a short denticle slightly lower than its posterior end. The second plica is slightly indented in place opposing the parietal curved lamella just before it becomes dichotomously bifurcated posteriorly, with its lower posterior arm slightly inclined away from the suture. The third, fourth and fifth exhibit an increasing tendency to be divided opposing the parietal lamella and inclined posteriorly away from the suture. The sixth is strongly, equally divided, having both parts equally inclined posteriorly away from the suture. The last one, unequally divided, consists of a long, not inclined anterior part and short, inclined posterior part.

Differential diagnosis. *Naggsia laomontana* resembles *Gudeodiscus* species in having the single parietal lamella, rounded body whorl and densely ribbed protoconch.
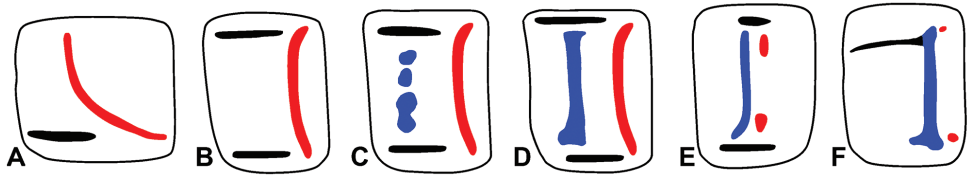
Figure 11. Parietal plication of Plectopylidae species (diagrammatic figures). **A** *Hunyadiscus saurini* sp. n. **B–E** main character states of *Endothyrella*, *Gudeodiscus*, *Halongella*, *Sicradiscus* and *Sinicola* (mainly after Páll-Gergely and Hunyadi 2013 and Páll-Gergely et al. 2015b and Páll-Gergely and Asami 2016) **F** *Hunyadiscus andersoni* (W. Blanford 1869). Blue colour indicates the anterior lamella and its homologous structures; red colour indicates the posterior lamella and its homologous structures.

Figure 12. Habitat of *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* Páll-Gergely & Hunyadi, 2015. The left side of Nam Khan ca. 18 km SE of Muang Xiang Ngeun. The red dot indicates the approximate place where the specimens were collected. Photo: Igor Muratov.

The protoconch of *N. laomontana* however reveals a unique surface structure, the riblets are comprised of slight waves that do not stand as regularly as those of *Gudeodiscus*. *Gudeodiscus* species that usually have a somewhat elevated spire, more whorls, two horizontal plicae in front of the parietal lamella, and simple (undivided) palatal plicae.

    Measurements (in mm). D= 28.3–32, H= 8.8–9.1 (n=3, syntypes); D= 18.6–21, H= 6.4–7.5 (n=5, specimens from Laos).

Figure 13. Habitat of *Naggsia laomontana* (L. Pfeiffer, 1862). Kuang Si waterfall. The red dot indicates the exact place where the specimens were collected. Photo: Igor Muratov.

Characters of the genital structure (Figs 7–8, 9A–C). Two specimens were anatomically examined (sample 16L06). The right retractor muscle crosses between the penis and vagina.

Penis long, its distal part is more slender than the proximal part, internally with 5–6 longitudinal folds aligned next to each other; only one of the folds reach the proximal end of the penis, the others are shorter; the penial wall (outside of the folded area) is wrinkled; the wrinkles are stronger near the distal end of the penis; many small, flat, lenticular calcareous granules were found in the penis lumen; epiphallic differentiation was not detected; retractor muscle slightly thinner than penis, shorter than it and connected to the apical end of penis; vagina shorter than half of penis; vas deferens has thick coiled portion just after coming out of spermoviduct, connects
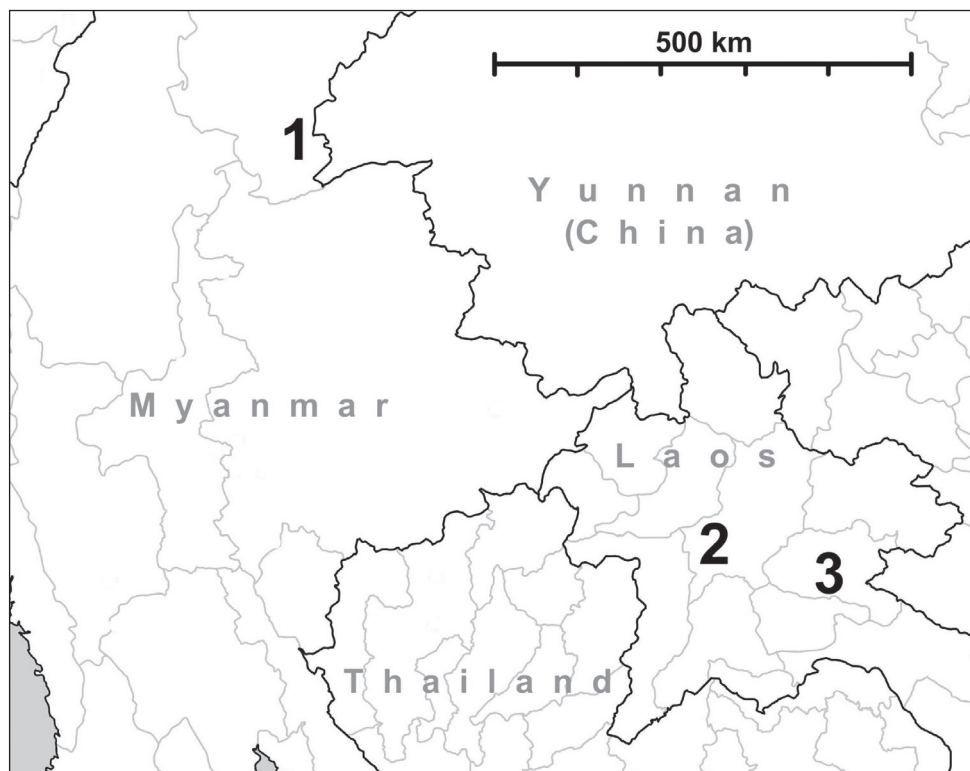
Figure 14. Map showing the distribution of *Gudeodiscus* Páll-Gergely, 2013. The small circles in Laos indicates the new localities of *Gudeodiscus* (*Gudeodiscus*) *messageri raheemi* Páll-Gergely & Hunyadi, 2015. After Páll-Gergely and Hunyadi (2013) and Páll-Gergely et al. 2015a.

to vaginal wall and forms part of penial wall, reaching the middle of the proximal part of penis; diverticulum short, oval, gametolytic sac with relatively thick, cylindrical stalk and thickened, rather quadrangular sac; there were five, well-developed embryos in the uterus.

Radula (Figure 10). Radula elongated, but not very slender; the basal plates of the centrals are present, but their cusps are absent; the teeth are arranged in rows; each row contains 18–19 teeth, the first nine are laterals, the remaining are marginals, but it is rather difficult to decide which teeth are the last laterals and the first marginals; lateral teeth stand in straight rows which are perpendicular to the central column; marginals stand in anteriorly pointed, slightly oblique rows; endocones of laterals are rhomboid, rather blunt; ectocones are small, pointed, triangular; endocones of marginals are slender ovoid, blunt; the ectocones are small, pointed, triangular.

Distribution. The species was described from "Lao Mountains, Camboja". We have seen material with more detailed geographical data only from the central part of Northern Laos (around Luang Prabang). In the collection of the Natural History Museum London, a single shell of *Ch. laomontana* is present with the locality "China" (NHMUK 20110363, Salisbury Collection Ex Beddome Ex Canon Hoisley coll. 1918; see Páll-Gergely and Hunyadi 2013). Its occurrence in China is not verified and the locality is possibly wrong.

Figure 15. Map showing the distribution of *Hunyadiscus andersoni* (W. Blanford, 1869) (**1**), *Naggsia laomontana* (L. Pfeiffer, 1862) (**2**) and *Hunyadiscus saurini* Páll-Gergely, sp. n. (**3**). For the accuracy of location no. 3. see under *Hunyadiscus saurini*.

Ecology and behaviour. This species can be found in primary or old secondary broad-leaved forests, but it inhabits some peculiar habitats as well. It can survive droughts as well as periodical floods and can be found in large numbers near waterfalls (Kuang Si waterfall, for example, is one very popular collecting spot) (Figure 13). Unlike most terrestrial snails that start to crawl when placed in water, snails of this species, when under water, retract deep into the shell, which is probably an adaptation that helps to survive frequent floods.

Acknowledgements

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RESEARCH ARTICLE

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Granopupa in Iran, monophyly, and the fossil Granariinae (Gastropoda, Pulmonata, Chondrinidae)

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Abstract

Indisputable Chondrinidae, Granariinae species, characterized by shell shape and apertural dentition, are known from Eocene deposits to the Recent. The generic classification of the extant species is based on conchological, anatomical and molecular data that are available now for most of the known species, including 'Granaria' persica as a representative of the once problematic group of so-called eastern *Granaria* species. According to molecular and anatomical characters, these eastern species have to be classified with *Granopupa granum* in *Granopupa*. *Graniberia* **gen. n.** is introduced for *G. braunii* on the basis of molecular and conchological data. For the pre-Pleistocene species, two generic names are equally well available now, viz. *Granopupa* and *Granaria*. Shell characters only do not enable a decision here. For the sake of nomenclatorial stability we propose to use *Granaria* for these species. Because both molecular and anatomical data most likely will never be known for the fossils, it will remain unclear whether the combined extant and extinct *Granaria* species form a monophyletic group.

Introduction

The genus-group taxa of the Chondrinidae Steenberg, 1925, are currently characterized by conchological, anatomical, and molecular characters (Gittenberger 1973, Kokshoorn and Gittenberger 2008, 2010). These data were not available for all the species, however, so that not all could be classified accordingly.

The extant *Granopupa granum* (Draparnaud, 1801), and *Granaria* Held, 1837, species, and all the fossil chondrinids known from Eocene to Pliocene, have similar shell shapes and, what is more distinctive, the same characteristic arrangement of the apertural teeth, i.e. the palatalis inferior is more prominently developed than the other palatals. The extant so-called *Granaria* species show a disjunct distribution, with a western group of four species occurring in Europe and an eastern group of three species in the Arabian peninsula and Iran. The western species, viz. *G. frumentum*, *G. variabilis*, *G. stabilei* and *G. braunii*, are relatively well-known, whereas the eastern group, viz. *G. lapidaria*, *G. persica* and *G. arabica*, was known from shells only.

The shells of *Granopupa granum*, measuring 3.1–6.0 × 1.4–1.8 mm, are smaller than those of the European *Granaria* species, measuring 5.6–9.3 × 2.1–2.6 mm in the smallest, i.e. *G. stabilei*, and 6.7–19.0 × 2.6–4.5 in the largest species, i.e. *G. variabilis* (see Gittenberger 1973). The generic classification of the species from the Middle East, with shells measuring 4.9–5.5 × 2.1–2.2 mm (see Gittenberger 1973), was questionable. They could be either relatively small *Granaria*, or large *Granopupa*, because an obvious gap in sizes does not exist. The use of the generic name *Granaria* for the extant species occurring in the disjunct eastern part of the alleged range of that genus, was based on tradition and on the lack of a clearly preferential alternative.

For the chondrinids from before the Pleistocene (Höltke and Rasser 2013) neither molecular nor anatomical data are likely to ever be known, so that their classification has to be based on shell morphology only.

Recently, one of us (U. B.) collected together with empty shells a live specimen of '*Granaria' persica*. As a consequence, both anatomical and molecular data are available for that species now. Therefore, a revised classification of this species is proposed in this article. Additionally, the generic classification of the remaining, so-called *Granaria* species is dealt with.

Material and methods

A single live animal of '*Granaria' persica* was collected by Ulrich Bößneck in Iran, province of Hormozgan, Banooband, Genu Mtn, at 740-1000 m altitude (Fig. 1). The specimen was transferred into ethanol 70%. During dissection, the proximal part of the genital tract was isolated and coloured with organic cochineal dye, subsequently hardened in ethanol 97%, then cleared in Euparal essence and eventually fixed in Euparal as a genital slide. Serial sections were not made, so that the structure of the lumen can only be described by studying the transparent parts of the genital slide with a

Figure 1. *Granopupa persica* (Gittenberger, 1973). Iran, province of Hormozgan, Kuh-e Bakhun, large valley, 980 m alt., 27°55'11"N 56°41'24"E, washed ashore; U. & K. Bößneck & A. Saboori leg. Shell height 5.2 mm (RMNH 336351). Scale bar 1 mm. Photograph by DSJG.

regular microscope. The buccal mass was partly dissolved in KOH to isolate the radula, which was cleaned, mounted on a stub and photographed with a SEM.

The material that is used here is housed in Naturalis Biodiversity Center, Leiden, The Netherlands.

DNA was extracted with a DNeasy blood & tissue kit (Qiagen). *COI* and *16S* were amplified using the procedure described in Kokshoorn and Gittenberger (2008). Products were sequenced in both directions (using the same primers) at BaseClear (The Netherlands) and edited in SEQUENCHER 5 (Genecodes Corp.). Sequences for *'Granaria' persica* have been deposited in GenBank (accession numbers KT948999 and KT949000 for *COI* and *16S*, respectively). Datamatrices with relevant reference sequences from GenBank were constructed in Geneious PRO 7.0.6. Because no *COI* sequence is available for *Granaria variabilis* it was coded as missing data. Both datasets were aligned with MAFFT v.7.017 (Katoh and Standley 2013) using default settings. Conserved regions of the *16S* alignment were selected with GBLOCKS v. 0.91b (Castresana 2000). PARTITIONFINDER (Lanfear et al. 2012) was used to check for the best partitioning scheme (*COI* codon positions and *16S* were considered as potential partitions) and substitution models. The translated amino acid sequene of *COI* was added as a fifth partition for a Bayesian phylogeny reconstruction. None of the suggested partitions could be combined and GTR+G, GTR+G, HKY+I+G, GTR+I+G and aa mixed were specified for *COI* codon position 1, 2, 3, *16S* and the *COI* amino acid partition, respectively. A phylogenetic analysis was carried out with MRBAYES (Ronquist and Huelsenbeck 2003) v.3.2.3 (10 M generations, 2 runs, 4 chains) hosted on the CIPRES science gateway (Miller et al. 2010). Trees were sampled every 1000 generations, the first 2500 trees were discarded as burnin (relburnin = yes, burninfrac = 0.25). To compare and further explore the *COI* and *16S* datasets, both were analysed separately as well (see Supplementary information). Except for the omission of *Granaria variabilis* (for which no *COI* data are available) the MrBayes analysis (partitioning and selected models) for *COI* was identical to that for the concatenated dataset. For *16S* the complete sequences (no characters omitted) were utilized. The selected model again was GTR+I+G.

Abbreviation: pp. = posterior probability.

Results

In *'Granaria' persica*, as in the other chondrinid species, the male part of the genital tract forms a loop because the proximal part of the vas deferens is fixed to the genital atrium (Gittenberger 1973). A prominent flagellum, as is present in both the *Granaria* and the *Solatopupa* species, is lacking. The male loop can be subdivided in five parts, which differ in the structure of the lumen and slightly in width; the transitional sites are more or less clearly distinguishable by irregularities in width or curvature of the duct. The segments are described from proximal (starting from the body wall) to distal. The most proximal segment of the loop, i.e. the penis (Fig. 2: 1), has a muscular wall with very fine, transverse striae and a short ridge in the distal third of the lumen. The adjoining epiphallic part (Fig. 2: 2) has a thin wall and a regular transverse structure, maybe with small papillae in the lumen. It is followed by the narrowest part of the loop (Fig. 2: 3), without any regular, luminal structure. The next part (Fig. 2: 4) is
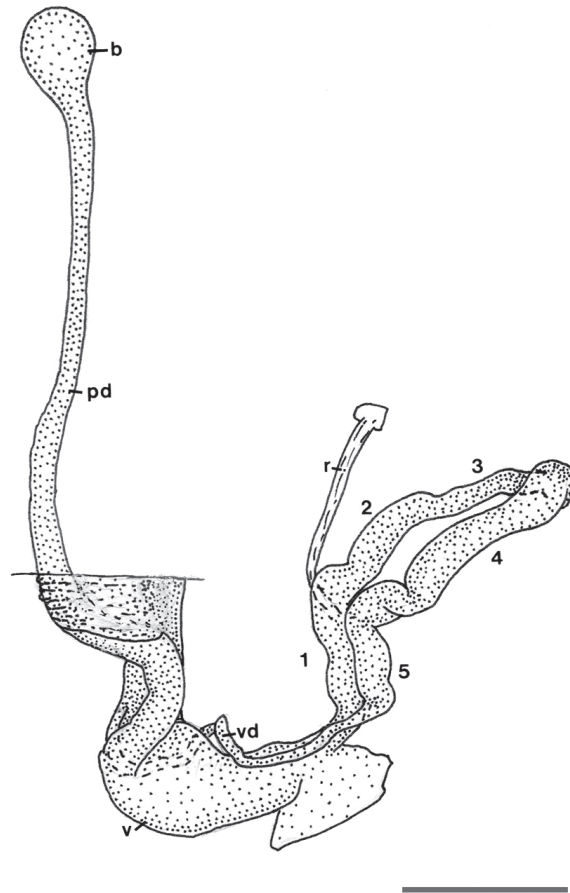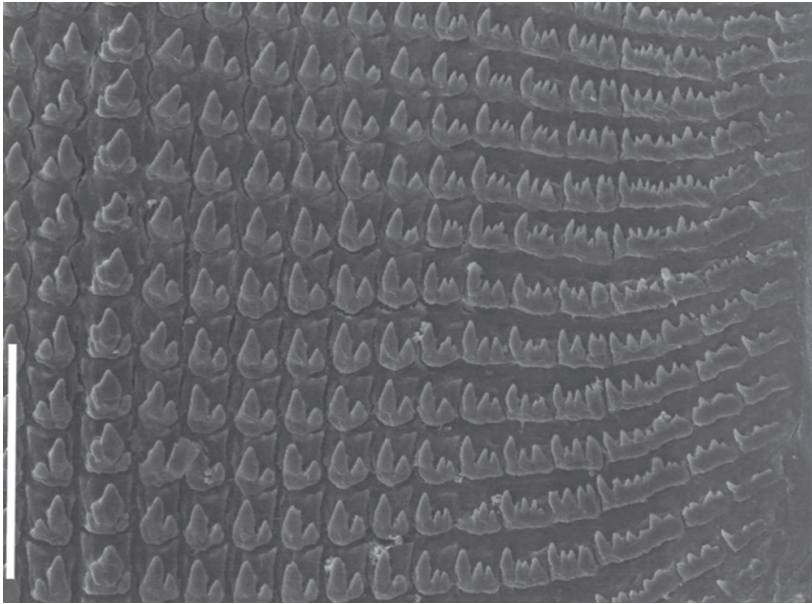
Figure 2. *Granopupa persica* (Gittenberger, 1973). Iran, province of Hormozgan, Genu Mt., Banoo-band, 740–1000 m alt., 27°22'01"N 56°09'45"E, dry rocky limestone slope with little vegetation; U. Bößneck leg. Genital tract. Abbreviations: b, bursa copulatrix; pd, pendunculus; r, retractor muscle; v, vagina; vd, vas deferens. The figures **1–5** refer to the segments of the male loop that are mentioned in the text. Scale bar 1 mm.

clearly broader again; the lumen has relatively large papillae, which gradually pass into a more transverse arrangement. The most distal part of the loop (Fig. 2: 5) has a thick wall with very fine transverse and longitudinal striae, and could be considered a part of the vas deferens; the longitudinal striae can be followed over some distance also more proximally.

The radula of '*Granaria*' *persica* has rows of teeth with a tricuspid central tooth, accompanied by adjoining bicuspid teeth, and teeth with more cusps, towards the margin of the radular ribbon. In the specimen that could be studied, the central tooth shows some individual irregularities. It is accompanied by 6 bicuspid teeth; from tooth 7 on, the side cusp is split into two, and more marginally in more, irregular, smaller cusps (Fig. 3).

Figure 3. *Granopupa persica* (Gittenberger, 1973). Iran, province of Hormozgan, Genu Mt., Banooband, 740–1000 m alt., 27°22'01"N 56°09'45"E, dry rocky limestone slope with little vegetation; U. Bößneck leg. Half row of radula teeth; scale bar 50 μm. SEM photograph by L.P. van Ofwegen.

The separate molecular analyses did not result in entirely congruent results (see Supplementary Information), so that the summarizing cladogram that is presented here (Fig. 4) has uncertainties in it. The phylogenetic relationships of all the genera remains unresolved. All reconstructions indicate, however, that *'Granaria' persica* is most closely related to *Granopupa granum*. The position of *G. braunii* is unclear, but none of the reconstructions assigns that species to a clade with *G. frumentum* or *G. stabilei*.

For data regarding the European representatives of the chondrinid species, we refer to Gittenberger (1973) and Kokshoorn and Gittenberger (2008, 2010).

Discussion

For genera and species of the extant Granariinae we refer to Kokshoorn and Gittenberger 2010. DNA sequence data from this study do not allow (and were neither intended) to test for the monophyly of Granariinae or Chondrininae.

In the *Granaria* species of which the genital tract had been studied in some detail (Gittenberger 1973), viz. the type species *G. frumentum* (Draparnaud, 1801), *G. variabilis* (Draparnaud, 1801), *G. stabilei* (E. von Martens, 1865), and *G. braunii* (Rossmässler, 1842), the male loop is provided with a prominent flagellum. In these species, the lumen of the entire proximal half of the loop, which may be considered homologous with the penis, has some longitudinal ridges only, which continue into
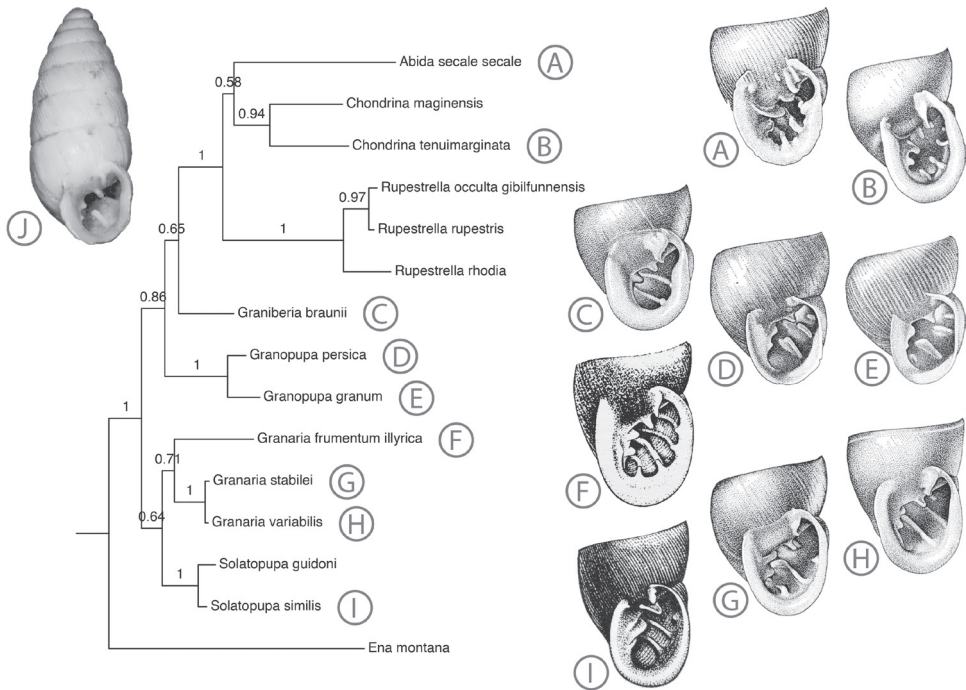
Figure 4. Bayesian phylogeny reconstruction of Chondrinidae based on a concatenated dataset of nucleotide (*COI* and *16S*) and amino acid (*COI*) sequences. All depicted apertures are after Gittenberger (1973), except **F** and **I** which are after Kerney, Cameron and Gittenberger (1980). Aperture **D** is not of *Granopupa persica*, but of the closely related *Granopupa arabica*. Inset J shows a photo of *Granaria grossecostata*, after Höltke and Rasser (2013: fig. 5) (with permission).

the flagellum; before the transition into the vas deferens, the lumen is covered with many fine papillae. The *Solatopupa* species, which differ in shell shape and the structure of the radula, have the same bauplan of the genital tract (Gittenberger 1973).

According to molecular analyses (Kokshoorn and Gittenberger 2008 and Fig. 4), the W European *Granaria variabilis* and *G. stabilei* are sistersperies (Fig. 4 and Suppl. material 1; pp. 1.0 and 0.86); they may have evolved from a common ancestor as lowland and alpine descendant, respectively. The polytypic Central & E European *G. frumentum* (see Fehér et al. 2010) could be the sistertaxon of their ancestral species (Fig. 4; pp. 0.71), but the *COI* and *16S* phylogenies (Suppl. materials 1, 2) are inconclusive. In the latter phylogeny *G. frumentum* is the sistertaxon of *Solatopupa* (Suppl. material 1; pp. 0.97), whereas with *COI Granaria* and *Solatopupa* are not monophyletic (though both are outside the clade Chondrininae-*Granopupa*). The summarizing cladogram indicates *Granaria* and *Solatopupa* as sistergroups (Fig. 4; pp. 0.64), but their monophyly is only supported by *16S* (Suppl. material 1; pp. 0.98).

The radula of '*Granaria*' *persica* has the bauplan that is considered plesiomorphic because it is known from *Granaria*, *Granopupa*, *Abida* and snails of many other pulmonate genera that are not feeding on algae or lichens and are not obligatory rock-

scraping (Gittenberger 1973, Breure and Gittenberger 1982). The radulae of both *Chondrina* and *Rupestrella* have the apomorphic rock-scraping condition, i.e. a series of virtually identical unicuspid teeth in the central part of the rows of teeth (Breure and Gittenberger 1982).

In *'Granaria' persica*, there is no flagellum and, according to the luminal structure, the penis is restricted to the proximal third of the male loop; the segment of the loop before the vas deferens is devoid of small papillae. According to the structure of the genitalia, *G. persica* and *G. granum* are sistergroups and, as a consequence, should be considered congeneric. This view is convincingly supported by the molecular phylogenies (Fig. 4, Suppl. material 1, 2; pp. 1.0, 1.0, 0.86), which also show *Granopupa granum* and *G. persica* as sistergroups. We suggest to classify in *Granopupa* the three chondrinid species from the eastern part of the range of the family, that were classified in *Granaria* by Gittenberger (1973), and considered closely related, viz. *Granopupa arabica* (Dohrn, 1860), *Granopupa lapidaria* (Hutton, 1849) and *Granopupa persica* (Gittenberger, 1973).

The Iberian *'Granaria' braunii* does belong to neither the otherwise monophyletic group *Granaria* (Fig. 4, Suppl. material 1; pp. 0.71, 0.75) nor to the clade *Granaria-Solatopupa* (Fig. 4, Suppl. material 2; pp. 0.64, 0.98), as defined above. Morphologically *'G.' braunii* cannot be distinguished from *Granaria* and *Solatopupa* on the basis of the structure of the genital tract, whereas the apertural armature of the shell has the *Granaria & Granopupa* bauplan with a most prominent palatalis inferior. Apart from that, however, *'G.' braunii* is not particularly similar to any of the other chondrinids, and therefore, short of molecular data, its closest relative was considered unknown by Gittenberger (1973: 62). The molecular phylogenetic analyses that could be performed now (Fig. 4, Suppl. material 1, 2) indicate that '*G.*' *braunii* should not be classified with *Granaria* (i.e. *G. frumentum*, *G. variabilis* and *G. stabilei*). Hence we introduce a monotypic genus for this species.

Graniberia Gittenberger, Groenenberg & Kokshoorn, gen. n.
http://zoobank.org/2B716941-2F7D-436A-A4B9-FA53835D5241

Diagnosis. Columellaris much more prominent than the infracolumellaris, which is not or hardly visible in frontal view; palatal lamellae reaching their maximum prominence clearly deeper than half a whorl inside the last whorl; apertural lip strongly reflected and broadly thickened.

Type species. *Graniberia braunii* (Rossmässler, 1842) Figure 4C.

Remarks. The three extant *Granaria* species, the fossil taxa that are currently classified with *Granaria*, and the four known *Granopupa* species, all differ from *Graniberia braunii* in the characters mentioned in the diagnosis.

In all *Granaria* and *Granopupa* species and in the other Chondrinidae species with an apertural dentition that is not reduced, viz. several *Abida* and *Chondrina* species, the infracolumellaris is clearly visible. Therefore, an obsolete infracolumellaris as in

Graniberia braunii, is considered the apomorphic character state. For both the location of the palatal lamellae and the prominence of the apertural lip this is also concluded. A similar reasoning is accepted here, with only the marginal note that very few *Abida* and *Chondrina* species have more or less clearly developed a thickened apertural border, whereas in only very few *Abida* species deep palatal folds occur. Two subspecies are currently recognized, viz. *Graniberia braunii braunii* (Rossmässler, 1842) and *G. braunii marcusi* (Gittenberger & Ripken, 1993).

Derivatio nominis. *Graniberia* after the distribution of a genus resembling *Granaria* in the Iberian peninsula.

Monophyly of the *Granaria* s.l. species

There are no conchological differences known to distinguish between *Granaria* and *Granopupa*. Even the disputable use of a difference in shell size in not tenable anymore. As a consequence, the generic classification of the fossil so-called *Granaria* species is problematic. Unless an overlooked diagnostic character of the shells will be discovered, it will remain impossible to decide in a particular case for either *Granopupa* or *Granaria* on the basis of shell morphology. The generic classification of the fossil chondrinids is problematic anyway, because the diversification of the chondrinid lineages may have taken place an unclear period of time after the Eocene, as is suggested by the fossil record, and by the application of a molecular clock model to the molecular phylogenetic reconstruction (Kokshoorn and Gittenberger 2008). Thus, the oldest '*Granaria*' species, which is known from the Eocene, and the taxa from younger deposits [see Höltke and Rasser (2013)], may be ancestral to the species in the combined six genera that are now considered to constitute the Chondrinidae. The genus *Granaria*, as it is actually accepted in the literature for both extant and extinct species might be polyphyletic.

Acknowledgements

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Supplementary material 1

Supplementary figure 1

Authors: Edmund Gittenberger, Bas Kokshoorn, Ulrich Bößneck, Bastian T. Reijnen, Dirk S.J. Groenenberg

Data type: PDF file

Explanation note: Bayesian phylogeny reconstruction of Chondrinidae based on *COI* (nucleotide + amino acid) sequence data.

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Supplementary material 2

Supplementary figure 2

Authors: Edmund Gittenberger, Bas Kokshoorn, Ulrich Bößneck, Bastian T. Reijnen, Dirk S.J. Groenenberg

Data type: PDF file

Explanation note: Bayesian phylogeny recontruction of Chondrinidae based on *16S* sequence data.

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REVIEW ARTICLE

Redescription of poorly known species of *Ceratothoa* Dana, 1852 (Crustacea, Isopoda, Cymothoidae), based on original type material

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Abstract

Due to the difficulty in accurately identifying cymothoids, these parasitic isopods are often incorrectly named or confused with other species. Within the genus *Ceratothoa*, a number of recent studies have aimed at clarifying some of the problematic species; however, several of the less studied species still require revision. This paper redescribes, from type material, several poorly known *Ceratothoa* species including *C. angulata*, *C. capri*, *C. carinata*, *C. collaris*, *C. gilberti*, *C. gobii*, *C. guttata*, *C. italica*, *C. oestroides*, and *C. verrucosa*, further resolving taxonomic uncertainties within the genus.

Contents

Introduction

Although being one of the physically larger parasitic isopods, cymothoids are still relatively understudied. Often easily observed, these isopods can be located inside the gills, mouths, body cavities and on external surfaces of their fish hosts (Hadfield et al. 2011). Originally, most studies of these parasites were limited to the more populated and accessible regions of the world, such as Europe and North America (Smit et al. 2014). Early taxonomists included the cymothoid isopods in their extensive monographs, but often these accounts were limited in descriptive information. Over the years, several scientists started focusing on this group in more detail and made significant contributions to knowledge on cymothoids. One such notable work is that of Joergen Christian Schioedte and Frederik Vilhelm August Meinert in their series of monographs from 1881 to 1884, where the different life stages, hosts and distributions were all observed (Schioedte and Meinert 1881, 1883, 1884).

However, several cymothoid species have not been studied in many years. A number of factors could be responsible for this lack of research, including a lack of cymothoid specialists, but it is highly probable that many of these species cannot be accurately identified from the original descriptions. This paper revises these poorly known species of *Ceratothoa* Dana, 1852 with redescriptions based on their type material.

Currently there are 30 *Ceratothoa* species known worldwide (according to the World List of Marine, Freshwater and Terrestrial Isopod Crustaceans database (Bruce and Schotte 2016). *Ceratothoa* is one of the more speciose genera within the family Cymothoidae, and is usually found residing in the buccal cavity of the fish host. Recent descriptions incorporate comprehensive descriptions and figures essential for accurately identifying specimens to species level, often absent in many of the original descriptions (Martin et al. 2013, 2015a, Hadfield et al. 2014a, 2014b). These papers have added new species and made several taxonomic changes (bringing species in and out of synonymy) within this genus, but more work is still required for the remaining species. Several of these species are considered questionable or no longer valid due to the lack of type material or inadequate descriptions. Here these lesser known *Ceratothoa* species are revised and updated, separating valid from invalid information and determining their correct taxonomic status where possible.

Methods

Type material for the *Ceratothoa* species was borrowed where available or drawn at their respective museums. Isopods were processed according to the techniques described in Hadfield et al. (2010, 2013). Species descriptions were prepared in DELTA (Descriptive Language for Taxonomy, see Coleman et al. 2010) using a general Cymothoidae character set. Classification follows that of Brandt and Poore (2003). Host authorities are not included in the text or references; host nomenclature and distribution being sourced from FishBase (Froese and Pauly 2015) and Catalog of Fishes (Eschmeyer 2016).

Abbreviations. MCZ – Museum of Comparative Zoology; MNHN – Muséum national d'Histoire naturelle, Paris; NHMUK – Natural History Museum, London; RMNH – Rijksmuseum voor Natuurlijke Historie (Naturalis Biodiversity Center); SAMC – South African Museum, Cape Town; USNM – National Museum of Natural History, Smithsonian Institution, Washington; ZMHB – Zoologisches Museum, Museum für Naturkunde, Humboldt-Universität Berlin; ZMUC – Zoological Museum, University of Copenhagen; TL – total length; W – width.

Taxonomy

Suborder Cymothoida Wägele, 1989
Superfamily Cymothooidea Leach, 1814
Family Cymothoidae Leach, 1814

Genus *Ceratothoa* Dana, 1852

Ceratothoa Dana, 1852: 303; 1853: 752.—Miers 1876: 104–105.—Haswell 1882: 282.—Schioedte and Meinert 1883: 322–323.—Richardson 1905: 233–234.—

Bowman 1978: 217–218.—Brusca 1981: 177–178.—Bruce and Bowman 1989:
   1–2.—Horton 2000: 1041.—Martin, Bruce and Nowak 2013: 396; 2015a: 253–
   254.—Hadfield, Bruce and Smit 2014a: 449–450; 2014b: 3–4.
Codonophilus Haswell, 1881: 471.— 1882: 283.—Hale 1926: 201, 223.
Rhexana Schioedte & Meinert, 1883: 289–290.
Cteatessa Schioedte & Meinert, 1883: 296–297.
Meinertia Stebbing, 1893: 354; 1900: 642; 1910: 103.—Richardson 1905: 236–
   237.— Menzies 1962: 116.—Schultz 1969: 156.
Rhexanella Stebbing, 1911: 179.
Not *Ceratothoa*.—Dana, 1853: 747.—Richardson 1905: 236.—Schultz 1969: 155.—
   Kussakin 1979: 287 [= *Glossobius* Schioedte & Meinert, 1883].

Type species. *Cymothoa parallela* Otto, 1828 (by subsequent designation, see Martin
et al. 2015a).

   Remarks. Diagnostic characters for *Ceratothoa* include the contiguous and swol-
len antennular bases, triangular cephalon, and the elongate body (2.1–2.9 times as
long as wide). *Ceratothoa* also has a pleotelson and pleonite 1 which are narrower than
the other pleonites, and subequal uropod rami which do not extend past the pleotel-
son. A full diagnosis of the genus is provided by Hadfield et al. (2014b).

Ceratothoa angulata (Richardson, 1910)
Figure 1

Meinertia angulata Richardson, 1910: 22, fig. 21.
Codonophilus angulatus.—Nierstrasz 1931: 132.
Ceratothoa angulata.—Bruce and Bowman 1989: 2–4, figs 1–2.—Trilles 1994: 116.—
   Williams, Bunkley-Williams and Pitlik 2000: 157–158.—Paulay, Kropp, Ng and
   Eldredge 2003: 479.—Ravichandran, Rameshkumar and Trilles 2011: 1–3.—
   Rameshkumar, Ravichandran and Sivasubramanian 2013: 99–105.

Material examined. *Holotype*. United States National Museum, USA (USNM 41008)
– female (21 mm TL; 8 mm W), from Port San Pio, Philippines, near mouth of a small
stream, host unknown, 11 Nov 1908 (Richardson 1910).
   Description. *Holotype female.* Length 21 mm, width 8 mm.
   Body oval, twice as long as greatest width, dorsal surfaces smooth and polished
in appearance, widest at pereonite 5, most narrow at pereonite 7, lateral margins
posteriorly ovate. *Cephalon* 0.5 times longer than wide, visible from dorsal view,
triangular. *Frontal margin* rounded to form blunt rostrum. *Eyes* oval with distinct
margins, one eye 0.3 times width of cephalon, 0.5 times length of cephalon. *Antennula*
more stout but same length as antenna, same length as antenna, with 7 articles. *Antenna*
with 7 articles; antennae extending to middle of the eye.
   Pereonite 1 with a slight dorsomedial projection, anterior border straight, antero-
lateral angles extending to anterior margin of eyes with wide truncated and dorsally
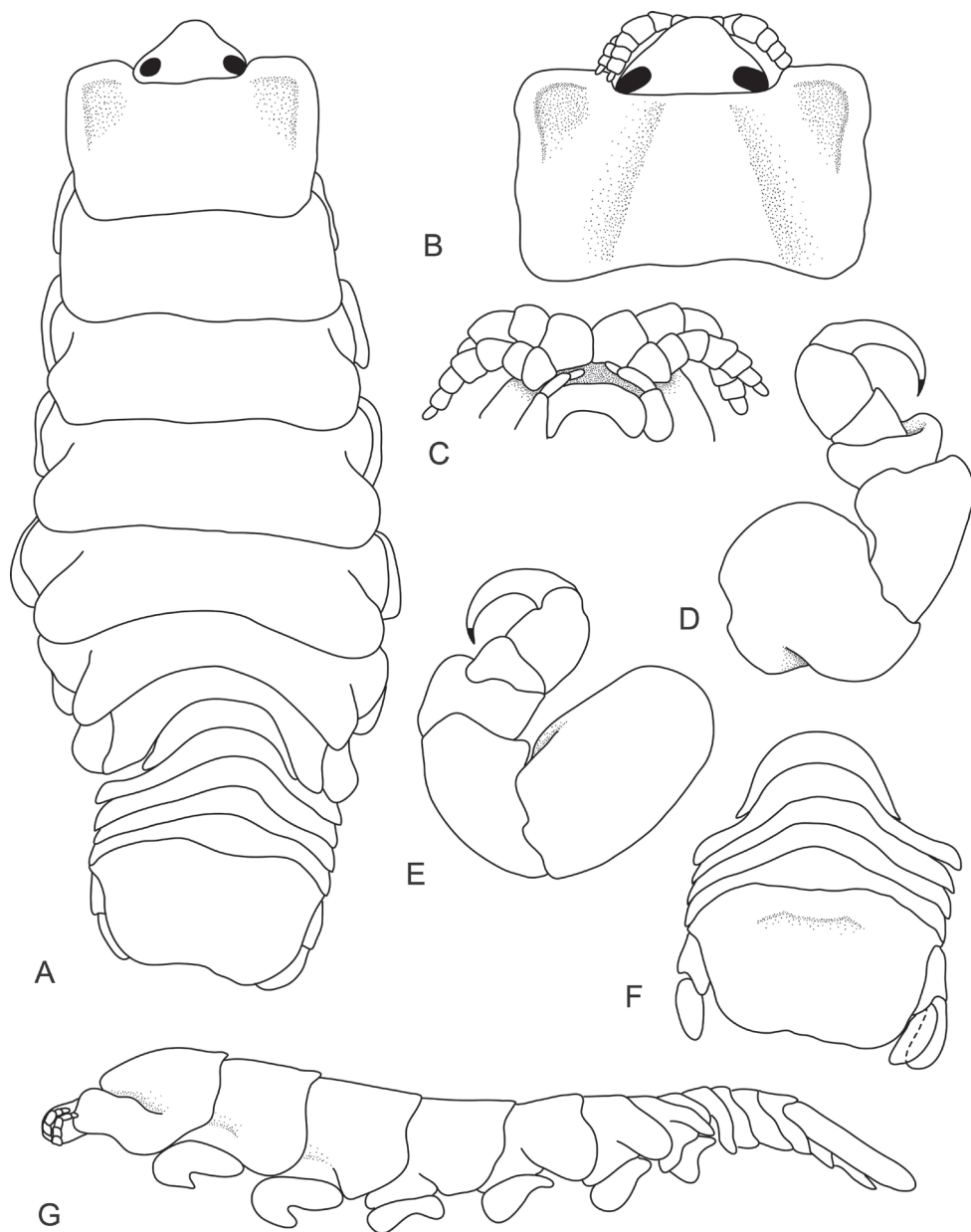
Figure 1. *Ceratothoa angulata* (Richardson, 1910), female holotype (21 mm) (USNM 41008). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** ventral view of cephalon **D** pereopod 1 **E** pereopod 7 **F** dorsal view of pleotelson **G** lateral view.

projected ridges, slight depression at base of each ridge. Posterior margins of pereonites smooth and straight, with posteroventral angles rounded. Coxae 4–7 rounded; not extending past pereonite margin. Pereonites 1–5 increasing in length and width, 6–7 decreasing in length and width, becoming more progressively rounded posteriorly.

Pleon with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin smooth, mostly concave; pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 posterior margin produced medially. *Pleotelson* 0.6 times as long as anterior width, dorsal surface smooth, lateral margins posteriorly narrow, posterior margin subtruncate and shallowly emarginate.

Pereopod 1 basis 1.6 times as long as greatest width; ischium 0.8 times as long as basis; merus proximal margin with bulbous protrusion; carpus with straight proximal margin; propodus 1.2 times as long as wide; dactylus slender, 1.5 as long as propodus, 3 times as long as basal width. *Pereopod 7* basis 0.8 times as long as greatest width; ischium as long as basis, with a large proximal bulbous protrusion; merus proximal margin with large bulbous protrusion, merus 0.5 times as long as wide, 0.3 times as long as ischium; carpus 0.8 times as long as wide, 1.1 times as long as ischium, without bulbous protrusion; propodus 1.3 times as long as wide, 0.5 times as long as ischium; dactylus slender, 1.7 times as long as propodus, 3.3 times as long as basal width.

Uropod same length or slightly longer than the pleotelson; peduncle 1.3 times longer than rami, peduncle lateral margin without setae; rami subequal, extending beyond pleotelson, marginal setae absent. *Endopod* 2.6 times as long as greatest width, straight medial margin, convex lateral margin, apically slightly pointed; e*xopod* 2.3 times as long as greatest width, extending to end of endopod, apically rounded.

Size. Female: 17.5–21.5 mm TL (9 mm W); male: 7 mm TL (Bruce and Bowman 1989, Williams et al. 2000).

Distribution. Known from the western and central Indo-Pacific region: Philippines (Richardson 1910); Indonesia (Nierstrasz 1931, Bruce and Bowman 1989); Guam, Micronesia (Williams et al. 2000); and India (Ravichandran et al. 2011, Rameshkumar et al. 2013). The record in Guam extends the range of this species by 2060 km and since this species has only ever been found on the one host species, the isopod range might extend even further as the host has a wider known geographic range in the Indo-Pacific. Ravichandran et al. (2011) recorded this species from India supporting suggestions by Bruce and Bowman (1989) and Williams et al. (2000) that *C. angulata* may have a similar distribution to its host.

Hosts. In the buccal cavity of Dussumier's halfbeak, *Hyporhamphus dussumieri* (previously *H. laticeps*) (Bruce and Bowman 1989, Williams et al. 2000, Ravichandran et al. 2011).

Remarks. The distinguishing characters of *C. angulata* include the truncate anterolateral margins of pereonite 1 which form distinct ridges on both lateral sides and two small medial depressions, the slightly emarginate and truncate pleotelson, and the broadly rounded uropodal exopod. The unusually large, quadrate pereonite 1 formed from the lateral ridges is very characteristic for this species.

Richardson's (1910) description was based on a single specimen, a female from an unidentified host in the Philippines, and consisted of a short description with a single figure. Bruce and Bowman (1989) provided a redescription based on the holotype

(with only two figures) and additional material from Borneo (a non-ovigerous female and male), including a short description of the male and figures for both specimens.

Ceratothoa angulata resembles *C. guttata* with the narrow pleon and pleotelson but the unique pereonite 1 makes it readily distinguishable from other species.

Ceratothoa capri (Trilles, 1964)
Figure 2

Meinertia capri Trilles, 1964a: 188–198, figs 1–41; 1972a: 1218–1220, figs 219–263, pl. II (17), pl. III (22); 1972b: 1256.—Trilles and Raibaut 1973: 277.
Ceratothoa capri.—Trilles 1986: 623, tab. 1; 1994: 116.—Horton 2000: 1045–1046, figs 5 (c–e).—Rodríguez-Sánchez, Serna and Junoy 2001: 154.—Junoy and Castello 2003: 307.—Kirkim, Kocataş, Katağan and Sezgin 2008: 382–385.—Kirkim, Ozcan and Katagan 2009: 1079–1085, figs 2B & 3B–E.—Innal and Kirkim 2012: A13–A16, figs 1A–B.—Al-Zubaidy and Mhaisen 2013: 166–172, fig. 5.

Material examined. *Lectotype* [here designated]. National Museum of Natural History, Paris (MNHN-IU-2014-17477) – female (16 mm TL, 8 mm W) collected in buccal cavity of *Capros aper* off coast of Nouvelle (Aude, France, Mediterranean), 400–500 m depth, sample (n°81) (Trilles 1964a, 1972a). Also noted: dissected maxilliped, P5–P7 damaged or missing, pleopod 1–2 missing, uropods missing, antennae on one side missing. *Paralectotype*. Male (6 mm TL), same data as lectotype (MNHN-IU-2007-4028) (Trilles 1964a, 1972a).

Description. *Lectotype female.* Length 16 mm, width 8 mm.

Body oval, 1.7 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1, lateral margins posteriorly ovate. *Cephalon* 0.5 times longer than wide, visible from dorsal view, triangular. Frontal margin rounded to form blunt rostrum. *Eyes* oval with distinct margins, one eye 0.3 times width and length of cephalon. *Antennula* more stout than antenna. *Antenna* with 8 articles.

Pereonite 1 smooth, anterior border straight, anterolateral angle acute, anteriorly produced, extend to anterior margin of eyes. Posterior margins of pereonites smooth and slightly curved laterally. *Coxae* 2–3 narrow; 4–7 with rounded point; not extending past pereonite margin. *Pereonites* 1–5 increasing in length and width; 6–7 decreasing in length and width; 6 and 7 narrower and becoming more progressively rounded posteriorly. *Pleon* with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin smooth, mostly concave. Posterolateral angles of pleonite 2 narrowly rounded.

Pereopod 1 basis 1.7 times as long as greatest width; ischium 0.8 times as long as basis; merus proximal margin with bulbous protrusion; carpus with straight proximal margin; propodus 1.6 times as long as wide; dactylus slender, 1.1 times as long as propodus, 2.9 times as long as basal width. *Pleonites* 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4, posterior margin
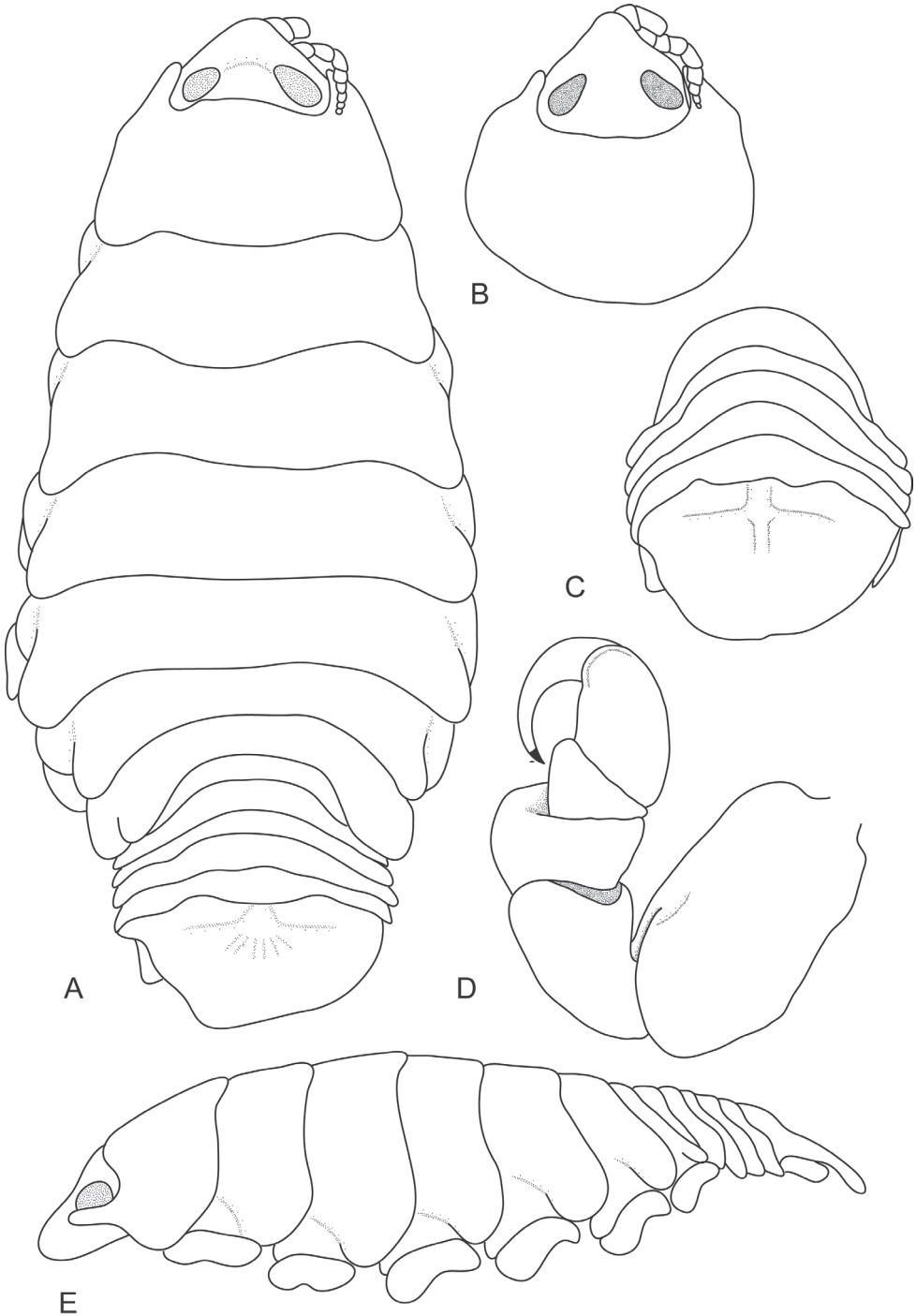
Figure 2. *Ceratothoa capri* (Trilles, 1964), female lectotype (16 mm) (MNHN-IU-2014-17477). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** dorsal view of pleotelson **D** pereopod 1 **E** lateral view.

produced medially. *Pleotelson* 0.6 times as long as anterior width, dorsal surface with 2 sub-medial depressions, lateral margins weakly convex, posterior margin subtruncate.

Size. Female: 13–20 mm TL; male: 6–7 mm TL; second pullus: 2.5–3.5 mm TL (Trilles 1964a, 1972a, b).

Distribution. Throughout the Mediterranean with records from France (Trilles 1964a); Tunisia (Trilles and Raibaut 1973); Straits of Gibraltar and the Alborán Sea (southern Iberian Peninsula) (Rodríguez-Sánchez et al. 2001); Turkey (Kirkim et al. 2008, Innal and Kirkim 2012); Cyprus (Kirkim et al. 2009); and Yemen (Al-Zubaidy and Mhaisen 2013).

Rodríguez-Sánchez et al. (2001) stated that *C. capri* was found in the Atlantic and Mediterranean Sea including the Bay of Biscay (Bolívar 1892), Canary Islands (Koelbel 1892) and the Iberian Mediterranean (Barceló Combis 1875, Balcells 1953, Trilles 1977). None of these papers quoted by Rodríguez-Sánchez et al. (2001) mention *C. capri*, and it is possible that these were erroneous reference entries for this species. These references were probably intended for *C. oestroides*, which is mentioned in each of these articles and was also collected by Rodríguez-Sánchez et al. (2001). Junoy and Castello (2003) repeated this *lapsus* of the references in their checklist for the Iberian Peninsula and Balearic Islands. Rodríguez-Sánchez et al. (2001) also made reference to specific GPS co-ordinates in their paper which appear inaccurate as they correspond to localities on land instead of the expected aquatic points necessary for an oceanographic expedition.

Hosts. On the branchio-spines in the gill and on the bottom of the buccal cavity of *Capros aper* (see Trilles 1964a, 1972a, Trilles and Raibaut 1973); from the buccal cavity of *Boops boops* and *Spicara smaris* (see Kirkim et al. 2008, Innal and Kirkim 2012); *Centracanthus cirrus* (see Kirkim et al. 2009); and *Chelon macroleps* (see Al-Zubaidy and Mhaisen 2013).

Remarks. *Ceratothoa capri* can be distinguished by the acute anterolateral margins which extend past the prominent eyes; body widest at pereonite 5; a narrow pleotelson; and no appendix masculina on the second pleopod in males.

There are a number of species of *Ceratothoa* in the Mediterranean; however *C. capri* differs from them all. There are several differences between *C. capri* and *C. gobii* but the most obvious is the bilobed pleotelson in *C. gobii* which is absent in *C. capri*. The defining pereonite 1 characters of *C. collaris* are absent in *C. capri* and differences between *C. capri* and *C. italica* include less developed eyes, acute and produced anterior margin of the cephalon and the more truncate body of *C. italica*. Similar characters separate it from *C. steindachneri* as well as the number of articles of the antennae and *C. oxyrrhynchaena* differs from *C. capri* in the shape of the 7th pereopod basis of the female. Lastly, *C. oestroides* is less globular or elliptical when compared to *C. capri*; is darker in the post-cephalic region due to more chromatophores; has shorter uropods; and a more stout body.

In the original description of this species, Trilles (1964a) did not designate a holotype; however, full descriptions of the female, male and second pullus were given along with figures of each. Several years later, Trilles (1972a) listed a male and female *C. capri*

located in the buccal cavity of *Capros aper* from the Gulf of Lion, Mediterranean, which he stated were the types for the species. Examination of these specimens confirms that they are the syntypes of *C. capri*. The female specimen is here designated as lectotype and redescribed. This lectotype is necessary to fix and stabilise the identity of this species and use of the name.

Ceratothoa carinata (Bianconi, 1869)
Figure 3

Cymothoa carinata Bianconi, 1869: 210–211, pl. II, figs 2 (a–b).—Gerstaecker 1901: 258.
Cymothoa (*Ceratothoa*) *carinata.*—Hilgendorf 1879: 846.
Ceratothoa carinata.—Schioedte and Meinert 1883: 327–329, pl. XIII (Cym. XX) figs 1–2.—Trilles 1986: 623, tab. 1; 1994: 116–117; 2008: 23.—Kensley 2001: 232.—Bruce 2007: 278.—Trilles 2008: 23.—Martin, Bruce and Nowak 2013: 397–401, figs 1–3.—Nagasawa, Fukuda and Nishiyama 2014: 59–61, fig. 1.— Martin, Bruce and Nowak 2015a: 266–267.
Meinertia carinata.—Lanchester 1902: 378.—Stebbing 1910: 103–104.—Trilles 1972b: 1244–1245, 1256, pl. I, photos 5–7; 1972c: 3–7, photos 1–4.—Avdeev 1979: 48, 50.
Codonophilus carinatus.—Nierstrasz 1931: 132.
*Ceratothoa curvicauda* Nunomura, 2006: 36–38, figs 12–13.
Ceratothoa sp.—Saito 2009: 7–9, photos 1–2.

Material examined. *Neotype* [here designated]. South African Musuem, Cape Town (SAMC-A085795) – female (33 mm TL; 15 mm W), collected from Maputo Bay, Mozambique, from the buccal-cavity of *Selar crumenophthalmus*, November 2013, coll. Wynand Vlok (HP 221). *Paratypes.* Three females (27–31 mm TL; 12–14 mm W), same data as holotype (SAMC-A085796).

Description. *Neotype female.* Length 33 mm, width 15 mm.

Body rectangular, 1.8 times as long as greatest width, dorsal surface with medial longitudinal ridge present, widest at pereonites 3–5, most narrow at pereonite 1, lateral margins slightly convex. *Cephalon* 0.6 times longer than wide, visible from dorsal view, subtriangular. *Frontal margin* rounded to form blunt rostrum. *Eyes* oval with distinct margins, one eye 0.1 times width of cephalon; 0.2 times length of cephalon. *Antennula* more stout and same length as antenna, with 7 articles; antennule peduncle articles 1 and 2 distinct and articulated. *Antenna* with 8 articles.

Pereonite 1 with median projection, anterior border straight, anterolateral angle with small distinct produced point, extend to middle of the eye. Posterior margins of pereonites smooth and straight. Coxae 2–3 narrow; with posteroventral angles rounded; 4–7 with rounded point; not extending past pereonite margin. Pereonites 6 and 7 narrower and becoming more progressively rounded posteriorly. *Pleon* with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin with irregular small
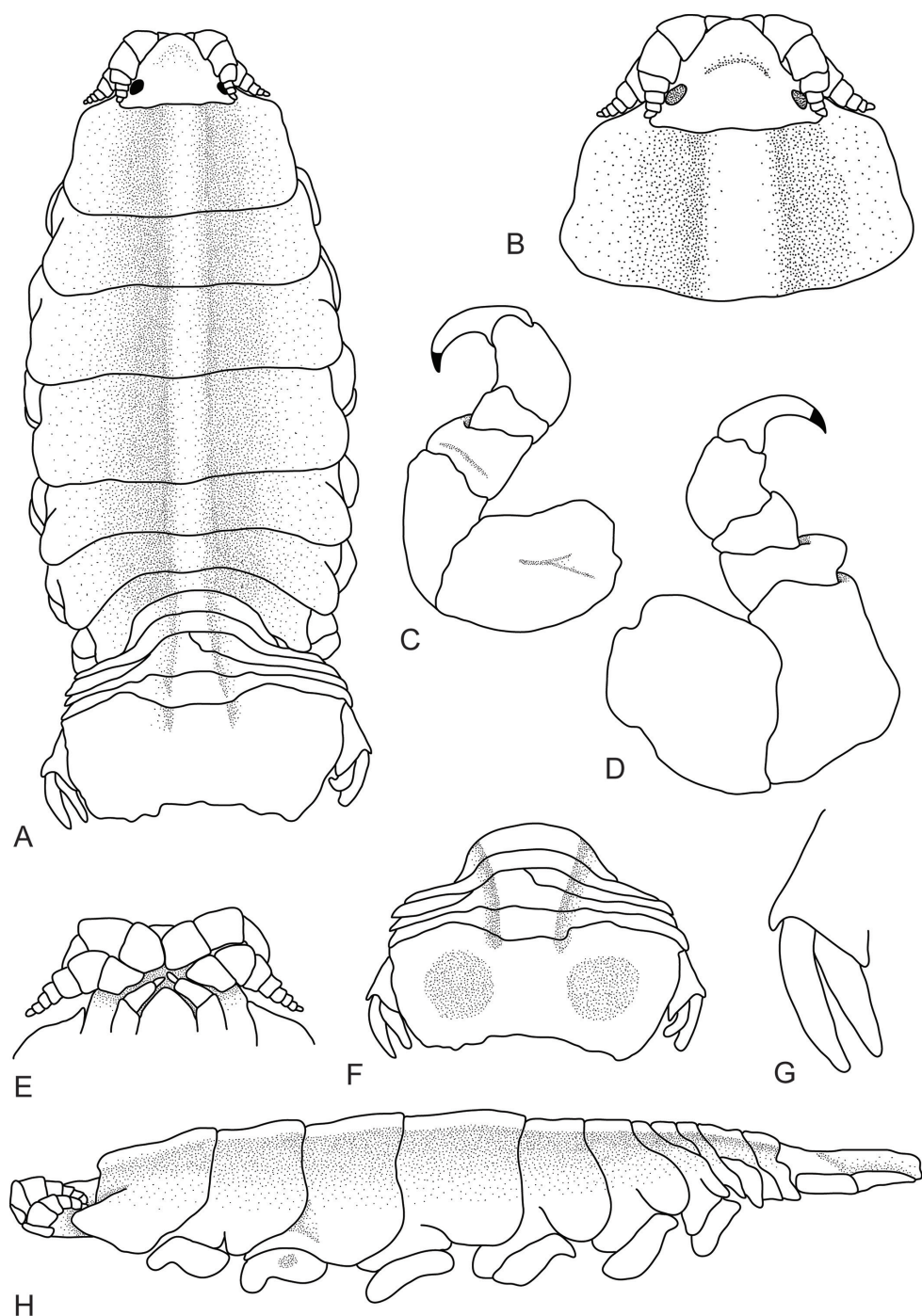
Figure 3. *Ceratothoa carinata* (Bianconi, 1869), female neotype (33 mm) (SAMC-A085795). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** pereonite 1 **D** pereopod 7 **E** ventral view of cephalon **F** dorsal view of pleotelson **G** uropod **H** lateral view.

nodules. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonite 5 widest, posterior margin produced medially. *Pleotelson* 0.5 times as long as anterior width, dorsal surface with 2 sub-medial depressions, lateral margins weakly convex, posterior margin subtruncate and shallowly emarginate.

Pereopod 1 basis 1.5 times as long as greatest width; ischium 0.8 times as long as basis; merus proximal margin without bulbous protrusion; carpus with straight proximal margin; propodus 1.7 times as long as wide; dactylus moderately slender, 1.2 times as long as propodus, 2.9 times as long as basal width. *Pereopod 7* basis as long as greatest width; ischium 1.2 times as long as basis, with a large proximal bulbous protrusion; merus proximal margin with large bulbous protrusion, merus 0.5 times as long as wide, 0.3 times as long as ischium; carpus 0.7 times as long as wide, 0.3 times as long as ischium, without bulbous protrusion; propodus 1.3 times as long as wide, 0.5 times as long as ischium; dactylus moderately slender, as long as propodus, 2.2 times as long as basal width.

Uropod same length as pleotelson, peduncle 0.8 times longer than rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices narrowly rounded. *Endopod* apically rounded, 3 times as long as greatest width, lateral margin straight, mesial margin straight, terminating without setae. *Exopod* extending to end of endopod, 4 times as long as greatest width, apically rounded, lateral margin weakly convex, mesial margin straight, terminating without setae.

Size. Ovigerous female: 28.5–38 mm TL (10–14 mm W); non-ovigerous female: 13–34 mm TL; male: 10–12 mm TL (Bianconi 1869, Schioedte and Meinert 1883, Stebbing 1910, Trilles 1972b, c).

Distribution. Western Indian Ocean and southwest Pacific Ocean: Mozambique (Bianconi 1869, Hilgendorf 1879, Schioedte and Meinert 1883); Great Redangs, Malay Peninsula (Lanchester 1902); Seychelles (Stebbing 1910); New Caledonia (Trilles 1972b, c, Bruce 2007); Red Sea (Trilles 2008); Japan (Nunomura 2006, Saito 2009, Nagasawa et al. 2014); and Australia (Martin et al. 2013).

Hosts. On *Lutjanus adetii* (previously *L. amabilis*) (Trilles 1972b, c); *Pseudocaranx dentex* (see Nunomura 2006); *Decapterus muroadsi* (see Nunomura 2006, Saito 2009, Nagasawa et al. 2014); and *Selar crumenophthalmus* (see Martin et al. 2013, present study).

Remarks. *Ceratothoa carinata* can be identified by the characteristic medial ridge extending longitudinally along the dorsal pereon surface. Furthermore, it has a laterally depressed and wider than long pleotelson; pereonite 7 with an enlarged carinate ischium and large bulbous protrusion on the merus; uropods reaching the distal edge of the pleotelson; as well as a concave posterior margin on the pleotelson.

Bianconi (1869) originally described this species from a single ovigerous female from Mozambique and stated that it was most similar to *C. gaudichaudii* and *C. trigonocephala*. Since then, another species, *C. trillesi* (Avdeev 1979) was also described from the Australia–New Zealand region, and shared many morphological characteristics. There has been some confusion surrounding the synonymy of *C. trillesi* with *C. carinata* (see Avdeev 1979, Trilles 1994), however, these species differ substantially with *C. trillesi* lacking the distinctive medial ridge, enlarged basis and ischium on pereopod 7, and a wide and depressed pleotelson seen in *C. carinata*.

Species and names within *Ceratothoa* have been moved in and out of synonymy, an indication of both the difficulty of identifying and characterising species. Furthermore, many species are variable (Hadfield et al. 2014a) and species morphological boundaries are often unclear. In addition, the Cymothoidae also have groups of cryptic species such as has been seen in *Mothocya* (Bruce 1986) and *Anilocra* (Bunkley Williams and Williams 1981, Bruce 1987) and therefore the designation of a neotype is necessary in the long-term interests of nomenclatural stability.

Schioedte and Meinert (1883) mention a specimen from the type locality (Mozambique) that was originally deposited into Zoologisches Museum, Museum für Naturkunde, Homboldt-Universität, Berlin, Germany. Enquiries to that museum, as well as Muséum National d'Histoire Naturelle, Natural History Museum, London, the Naturalis Biodiversity Center and the Zoological Museum, University of Copenhagen, failed to locate any material that could be identified as the type for *C. carinata*. It is highly probable that this specimen was destroyed in World War II or lost during relocation of the material. As cymothoid isopods are among the most misunderstood and difficult isopods to identify (Brusca 1981), a complete description (or redescription) of the type material is essential for accurate identifications and research on the species. The current material of *C. carinata* was obtained from the type locality and corresponds with the original drawings of the species (Bianconi 1869). Both specimens have the noticeable medial ridge or hump running longitudinally down the length of the pereon. The pleotelson is medially concave and is wider than long. Furthermore, the uropods do not extend past the end of the pleotelson and the posterior margin of the pleotelson is indented medially; the eyes are small but clearly visible; and the antennae are stout and extend to the anterior margin of pereonite 1.

As the current specimen is undoubtedly *C. carinata*, it is hereby designated as the neotype for the species, fulfilling all of the requirements necessary in the International Code of Zoological Nomenclature (Anon 1999, ICZN, Article 75).

Ceratothoa collaris Schioedte & Meinert, 1883
Figure 4

Cymothoa oestroïdes.—Lucas 1849: 78, pl. 8, figs 4a–c (see also page notes in Trilles 1972a p 1201). [not *Ceratothoa oestroides* (Risso, 1826)].

Ceratothoa collaris Schioedte & Meinert, 1883: 366–368, tab. XVI (Cym. XXIII) figs 8–9.—Carus 1885: 443.—Rokicki 1984a: 73; 1984b: 44–60, figs 9–12; 1985: 95–119, tabs. 1–3, fig. 8.—Trilles 1986: 623, tab. 1; 1994: 117.—Horton 2000: 1046–1047, figs 6a–c.—Ramdane and Trilles 2008: 173–178.—Bariche and Trilles 2008: 85–93, figs 1–5.

Meinertia collaris forma *typica*.—Monod 1924a: 31–34; 1924b: 430–432.—Trilles and Raibaut 1973: 277–278.—Capapé and Pantoustier 1976: 203.

Meinertia collaris forma *africana*.—Monod 1924a: 31–34; 1924b: 430–432; 1925: 103–104.—Trilles 1977: 10.

Meinertia collaris forma *globuligera*.—Monod 1924a: 31–34; 1924b: 430–432.
Meinertia collaris.—Trilles 1972b: 1240–1241, pl. I (1–2).—Dollfus and Trilles 1976: 822.—Moreira and Sadowsky 1978: 100, 110, 113–114, 120, 134.
Ceratothoa collaris forma *africana*.—Trilles 1979: 515, 522.
Ceratothoa collaris forma *typica*.—Trilles 1979: 521.

Material examined. *Holotype.* National Museum of Natural History, Paris (MNHN-Is386) – ovigerous female specimen (40 mm TL) collected in Algeria by Lucas (Schioedte and Meinert 1883), host unknown, registered as *Meinertia collaris*, J.P. Trilles det. 17.12.1971 (n°40) (Trilles 1972b). Also noted: both right antennae are missing and some appendages are broken.
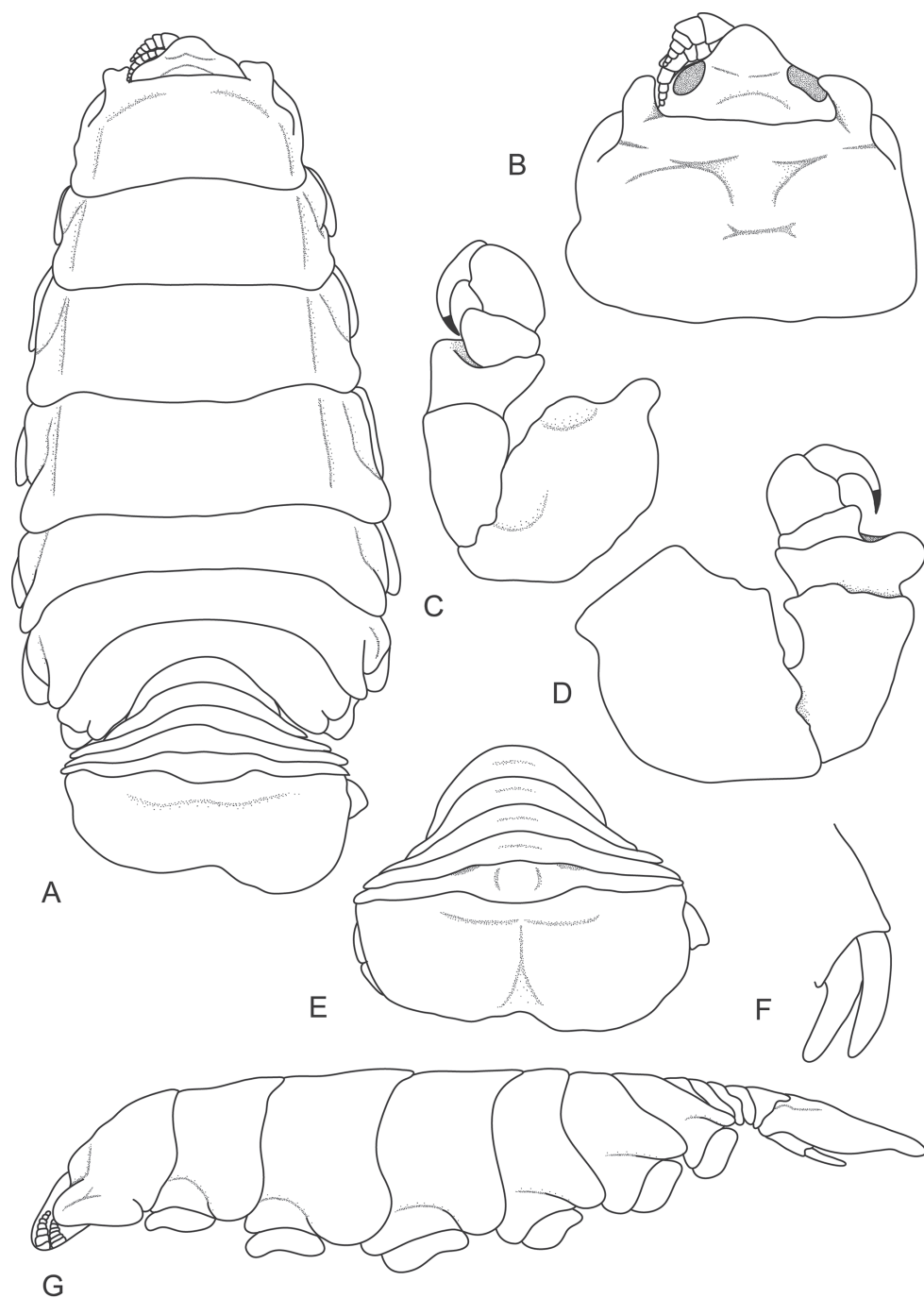
Description. *Holotype female.* Length 40 mm, width 18 mm.

Body oval, 1.8 times as long as greatest width, dorsal surfaces slightly bumpy, widest at pereonite 4 and pereonite 5, most narrow at pereonite 1, lateral margins posteriorly ovate. *Cephalon* 0.6 times longer than wide, visible from dorsal view, triangular. *Frontal margin* rounded to form blunt rostrum. *Eyes* oval with distinct margins. *Antennula* more stout than antenna, shorter than antenna, with 7 articles. *Antenna* with 8 articles.

Pereonite 1 with slight indentations, anterior border straight, anterolateral angle with distinct anterior projection, extend to middle of the eye. Posterior margins of pereonites smooth and slightly curved laterally. Coxae 2–3 narrow; with posteroventral angles rounded; 4–7 acute, posteriorly pointed; not extending past pereonite margin. Pereonites 1–4 increasing in length and width; 5–7 decreasing in length and width; becoming more progressively rounded posteriorly. *Pleon* with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin smooth, mostly concave. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4, posterior margin produced medially. *Pleotelson* 0.4 times as long as anterior width, dorsal surface with medial furrow, lateral margins weakly convex, posterior margin damaged and shallowly emarginate.

Pereopod 1 basis 1.7 times as long as greatest width; ischium 0.6 times as long as basis; merus proximal margin with bulbous protrusion; carpus with rounded proximal margin; propodus 1.6 times as long as wide; dactylus slender, 0.9 times as long as propodus, 2.1 times as long as basal width. *Pereopod 7* basis 1.4 times as long as greatest width; ischium 0.8 times as long as basis, without protrusions; merus proximal margin with large bulbous protrusion, merus 0.4 times as long as wide, 0.3 times as long as ischium; carpus 0.4 times as long as wide, 0.2 times as long as ischium, without bulbous protrusion; propodus 0.9 times as long as wide, 0.4 times as long as ischium; dactylus slender, 1.5 times as long as propodus, 2.3 times as long as basal width.

Uropod more than half the length of pleotelson, peduncle 0.9 times longer than rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices narrowly rounded. *Endopod* apically rounded, 3.6 times as long as greatest width. *Exopod* extending to end of endopod, 4 times as long as greatest width, apically rounded.

Figure 4. *Ceratothoa collaris* Schioedte & Meinert, 1883, female holotype (40 mm) (MNHN-Is386). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** pereopod 1 **D** pereopod 7 **E** dorsal view of pleotelson **F** uropod **G** lateral view.

Size. Female: 18–40 mm TL (9–18 mm W); male: 9–22 mm TL (4–10 mm W); second pullus: 2.7–2.8 mm TL (0.7–0.8 mm W) (Monod 1924b, Capapé and Pantoustier 1976, Dollfus and Trilles 1976, Trilles 1977, 1979, Rokicki 1984a, b, Bariche and Trilles 2008).

Distribution. Mediterranean and eastern Atlantic Ocean: Algeria (Schioedte and Meinert 1883, Lucas 1849, Trilles 1972b, 1979, Ramdane and Trilles 2008); Morocco (Monod 1924a, b, Trilles 1972b, Dollfus and Trilles 1976); Mauritania (Monod 1924a, b, Trilles 1972b, 1977, Dollfus and Trilles 1976); Tunisia (Trilles and Raibaut 1973, Capapé and Pantoustier 1976); Senegal (Trilles 1979); and Lebanon (Bariche and Trilles 2008).

Ceratothoa collaris is common in Tunisia (Trilles and Raibaut 1973) and Mauritania (Monod 1924a, Trilles 1977). This species has not been collected from the north or north-western Mediterranean countries despite many recent studies there. It has been found in southern areas of the Mediterranean, but never from Libya, Egypt, or Israel.

Hosts. Frequently in the mouth of sparids from the genera *Dentex* and *Pagellus* (especially *Dentex gibbosus* and *Pagellus erythrinus*): *D. gibbosus* (previously *D. filosus*) (see Monod 1924a, b, Trilles 1972b, Trilles and Raibaut 1973, Rokicki 1984b), in *Pagellus erythrinus* (see Monod 1925, Bariche and Trilles 2008); mouth of *Pagellus acarne* (see Trilles 1972b, Dollfus and Trilles 1972); buccal cavity of *Dentex dentex*, *Dentex maroccanus*, *Spicara* sp., *Smaris* sp. and on ventral disc of *Raja miraletus* (see Trilles and Raibaut 1973); on *Sargus sargus*, *Pagellus bogaraveo*, pharynx of *Pagellus erythrinus*, and in gill cavity of a sparid (see Dollfus and Trilles 1972); on gill slits of *Torpedo marmorata* (see Capapé and Pantoustier 1976); in the mouth of *Pseudotolithus moorii* (previously *Corvina camaronensis*) (see Trilles 1977); in the mouth of *Smaris vulgaris* and on gills of *Pagellus* sp. (see Trilles 1979); *Dentex macrophthalmus*, *Pagrus pagrus* (see Rokicki 1984b); in the branchial cavity of *Pagrus caeruleostictus* (see Ramdane and Trilles 2008, Bariche and Trilles 2008); less frequent on *Dentex macrophthalmus*, *Pagellus acarne*, *Pagrus* sp., and rarely on *Dicentrarchus labrax* and *Epinephelus aeneus* (see Bariche and Trilles 2008).

Lucas (1849) considered *C. collaris* to have a low host specificity (euryxenic) but Bariche and Trilles (2008) showed that there is a clear preference for Sparidae fish, particularly *Pagellus erythrinus*, which is commonly parasitised in Lebanon and Africa (Morroco and Algeria). Monod (1925) also stated how most of these isopods recorded from *Dentex filosus* were actually removed from *Pagellus erythrinus*, especially in the case of *C. collaris* forma *africana*.

Remarks. *Ceratothoa collaris* can be distinguished by the prominent anterolateral projections which do not extend past the eyes and form a collar-like structure from where it gets its name. It also has a wide pleotelson (same width or wider than pleon), uropods that do not extend past the pleotelson and a large bulbous protrusion on the pereopod 7 merus.

Ceratothoa collaris was described from Algeria, originally misidentified as *C. oestroides* by Lucas (1849). Later, Monod (1924a) described three different forms of this species, namely *C. collaris* forma *globuligera*, *C. collaris* forma *africana* and *C. collaris* forma *typica* based on morphological differences of their cephlon and antennae (Monod 1924a). Over

the years, many researchers have identified other species where many forms are common, such as *C. steindachneri* (see Horton 2000), but naming the different forms are not necessary, thus Bariche and Trilles (2008) removed the three *C. collaris* forms.

Ceratothoa gilberti (Richardson, 1904)
Figure 5

Meinertia gilberti Richardson, 1904: 53, figs 32–33; 1905: 241–242, figs 247–249.—
    Schultz 1969: 157–158, fig. 237.
Codonophilus gilberti.—Nierstrasz 1931: 132.—Brusca 1977: 130; 1980: 230, 232,
    fig. 12.17.
Meinertia sp.—MacGinitie 1937: 1031, 1035, pl. I, fig. 1.
Ceratothoa gilberti.—Wallerstein 1980: 232.—Brusca 1981: 178–182, figs 21a–d, figs
    22a–l.—Avdeev 1982a: 65–67; 1982b: 69–77.—Brusca and Iverson 1985: 49.—
   Trilles 1994: 119.—Espinosa-Pérez and Hendrickx 2001: 48.

Material examined. *Lectotype* [here designated]. United States National Museum, USA (USNM 1254761) – female (22 mm TL; 9.5 mm W) collected from Mazatlan, Sinaloa (Mexico) in mouth of *Mugil hospes* (see Richardson 1904). The left side of pereonites 3–5 were damaged. *Paralectotypes*. Two males (11–12 mm TL; 4–5 mm W), same data as lectotype (USNM 29080).

Description. *Lectotype female.* Length 22 mm, width 9.5 mm.

Body oval, 1.8 times as long as greatest width, dorsal surfaces slightly bumpy, widest at pereonite 4 and pereonite 5, most narrow at pereonite 1, lateral margins posteriorly ovate. *Cephalon* 0.6 times longer than wide, visible from dorsal view, triangular. *Frontal margin* rounded to form blunt rostrum. *Eyes* oval with distinct margins. *Antennula* more stout than antenna, shorter than antenna, with 7 articles. *Antenna* with 8 articles.

Pereonite 1 with slight indentations, anterior border straight, anterolateral angle with distinct anterior projection, extend to middle of the eye. Posterior margins of pereonites smooth and slightly curved laterally. Coxae 2–3 narrow; with posteroventral angles rounded; 4–7 acute, posteriorly pointed; not extending past pereonite margin. Pereonites 1–4 increasing in length and width; 5–7 decreasing in length and width; becoming more progressively rounded posteriorly. *Pleon* with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin smooth, mostly concave. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4, posterior margin produced medially. *Pleotelson* 0.4 times as long as anterior width, dorsal surface with medial furrow, lateral margins weakly convex, posterior margin damaged and shallowly emarginate.

Pereopod 1 basis 1.7 times as long as greatest width; ischium 0.6 times as long as basis; merus proximal margin with bulbous protrusion; carpus with rounded proximal margin; propodus 1.6 times as long as wide; dactylus moderately slender, 0.9 times as
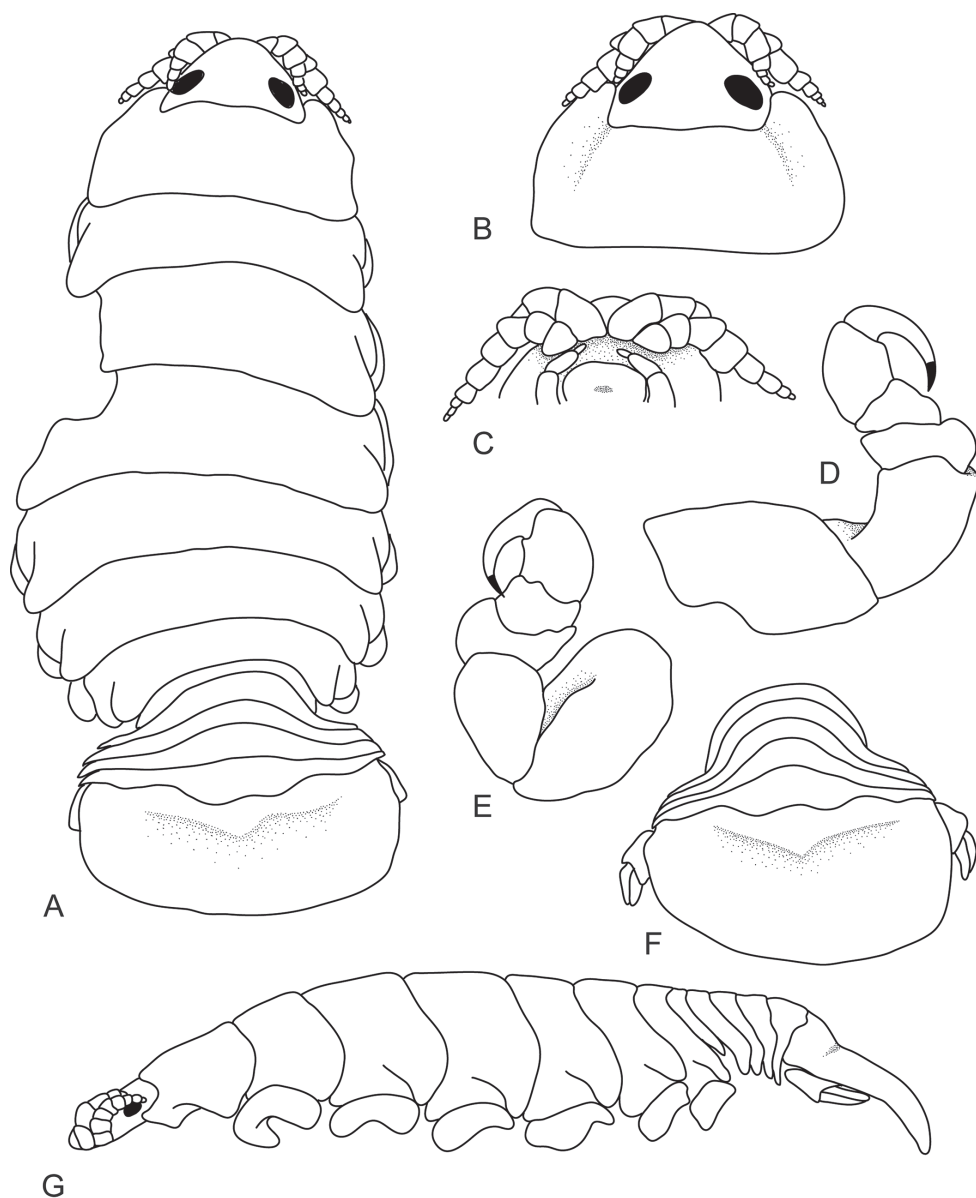
Figure 5. *Ceratothoa gilberti* (Richardson, 1904), female lectotype (22 mm) (USNM 1254761). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** ventral view of cephalon **D** pereopod 1 **E** pereopod 7 **F** dorsal view of pleotelson **G** lateral view.

long as propodus, 2.1 times as long as basal width. *Pereopod 7* basis 1.4 times as long as greatest width; ischium 0.8 times as long as basis, without protrusions; merus proximal margin with large bulbous protrusion, merus 0.4 times as long as wide, 0.3 times as long as ischium; carpus 0.4 times as long as wide, 0.2 times as long as ischium, without

bulbous protrusion; propodus 0.9 times as long as wide, 0.4 times as long as ischium; dactylus slender, 1.5 times as long as propodus, 2.3 times as long as basal width.

Uropod more than half the length of pleotelson, peduncle 0.9 times longer than rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices narrowly rounded. *Endopod* apically rounded, 3.6 times as long as greatest width. *Exopod* extending to end of endopod, 4 times as long as greatest width, apically rounded.

Size. Female: 16–29 mm TL (8–14 mm W) (Brusca 1981).

Distribution. Known from the south-western coast of northern America in the Gulf of California region: from southern California, USA (MacGinitie 1937, Brusca 1981, Espinosa-Pérez and Hendrickx 2001) and the west coast of Baja California to Puerto Penasco and Mazatlan, Mexico (Richardson 1904, Nierstrasz 1931, Brusca 1977, Brusca 1981, Espinosa-Pérez and Hendrickx 2001).

Hosts. On tongue of the mullet *Mugil cephalus* (see MacGinitie 1937, Brusca 1977, 1981); from the mullet, *Mugil hospes* (see Richardson 1904, Brusca 1977, 1981); and from a "flatfish" (Brusca 1981).

Remarks. *Ceratothoa gilberti* has an elongate body; pleon as wide as pereon; short uropods; a elongate, triangular cephalon; short anterolateral projections on pereonite 1; and a large pleotelson with a rounded posterior margin. Furthermore, Brusca (1981) previously noted that *C. gilberti* lacks an appendix masculina in the male. The female specimen is here designated as the lectotype and redescribed.

Ceratothoa gilberti has been infrequently collected and seems to be confined to the region around the Gulf of California. It has only been found on mullet species and has often been compared to *C. gaudichaudii* (which has recently been placed into *species inquirenda* by Martin et al. [2015]).

Ceratothoa gobii Schioedte & Meinert, 1883
Figure 6

Ceratothoa Gobii Schioedte & Meinert, 1883: 356–358, tab. XV (Cym. XXII) figs 12–13.
*Ceratothoa gobii*.—Carus 1885: 443.—Trilles 1994: 119.—Horton 2000: 1042–1043.
Meinertia gobii.—Montalenti 1948: 36.

Material examined. *Holotype.* Museum of Comparative Zoology, USA (MCZ 3707) – female (12 mm TL; 5 mm W), from the sand goby, *Gobius minutus*, from Messina, Italy, coll. Haeckel. Specimen with broken antennae and damaged pereonite 2.

Description. *Holotype female.* Length 12 mm, width 5 mm.

Body elongate, 1.9 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1, lateral margins slightly convex. *Cephalon* 0.7 times longer than wide, visible from dorsal view, triangular. *Frontal margin* rounded to form blunt rostrum. *Eyes* oval with distinct margins, one eye 0.3 times width of cephalon; 0.6 times length of cephalon.
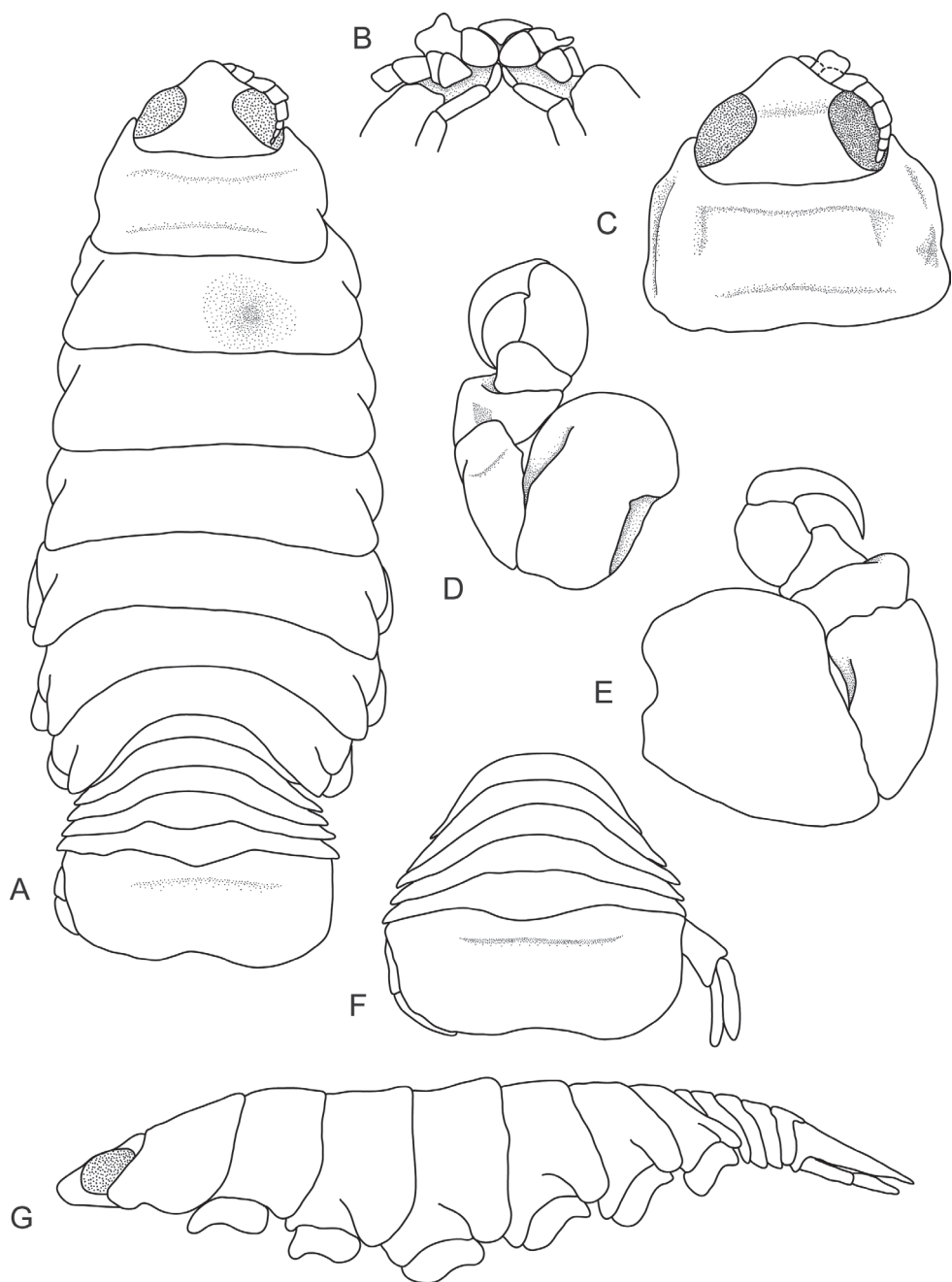
Figure 6. *Ceratothoa gobii* Schioedte & Meinert, 1883, female holotype (12 mm) (MCZ 3707). **A** dorsal view **B** ventral view of cephalon **C** dorsal view of pereonite 1 and cephalon **D** pereopod 1 **E** pereopod 7 **F** dorsal view of pleotelson **G** lateral view.

Pereonite 1 smooth, anterior border straight, anterolateral angle with small distinct produced point and produced past frontal margin of cephalon, extend to middle of the eye. Posterior margins of pereonites smooth and straight. With posteroventral angles rounded; coxae 4–7 rounded; not extending past pereonite margin. Pereonites 1–5 increasing in length and width; 6–7 decreasing in length and width; becoming more progressively rounded posteriorly. *Pleon* with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin smooth, mostly concave. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4, posterior margin produced medially. *Pleotelson* 0.4 times as long as anterior width, dorsal surface smooth, lateral margins weakly convex, posterior margin subtruncate and shallowly emarginate.

Pereopod 1 basis 1.4 times as long as greatest width; ischium 0.7 times as long as basis; merus proximal margin with bulbous protrusion; carpus with rounded proximal margin; propodus 1.7 times as long as wide; dactylus slender, as long as propodus, 3 times as long as basal width. *Pereopod 7* basis as long as greatest width; ischium 0.8 times as long as basis, without protrusions; merus proximal margin with large bulbous protrusion, merus 0.4 times as long as wide, 0.3 times as long as ischium; carpus 0.7 times as long as wide, 0.3 times as long as ischium, with slight bulbous protrusion; propodus 1.1 times as long as wide, 0.4 times as long as ischium; dactylus slender, 1.6 times as long as propodus, 2.7 times as long as basal width.

Uropod same length or slightly longer than the pleotelson, peduncle 0.6 times longer than rami, peduncle lateral margin without setae; rami extending beyond pleotelson, marginal setae absent, apices narrowly rounded. *Exopod* extending to end of endopod.

Remarks. *Ceratothoa gobii* has a triangular cephalon with a sub-truncate rostrum; large eyes which together take up more than half of the cephalon; uropods which extend past the posterior margin of the pleotelson; short anterolateral projections on pereonite 1; and pleonites 1–5 gradually becoming wider.

This species is based on the description of a single specimen by Schioedte and Meinert (1883) without mention of a type host; however, *Gobius minutus* (now *Pomatoschistus minutus*) is listed on the information in the museum bottle. This species has only been collected once.

Ceratothoa guttata (Richardson, 1910)
Figure 7

Meinertia guttata Richardson, 1910: 20–21, fig. 19.
Codonophilus guttatus.—Nierstrasz 1931: 132.
Meinertia venusta Avdeev, 1978: 30–32, pl. 1.
Ceratothoa venusta.—Avdeev 1981: 1160–1167, fig. 3; 1990: 32–42, figs 1–6.—
     Trilles 1986: 625, tab. 1; 1994: 129–130.

Ceratothoa guttata.—Bruce and Bowman 1989: 4–7, figs 3–4.—Trilles 1994: 119–120.—Kensley 2001: 232.—Bruce, Lew Ton and Poore 2002: 172.—Martin, Bruce and Nowak 2015a: 271–272.

Material examined. *Lectotype* [here designated]. United States National Museum, USA (USNM 1254762) – female (17 mm TL; 7 mm W), 7 Feb 1908, obtained off Jolo Island, Philippines; from a flying fish 4–5 inches long (Richardson 1910). Specimen's left side slightly damaged from pereonite 4 to 6. *Paralectotypes*. Five females (13.5–16 mm TL; 5–6 mm W), same data as lectotype (USNM 40914).

Description. *Lectotype female.* Length 17 mm, width 7 mm.

Body oval and elongate, twice as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5, most narrow at pereonite 1, lateral margins ovate. *Cephalon* 0.6 times longer than wide, visible from dorsal view, triangular. *Frontal margin* rounded to form blunt rostrum. *Eyes* irregular in outline. *Antennula* and antenna stout and same length. Antennula with 7 articles, *antenna* with 8 articles.

Pereonite 1 smooth, anterior border straight, anterolateral angle with small distinct anterior projection extending to base of eyes. Posterior margins of pereonites slightly produced medially. Coxae 2–3 wide, with posteroventral angles rounded; 4–7 large and produced on pereonite margins, not extending past pereonite margin. Pereonites 1–5 increasing in length and width; 6–7 decreasing in length and width; 2–5 subequal. *Pleon* with pleonite 1 most narrow, visible in dorsal view; pleonites posterior margin smooth, mostly concave. Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 forming acute point. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4, posterior margin produced medially. *Pleotelson* 0.6 times as long as anterior width, dorsal surface smooth, lateral margins weakly convex, posterior margin shallowly emarginate.

Pereopod 1 basis 1.5 times as long as greatest width; ischium 0.8 times as long as basis; merus proximal margin with large bulbous protrusion; carpus with straight proximal margin; propodus as long as wide; dactylus slender, 1.4 times as long as propodus, 2.4 times as long as basal width. *Pereopod 7* basis 0.8 times as long as greatest width; ischium 1.3 times as long as basis, with a large proximal bulbous protrusion overlapping merus; merus proximal margin with large distal bulbous protrusion, merus 0.7 times as long as wide, 0.3 times as long as ischium; carpus 0.7 times as long as wide, 0.3 times as long as ischium, without bulbous protrusion; propodus 1.4 times as long as wide, 0.5 times as long as ischium; dactylus slender, 1.4 times as long as propodus, 2.6 times as long as basal width.

Uropod more than half the length of pleotelson, peduncle 0.8 times longer than rami.

Other material. Holotype of *Ceratothoa venusta.* Russian Pacific Federal Fisheries Research Institute (AGK 75054) – on flying fish, *Parexocoetus brachypterus*, from the Red Sea (Avdeev 1978).

Size. Ovigerous females: 14.5–23.0 mm TL; non-ovigerous females: 15.5–16.5 mm TL; males: 5.4–7.4 mm TL (Bruce and Bowman 1989).
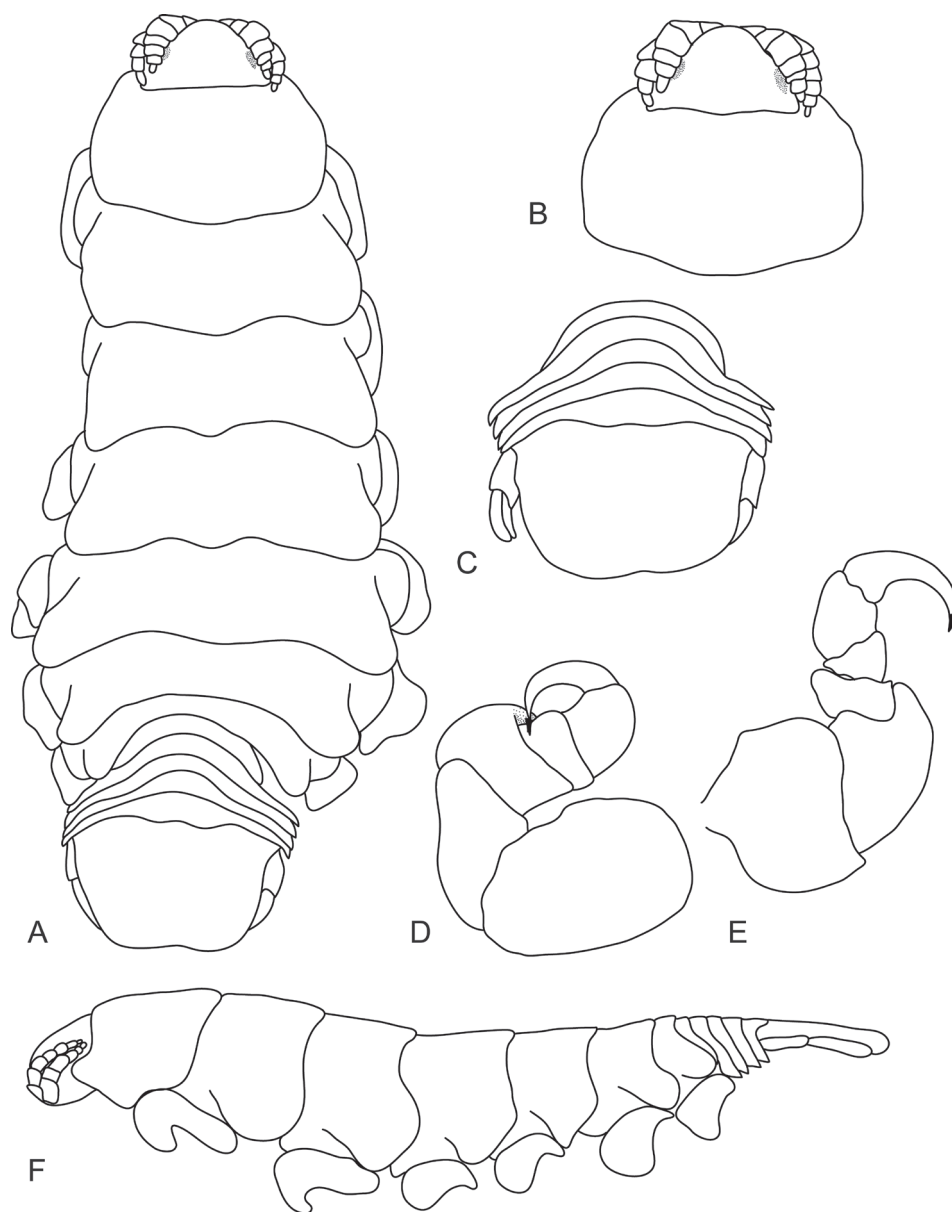
Figure 7. *Ceratothoa guttata* (Richardson, 1910), female lectotype (17 mm) (USNM 1254762). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** dorsal view of pleotelson **D** pereopod 1 **E** pereopod 7 **F** lateral view.

Distribution. Central and Western Indo-Pacific: Philippines (Richardson 1910, Nierstrasz 1931, Bruce and Bowman 1989, Kensley 2001); Red Sea (Avdeev 1978, Bruce and Bowman 1989, Kensley 2001); Madagascar; Australia; and Taiwan (Bruce and Bowman 1989, Kensley 2001).

Hosts. In mouths of flying fish, *Parexocoetus brachypterus* (see Bruce and Bowman 1989, Avdeev 1978).

Remarks. *Ceratothoa guttata* is distinguished by the elongate body widest at pereonite 5; uropods which do not extend past the posterior margin of the pleotelson; a narrow pleon; an expanded merus on pereopod 1; and an expanded ischium and merus on pereopod 7.

Ceratothoa guttata is considered to be highly host specific as it has, to date, only been reported from a single host, *Parexocoetus brachypterus*. Avdeev (1978) briefly described *Meinertia venusta* from the Red Sea on *Parexocoetus brachypterus*, comparing it to *C. guttata*. Bruce and Bowman (1989) revised *C. guttata* and synonymised *C. venusta* with *C. guttata* after comparing drawings of the two species. Similar morphology of pereopods 1 and 7, coxae, the pleon and the pereon, as well as the host specificity all confirm this synonymy. The largest female is here redescribed and reillustrated and designated as lectotype.

Ceratothoa italica Schioedte & Meinert, 1883
Figure 8

Ceratothoa italica Schioedte & Meinert, 1883: 347–350, tab. XV (Cym. XXII), figs 1–4.—Carus 1885: 442.—Trilles 1979: 521; 1986: 624, tab. 1; 1994: 121; 2008: 23.—Rokicki 1984b: 129–131, fig. 32; 1985: 95–119, tab. 1–3, fig. 6.—Trilles, Radujković and Romestand 1989: 289, fig. 8.—Horton 2000: 1047, figs 7c–e.—Öktener and Trilles 2004: 145–154.—Ramdane, Bensouilah and Trilles 2007: 67–74.
*Meinertia italica*.—Monod 1924a: 34; 1924b: 432–434.—Montalenti 1948: 42–46, figs 11–14, tab. 5, pl. 3.—Trilles 1964b: 106; 1972a: 1212–1215, figs 156–187, pl. II, figs 10–12; 1972b: 1238–1240.—Dollfus and Trilles 1976: 823.
Identity uncertain: *Ceratothoa italica*.—Ateş, Trilles, İşmen and Yiğin 2006: 375–380.—Trilles 2008: 23.

Material examined. *Lectotype* [here designated]. Zoological Museum, University of Copenhagen (ZMUC CRU-6914) – female specimen (36 mm TL, 17 mm W) collected in Rijeka, Croatia (previously called Fiume), Adriatic Sea by Budde-Lund (Schioedte and Meinert 1883), host unknown. Also noted: the female has a broken pereonite 1, pleonite 2 and antenna; pereopod 1 missing and other pereopods are damaged and missing dactylii. *Paralectotypes.* Thirty-seven pullus stage (4–5 mm TL), same data as lectotype (ZMUC CRU-8669); Eighty-two pullus stage (4–5 mm TL), same data as lectotype, label reads "Stor female udt. Som pectotype" (ZMUC CRU-9124).

Description. *Lectotype female.* Length 36 mm, width 17 mm.

Body rectangular and elongate, 1.7 times as long as greatest width, dorsal surfaces smooth and polished in appearance, widest at pereonite 5 and pereonite 6, most
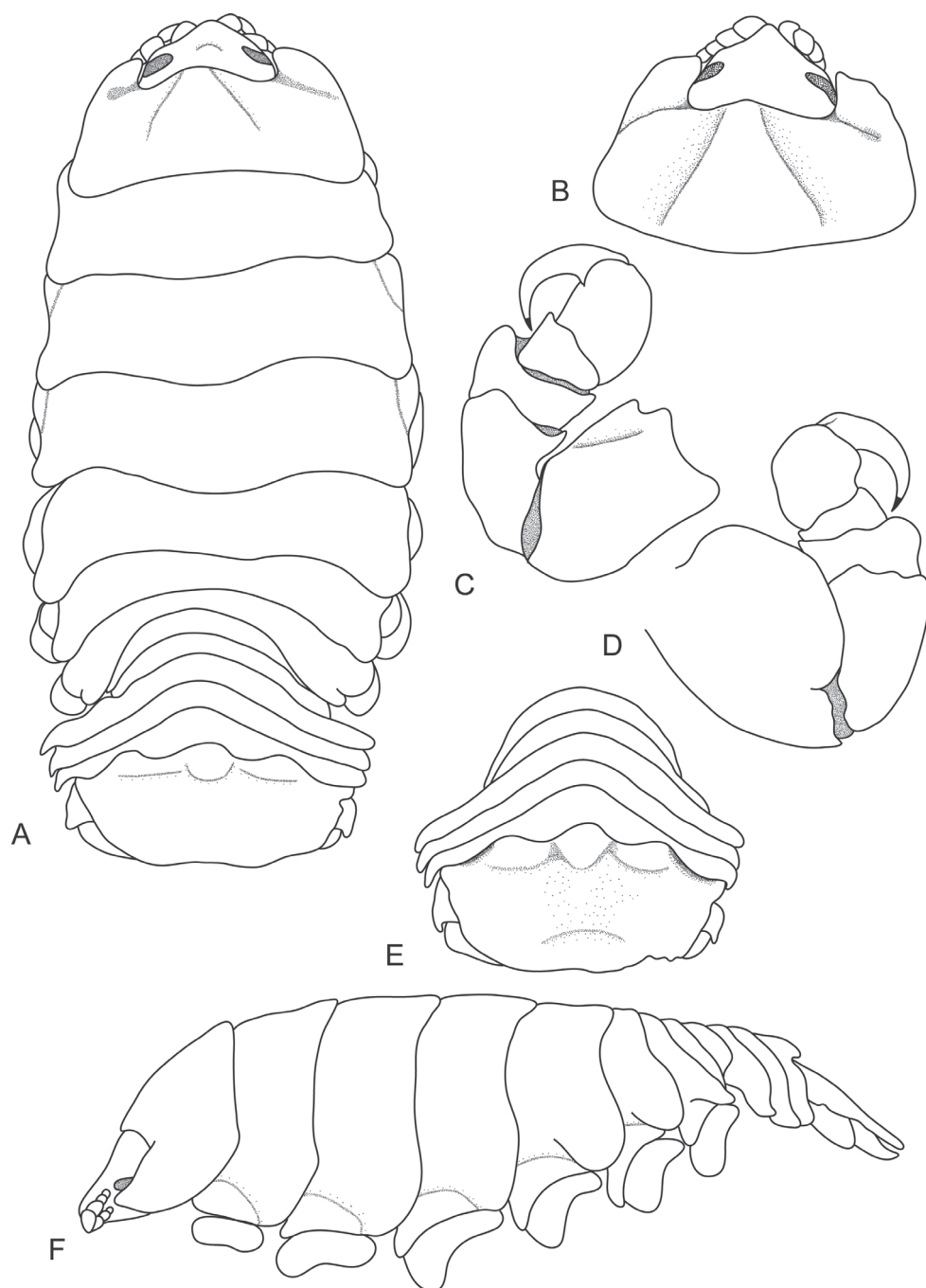
Figure 8. *Ceratothoa italica* Schioedte & Meinert, 1883, female lectotype (36 mm) (ZMUC CRU-6914). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** pereopod 1 **D** pereopod 7 **E** dorsal view of pleotelson **F** lateral view.

narrow at pereonite 1, lateral margins subparallel. *Cephalon* 0.5 times longer than wide, visible from dorsal view, triangular. *Eyes* irregular in outline, one eye 0.2 times width of cephalon; 0.2 times length of cephalon.

Pereonite 1 with unique bulbous orientation, anterior border anteriorly produced medially, anterolateral angle wide, with inwardly produced point, extend to anterior margin of eyes. Posterior margins of pereonites slightly produced medially. Coxae 4–7 rounded, not extending past pereonite margin. Pereonites 1–4 increasing in length and width; 5–7 decreasing in length and width; 6 and 7 narrower and becoming more progressively rounded posteriorly. *Pleon* with pleonite 1 same width as other pleonites (except pleonite 5), visible in dorsal view; pleonites posterior margin smooth, mostly concave. Posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4, posterior margin with 2 indented points. *Pleotelson* 0.5 times as long as anterior width, dorsal surface with medial furrow, lateral margins weakly convex, posterior margin subtruncate.

Pereopod 1 basis 1.5 times as long as greatest width; ischium 0.9 times as long as basis; merus proximal margin with large bulbous protrusion; carpus with straight proximal margin; propodus 1.1 times as long as wide; dactylus slender, 1.2 times as long as propodus, 2.3 times as long as basal width. *Pereopod 7* basis 1.2 times as long as greatest width; ischium 0.8 times as long as basis, without protrusions; merus proximal margin with slight bulbous protrusion, merus 0.5 times as long as wide, 0.4 times as long as ischium; carpus 0.5 times as long as wide, 0.3 times as long as ischium, without bulbous protrusion; propodus 1.1 times as long as wide, 0.5 times as long as ischium; dactylus slender, 1.4 times as long as propodus, 2.5 times as long as basal width.

Uropod same length as pleotelson, peduncle 0.5 times longer than rami, peduncle lateral margin without setae; rami extending to pleotelson apex, marginal setae absent, apices narrowly rounded.

Size. Ovigerous female: 15–30 mm TL; male: 8–15 mm TL; second pullus: 3 mm TL (Montalenti 1948, Trilles 1972a, Rokicki 1984b).

Distribution. Mediterranean region and north-western Africa: Adriatic Sea (Schioedte and Meinert 1883, Carus 1885, Öktener and Trilles 2004); Mauritania (Monod 1924a, 1924b, Trilles 1972b); Italy (Montalenti 1948); Galite Islands, Tunisia, France, Morocco (Trilles 1972b); Montenegro (Trilles et al. 1989); Aegean Sea (Ateş et al. 2006); and Algeria (Ramdane et al. 2007).

Hosts. In mouth of *Spondyliosoma cantharus* (previously *Cantharus lineatus*) (Monod 1924b, Trilles 1972b); mouth of *Lithognathus mormyrus* (previously *Pagellus mormyrus*) and other bream (Montalenti 1948, Trilles 1964b); on *Pagellus erythrinus* (see Trilles 1964b); on *Oblada melanura*; on Mustèle; in mouth of *Sargus* (see Trilles 1972b); occurs in Sparidae fishes (Rokicki 1985); in the mouth of *Diplodus sargus* (see Trilles et al. 1989); in the buccal cavity of *Diplodus annularis* (see Ramdane et al. 2007).

Remarks. *Ceratothoa italica* can be distinguished by the arched body; large bulbous protrusion on the merus of pereopod 1; a pointed rostrum; and uropods that do not extend past the pleotelson. This species also has a prominent projection in the middle of pereonite 1 (hump-like) and a pleon which is usually as wide as the pereon.

Ateş et al. (2006) stated that cymothoid isopods identified as *C. italica* were collected from the eggs of *Nephrops norvegicus*. As cymothoids are fish parasites, this interaction is an unusual association and is most likely accidental. Similarly, the record of *C. italica* (originally labelled as a *Cymothoa* sp. [SMF-3515]) from Senckenberg Research Institute, Frankfurt, Germany (Trilles 2008) seems doubtful as the specimen was collected from Norway and this species has only previously been recorded from the Mediterranean Sea region.

The female ZMUC CRU-6914 is here designated as the lectotype and the pullus stages in the same bottle and the other sample are paralectotypes (ZMUC CRU-8869, 9124).

Ceratothoa oestroides (Risso, 1826)
Figure 9

Canolira œstroïdes Risso, 1826: 123.

Cymothoa oestroides.—Milne Edwards 1840: 272.—White 1847: 110.—Lucas 1849: 78, pl. 8 (fig. 4).—Hope 1851: 33.—Heller 1866: 737–738.—Barcelo Combis 1875: 68.—Stalio 1877: 236.—Stossich 1880: 45.—Bonnier 1887: 133–134.—Monticelli 1890: 420–421.—Gerstaecker 1901: 181, 256–257.—Sanada 1941: 209.

Cymothoa (*Meinertia*) *oestroides*.—Taschenberg 1879: 245.—Dollfus 1922: 287.

Ceratothoa oestroides.—Schioedte and Meinert 1883: 350–356, tab. XV (Cym. XXII) figs 5–11.—Carus 1885: 442.—Barrois 1888: 12.—Gourret 1891: 14–15, pl. 4, figs 10–11.—Bolivar 1892: 133.—Koelbel 1892: 107, 115.—Norman 1905: 13.—Dudich 1931: 18.—Trilles 1979: 515, 521; 1986: 624, tab. 1; 1994: 122–124; 2008: 23.—Renaud, Romestand and Trilles 1980: 467–476.—Brusca 1981: 178.—Romestand, Thuet and Trilles 1982: 79–89.—Rokicki 1984b: 116–119, fig. 28; 1985: 95–119, tab. 1–3, fig. 7.—Radujković, Romestand and Trilles 1984: 164–166, figs 2–3; 1985: 106.—Trilles, Radujković and Romestand 1989: 289–291, fig. 9.—Šarušić 1999: 110–112.—Charfi-Cheikhrouha, Zghidi, Ould Yarba and Trilles 2000: 143–150, tab. 4.—Horton 2000: 1047–1048, fig. 7a–b.—Horton and Okamura 2001: 181–187.—Rodríguez-Sánchez, Serna and Junoy 2001: 154.—Mladineo 2002: 97–102; 2003: 97–101; 2006: 438–442.—Mladineo and Valic 2002: 304–310.—Junoy and Castello 2003: 307.—Korun and Akayli 2004: 123–132.—Öktener and Trilles 2004: 145–154.—Ramdane, Bensouilah and Trilles 2007: 67–74.—Pérez-Del Olmo, Fernández, Gibson, Raga and Kostadinova 2007: 152, 154.—Solak, Öktener, Trilles and Solak 2007: 237–238.—Oguz and Öktener 2007: 79–83.—Matašin and Vučinić 2008: 363–367.—Ramdane and Trilles 2008: 173–178.—Kirkim, Kocataş, Katağan and Sezgin 2008: 382–385.—Gökpinar, Özgen and Yildiz 2009: 188–190.—Mladineo, Šegvić and Grubišić 2009: 160–167.—Innal and Kirkim 2012: A13–A16.

Ceratothoa sargorum Gourret, 1891: 16, pl. 1, fig. 17; pl. 5, figs 1–4.

Meinertia oestroides.—Thielemann 1910: 36.—Nierstrasz 1915: 8.—Monod 1923a: 82–83; 1923b: 18–19; 1924a: 34; 1924b: 432.—Montalenti 1948: 47–50, figs

15–17, tab. 6, pl. 4.—Amar 1951: 530.—Balcells 1953: 548–552.—Euzet and
Trilles 1961: 190–192.—Trilles 1962: 118–123; 1964b: 107; 1972a: 1201–
1208, figs 90–136, pl. 1 figs 6–9, pl. 3 fig 20; 1972b: 1233–1235 (part); 1977:
8–9.—Vu–Tân–Tûe 1963: 225–232.—Berner 1969: 93–94.—Roman 1970:
163–208; 1979: 501–514.—Trilles and Raibaut 1971: 73–75, photos 2–3; 1973:
274.—Thampy and John 1974: 575, 580–582.—Romestand 1974: 571–591;
1979: 423–448, pl. 1–4.—Romestand and Trilles 1976a: 87–92, fig. 1; 1976b:
663–665; 1977a: 91–95, figs 1–2; 1977b: 47–53, figs 1–11; 1979: 195–202.—
Romestand, Voss–Foucart, Jeuniaux and Trilles 1976: 981–988.—Dollfus and
Trilles 1976: 822.—Chaigneau 1977: 403–418.—Romestand, Janicot and Tril-
les 1977: 171–172, 178–179, pl. 3, figs 10–14.—Quignard and Zaouali 1980:
357.—Thuet and Romestand 1981: 15–33.—Brusca 1981: 125.—Radujković
1982: 153–161.—Wägele 1987: 1–398.

Cymothea oestroides [sic].—Odon de Buen 1916: 363.
Ceratothoa (*Meinertia*) *oestroides*.—Brusca 1981: 123.
Not *Cymothoa oestroides*.—Gibert i Olivé 1920: 88.
Excluded (identity uncertain): *Meinertia oestroides*.—Trilles 1972b: 1233–1235 (part).
*Ceratothoa oestroides*.—Trilles 1981: 587.

Material examined. *Lectotype* [here designated]. National Museum of Natural History,
Paris (MNHN-IU-2014-17478) – female specimen (22 mm TL; 8 mm W), collect-
ed from the Mediterranean Sea; J.P. Trilles checked 17.12.1971, host unknown (n°6)
(Trilles 1972b). *Paralectotype*. Female specimen (21 mm TL; 8 mm W), same data as hol-
otype (Trilles 1972b) (MNHN-IU-2007-4240). Also noted: the two females were in the
same bottle as a female *C. parallela* (19 mm TL; 6 mm W) (MNHN-IU-2014-17479).

   Description. *Lectotype female.* Length 22 mm, width 8 mm.

   Body oval and elongate, 1.9 times as long as greatest width, dorsal surfaces smooth
and polished in appearance, widest at pereonite 4 and pereonite 5, most narrow at
pereonite 1, lateral margins posteriorly ovate. *Cephalon* 0.6 times longer than wide,
visible from dorsal view, triangular. *Frontal margin* rounded to form blunt rostrum.
Eyes oval with distinct margins, one eye 0.3 times width of cephalon; 0.4 times length
of cephalon. *Antennula* more stout than antenna, comprised of 7 articles. *Antenna*
comprised of 8 articles.

   Pereonite 1 smooth, anterior border straight, anterolateral angle with small distinct
produced point, extend to middle of the eye. Posterior margins of pereonites smooth
and slightly curved laterally. Coxae 2–3 narrow, with posteroventral angles rounded;
4–7 rounded, not extending past pereonite margin. Pereonites 1–4 increasing in length
and width; 5–7 decreasing in length and width; 6 and 7 narrower. *Pleon* with pleonite
1 most narrow and same width as other pleonites, visible in dorsal view; pleonites
posterior margin smooth, mostly concave. Pleonite 2 not overlapped by pereonite 7;
posterolateral angles of pleonite 2 narrowly rounded. Pleonites 3–5 similar in form to
pleonite 2; pleonite 5 free, not overlapped by lateral margins of pleonite 4, posterior
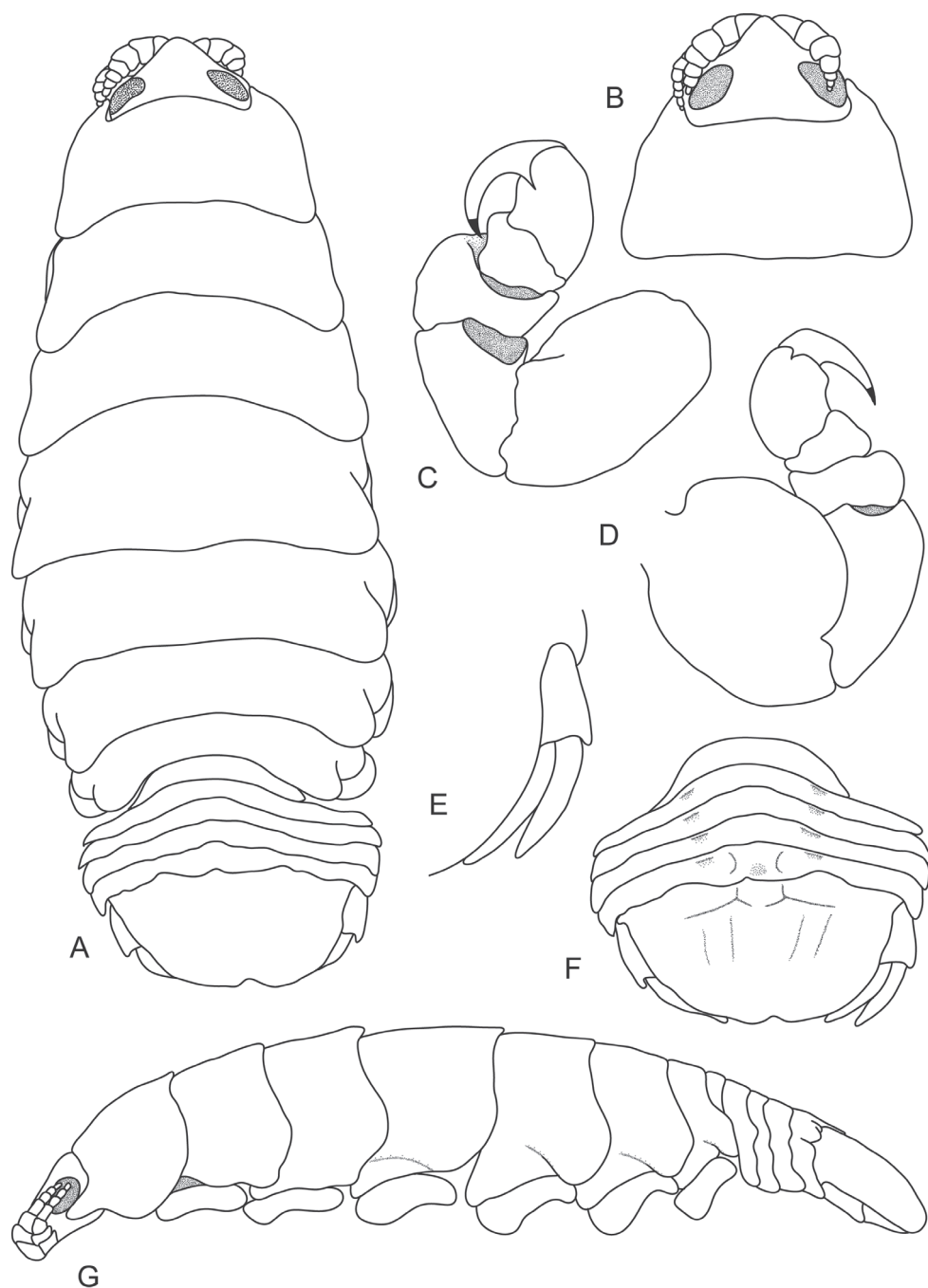
Figure 9. *Ceratothoa oestroides* (Risso, 1826), female lectotype (22 mm) (MNHN-IU-2014-17478). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** pereopod 1 **D** pereopod 7 **E** uropod **F** dorsal view of pleotelson **G** lateral view.

margin with 2 indented points or produced medially. *Pleotelson* 0.5 times as long as anterior width, dorsal surface with lateral indent, lateral margins weakly convex, posterior margin rounded with medial indent.

Pereopod 1 basis 1.7 times as long as greatest width; ischium 0.6 times as long as basis; merus proximal margin with large bulbous protrusion; carpus with rounded proximal margin; propodus 1.5 times as long as wide; dactylus slender, 0.9 times as long as propodus, 2.3 times as long as basal width. *Pereopod 7* basis 1.1 times as long as greatest width; ischium 0.9 times as long as basis, with slight bulbous protrusion; merus proximal margin with large bulbous protrusion, merus 0.6 times as long as wide, 0.3 times as long as ischium; carpus 0.7 times as long as wide, 0.3 times as long as ischium, without bulbous protrusion; propodus 1.4 times as long as wide, 0.6 times as long as ischium; dactylus slender, 0.9 times as long as propodus, 2.4 times as long as basal width.

Uropod same length or slightly longer than the pleotelson, peduncle 0.8 times longer than rami, peduncle lateral margin without setae; rami extending to pleotelson apex, marginal setae absent, apices narrowly rounded.

Size. Ovigerous female: 12–30 mm; non-ovigerous female: 11–24.5 mm TL; male: 3.5–13 mm TL; second stage pullus: 3.3–4 mm TL; first stage pullus: 3.1 mm TL (Schioedte and Meinert 1883, Montalenti 1948, Trilles 1972a, 1977, 1979, Rokicki 1984b, Radujković et al. 1985).

Distribution. Throughout the Mediterranean and eastern Atlantic: especially France and Algeria (Risso 1826, Milne Edwards 1840, Schioedte and Meinert 1883, Thielemann 1910, Trilles 1964b); Adriatic (Heller 1866, Stalio 1877); Aegean Sea (Horton and Okamura 2001, Trilles 2008); Straits of Gibraltar and the Alborán Sea (Rodríguez-Sánchez et al. 2001); Turkey (Öktener and Trilles 2004, Solak et al. 2007, Innal and Kirkim 2012); and the eastern Atlantic islands and north-west Africa (Barrois 1888, Koelbel 1892, Monod 1924b, Trilles 1972b, 1979, Dollfus and Trilles 1976).

Hosts. Common in the mouth and branchial regions of the bogue, *Boops boops* (see Schioedte and Meinert 1883, Gourret 1891, Monod 1923b, Balcells 1953, Vu–Tân–Tûe 1963, Berner 1969, Trilles and Raibaut 1971, 1973, Romestand and Trilles 1976a, 1977a, b, 1979, Roman 1979, Taschenberg 1879, Renaud et al. 1980, Radujković 1982, Trilles et al. 1989, Charfi-Cheikhrouha et al. 2000, Pérez-Del Olmo et al. 2007, Ramdane et al. 2007, Matašin and Vučinić 2008, Ramdane and Trilles 2008, Kirkim et al. 2008); "rare parasite of wrasses (Labres)" (Bonnier 1887); in the mouth of *Diplodus sargus* (see Gourret 1891); in buccal cavity of *Spicara maena* (see Gourret 1891, Trilles 1962, Berner 1969, Roman 1979, Radujković 1982, Charfi-Cheikhrouha et al. 2000, Kirkim et al. 2008); on the gills of two *Phycis phycis* (recorded as *Phycis mediterraneas*) (see Koelbel 1892); from the mouth of *Trachurus trachurus* (see Dollfus 1922, Trilles and Raibaut 1971, 1973, Dollfus and Trilles 1976, Charfi-Cheikhrouha et al. 2000, Ramdane et al. 2007); in the mouth of *Spicara* and "*Box*" sp. (see Montalenti 1948, Amar 1951); in the buccal cavity of *Diplodus vulgaris* (see Monod 1923b, Amar 1951); buccal and gill cavity of *Diplodus annularis* (see Monod 1923b, Trilles and Raibaut

1971, 1973, Trilles 1972b, Dollfus and Trilles 1976, Radujković 1982, Trilles et al. 1989, Charfi-Cheikhrouha et al. 2000); in the mouth of red mullet, *Mullus barbatus* (see Trilles 1962, Roman 1970); on sardine, *Sardina pilchardus* (see Trilles 1962, 1979, Charfi-Cheikhrouha et al. 2000); buccal cavity of *Spicara* sp. and gill cavity of *Uranoscopus scaber* (see Trilles and Raibaut 1973); in mouth cavity of *Pagellus erythrinus* (see Romestand and Trilles 1979, Radujković et al. 1985, Trilles et al. 1989); in mouth of *Spicara melanurus* (previously *Smaris melanurus*), *Sargus bellottii*, and *Abudefduf saxatilis* (see Trilles 1979); *Trachurus mediterraneus* (see Radujković 1982, Trilles et al. 1989); picarels, *Spicara smaris* (see Trilles et al. 1989, Ramdane et al. 2007, Matašin and Vučinić 2008, Ramdane and Trilles 2008); *Pagellus acarne* (see Ramdane et al. 2007, Ramdane and Trilles 2008); *Dicentrarchus labrax* (see Šarušić 1999, Horton and Okamura 2001, Mladineo 2002, 2003); *Sparus aurata* (see Šarušić 1999, Horton and Okamura 2001, Mladineo 2003); on the tongue of *Scorpaena notata*, *Liza aurata* and *Scorpaena porcus* (see Charfi-Cheikhrouha et al. 2000); in the black seabream, *Spondyliosoma cantharus* (see Charfi-Cheikhrouha et al. 2000, Gökpinar et al. 2009); from *Rostroraja alba* and *Zeus faber* (see Kirkim et al. 2008).

Remarks. *Ceratothoa oestroides* can be distinguished by having an acute rostrum; short antennae; prominent eyes; uropods which extend to or past the posterior pleotelson margin; large protrusion on the merus of pereonite 1; and a large carina on pereopod 7 in female specimens, as well as the appendix masculina absent in male specimens.

Ceratothoa sargorum Gourret, 1891, found on *Sargus rondeletii*, was described from a single female with large eggs, almost a millimetre in diameter (Gourret 1891). This species was later synonymised with *C. oestroides* as seen in Radujković et al. (1984). The original drawings by Gourret (1891) resemble the syntypic material of *C. oestroides* and confirm this synonymy.

There have been reported cases of *C. oestroides* involved in hyperparasitism. Dollfus (1922) recorded an unusual association with *C. oestroides* and a monogenean, *Allodiclidophora charcoti* (Dollfus, 1922) (Diclidophoridae) after being collected from *C. oestroides* in the mouth of *Trachurus trachurus* from Oviedo. Similarly, Monod (1923b) stated that the ectoparasite was found on one *C. oestroides* specimen from the mouth of *Box vulgaris*.

Ceratothoa oestroides has often been misidentified as *C. oxyrrhynchaena*. Both species use similar host fish and have an overlapping distribution range, but they are distinguished by the morphology of the seventh pair of pereopods in the female. It should be noted that male *C. oestroides* does not possesses an appendix masculina. We regard the records of *Ceratothoa oestroides* from the Caribbean (Trilles 1972b, 1981) as unconfirmed, and are not included in the synonymy and distribution for the species.

Horton (2000) recently revised this species including full synonymy, host and distribution notes for *C. oestroides* and listed the two female syntypes from MNHN (sample No. 6) as the type material. The type material had not previously been redescribed and no holotype had been designated by Risso (1826), so one female was hereby designated as a lectotype in order to provide a precise type-based description for the species.

Ceratothoa verrucosa (Schioedte & Meinert, 1883)
Figure 10

Oniscus Ceti Spengler, 1775: 312 [*nomen nudum*].
Rhexana verrucosa Schioedte & Meinert, 1883: 291–296, tab. XI (Cym. XVIII) figs
    5–10.—Thielemann 1910: 34–35, tab. 3.—Sanada 1941: 209–217.—Bruce and
    Bowman 1989: 2.—Trilles 1994: 134–135.
Rhexanella verrucosa.—Stebbing 1911: 179.—Nierstrasz 1915: 87.—Shiino 1951:
    83, figs 1a–b.—Trilles 1972b: 1255–1256, pl. II, figs 17–18.—Nunomura 1981:
    52.—Avdeev 1982b: 69–77.—Bruce and Bowman 1989: 2.—Yamaguchi 1993:
    193–194, fig. 21.
*Ceratothoa verrucosa*.—Yamauchi and Nunomura 2010: 73, figs 7–8.
Identity uncertain: *Rhexanella verrucosa*.—Nierstrasz 1931: 131.

Material examined. *Lectotype*. National Museum of Natural History (Naturalis), Lei-
den, Netherlands (RMNH.CRUS.I.7706) – ovigerous female (40 mm TL; 21 mm W),
collected from Japan, unknown host, 1823-1829, coll: Siebold, Ph.F.v (designated by
Yamaguchi 1993). *Paralectotypes*. Immature female (26 mm TL; 10.5 mm W), two males
(16–19 mm TL; 7–8 mm W), same data as holotype (RMNH.CRUS.I.39). Female
slightly twisted, non-ovigerous, damaged pereopods and pleopods with uropods missing.
    Description. *Lectotype female.* Length 40 mm, width 21 mm.
    Body oval, 1.9 times as long as greatest width, dorsal surfaces slightly bumpy, wid-
est at pereonite 4, most narrow at pereonite 1, lateral margins slightly convex. *Cepha-
lon* 0.7 times longer than wide, visible from dorsal view, subtriangular. *Frontal margin*
rounded to form blunt rostrum. *Eyes* irregular in outline. *Pereonite 1* with unique bul-
bous orientation, anterior border slightly indented, anterolateral angle with large wide
projections, extend to anterior margin of eyes. Posterior margins of pereonites slightly
damaged and bumpy. Coxae 2–3 wide; 4–7 large and produced on pereonite margins,
not extending past pereonite margin. Pereonites subequal. *Pleon* with pleonite 1 most
narrow, visible in dorsal view; pleonites posterior margin not smooth, mostly concave.
Pleonite 2 not overlapped by pereonite 7; posterolateral angles of pleonite 2 narrowly
rounded. Pleonites 3–5 similar in form to pleonite 2; pleonite 5 free, not overlapped
by lateral margins of pleonite 4, posterior margin produced medially. *Pleotelson* 0.5
times as long as anterior width, dorsal surface with lateral indent, lateral margins weak-
ly convex, posterior margin evenly rounded. *Antennula* more stout than antenna, same
length as antenna, consisting of 7 articles. *Antenna* consisting of 9 articles.
    Pereopod 1 basis 1.4 times as long as greatest width; ischium 0.7 times as long as
basis; merus proximal margin with large bulbous protrusion; carpus with rounded
proximal margin; propodus 1.2 times as long as wide; dactylus slender, 0.9 times as
long as propodus, 1.9 times as long as basal width. *Pereopod 7* basis 0.8 times as long as
greatest width; ischium as long as basis, with slight bulbous protrusion; merus proxi-
mal margin with large bulbous protrusion, merus 0.4 times as long as wide, 0.3 times
as long as ischium; carpus 0.6 times as long as wide, 0.9 times as long as ischium,
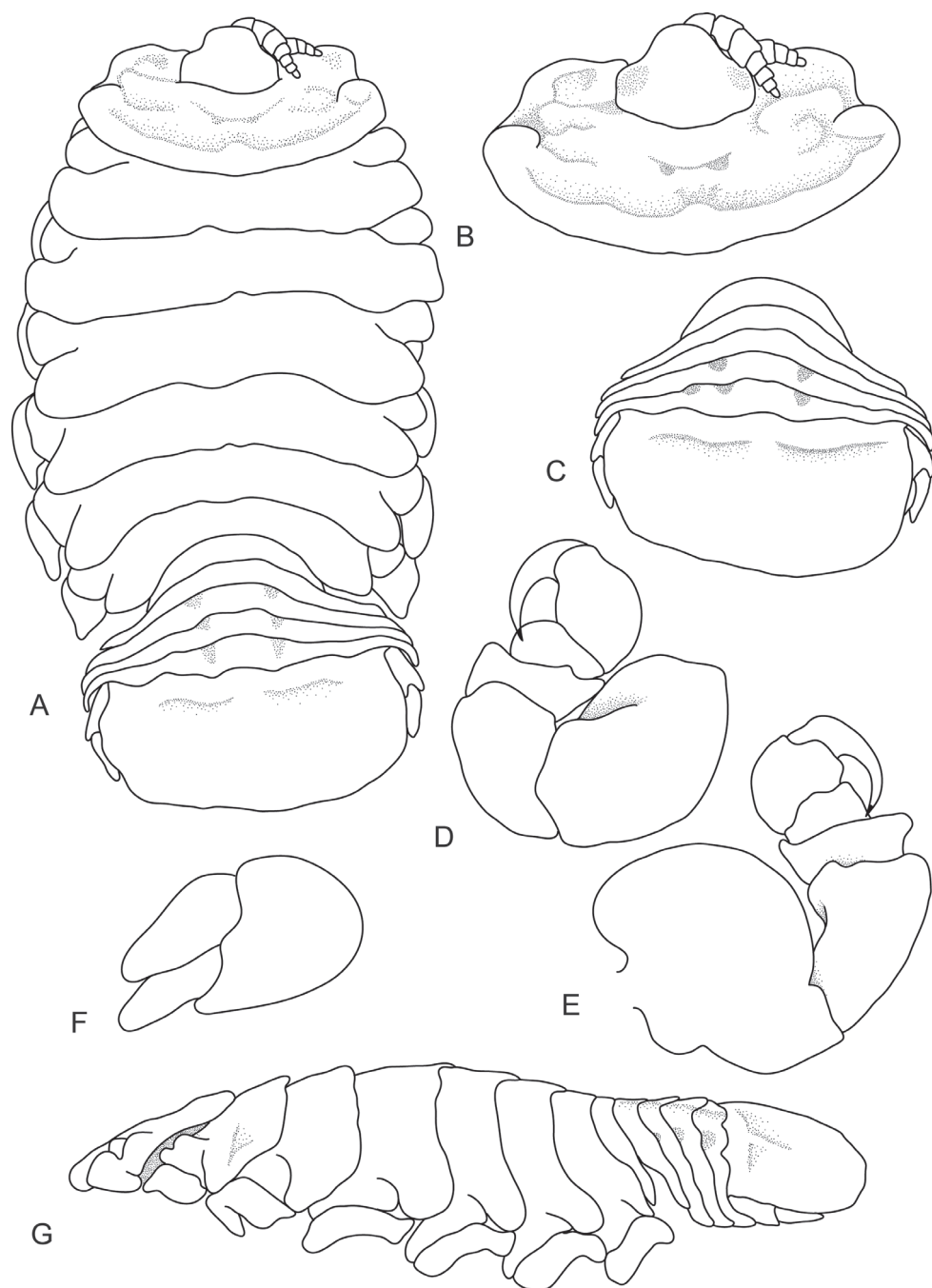
Figure 10. *Ceratothoa verrucosa* (Schioedte & Meinert, 1883), female lectotype (40 mm) (RMNH. CRUS.I.7706). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** dorsal view of pleotelson **D** pereopod 1 **E** pereopod 7 **F** uropod **G** lateral view.

without bulbous protrusion; propodus 0.7 times as long as wide, 0.4 times as long as ischium; dactylus slender, 1.8 times as long as propodus, twice as long as basal width.

Uropod half the length of pleotelson, peduncle as long as rami, peduncle lateral margin without setae; rami not extending beyond pleotelson, marginal setae absent, apices narrowly rounded.

Size. Ovigerous females: 27–50 mm TL (15.5–25.5 mm W); non-ovigerous females 20.5–40 mm TL; males 15–35 mm TL (11 mm W); larvae 3.5 mm TL (Schioedte and Meinert 1883, Thielemann 1910, Nierstrasz 1931, Trilles 1972b, Nunomura 1981, Yamauchi and Nunomura 2010).

Distribution. Known from Japan and surrounding islands (Schioedte and Meinert 1883, Thielemann 1910, Sanada 1941, Trilles 1972b, Nunomura 1981, Yamauchi and Nunomura 2010).

Hosts. In the mouth of *Pagrus major* (previously *Pagrosomus major*) ("Tai" in Japanese) (Sanada 1941, Shiino 1951, Nunomura 1981, Yamauchi and Nunomura 2010).

Remarks. *Ceratothoa verrucosa* is distinguished by the large, oval body; wide anterolateral projections on pereonite 1; pleon as wide as pereon; and short uropods not extending to the posterior margin of the pleotelson.

This species was originally thought to infect a Greenland whale (Spengler 1775) but Spengler (1775) may have tried to relate this isopod to the whale lice *Cyamus ceti*. Cymothoid isopods had not been mentioned often before this time and researchers had some confusion with their identification. Schioedte and Meinert (1883), however, stated that this species (referred as "*Oniscus Ceti*") was undoubtedly the same as their "*Rhexana*" species. There is no detailed description and no type material for Spengler's (1775) species, so the original description is a *nomen nudum*; the correct authority for the species is Schioedte and Meinert (1883) who first made the name available. The name *Rhexana* was preoccupied, and the genus was then changed to *Rhexanella* Stebbing, 1911, this name being later synonymised with *Ceratothoa* (see Hadfield et al. 2014b).

Nierstrasz (1931) listed this species from the East Indian Archipelago, specifically from Nangamessi Bay, Sumba (Indonesia) after being collected during the H.M. *Siboga* expeditions. If confirmed, this distribution range would increase the distribution of *Ceratothoa verrucosa* (previously only found from Japan and only from one host, *Pagrus major*).

Species excluded from *Ceratothoa*

Ceratothoa argus (Haswell, 1881), *nomen dubium*

Codonophilus argus Haswell, 1881: 471, pl. XVI, fig. 1; 1882: 283; 1885: 1001.—
    Stebbing 1893: 356.—Hale 1926: 223–226.—Barnard 1940: 404.—Trilles
    1972c: 5, 7.

Holotype. Deposition unknown.
    Distribution. Australia (Haswell 1881).
    Hosts. Under the bell of a *Rhizostoma* (see Haswell 1881).

Remarks. This species was described from an immature specimen (4 mm in length) in only a few sentences and a single figure. It was found under the bell of a *Rhizostoma* in Port Jackson (Sydney, New South Wales) and noted as being similar to *Aegathoa* in many ways, but differed in the sudden narrowing of the body at the commencement of the pleon, and the uniramous character of the caudal appendages. Hale (1926) synonymised *C. argus* with *C. imbricata* as it appeared similar to the brood young of *C. imbricata* and according to the label it was also reported as coming from the jelly blubber, *Catostylus mosaicus* (recorded as *Catostylus mosaicus*).

Due to the species being based on a single immature specimen (as well as a lack of a type specimen and an incomplete description), this species is hereby considered *nomen dubium*.

Ceratothoa poutassouiensis (Penso, 1939), *nomen dubium*

Meinertia poutassouiensis Brian, 1939: 20–24 [*nomen nudum*].
Meinertia (*Ceratothoa*) *potassoniensis*.—Penso 1939: 1, figs 1–2 [*lapsus*].
Ceratothoa poutassouiensis.—Trilles 1994: 127.—Horton 2000: 1042.

Hosts. *Micromesistius poutassou* (previously *Gadus potassoa*).

Remarks. These two species names, published in the same year, refer to the same species. Brian (1939) stated that "… *this isopod circa 2 cm in length … a species of* Meinertia *deserves to be described as it seems to be a new species, I hope to be able to publish the description of this species which I call* Meinertia poutassouiensis". Horton (2000) added that both authors cited the species as found on *Micromesistius poutassou* (as *Gadus potassoa*), but it was inadequately described with two uninformative figures in Penso (1939). This lack of sufficient information, lack of types to redescribe the species and lack of the location of the type material lead Horton (2000) to place *C. poutassouiensis* (Brian, 1939) into *nomen nudum*. The mention of size alone by Brian (1939) does not meet the criteria of availability, specifically ICZN Article 13.1.1, as it does not differentiate or define the species; Brian's name is therefore not available so the authority has to be Penso (1939) as Penso provided two figures of the species thereby validating the name. The correct spelling of the epithet remains that proposed by Brian (1939). Given the lack of a descriptive data, lack of types and lack of a specific type locality, the species is here regarded as *nomen dubium*.

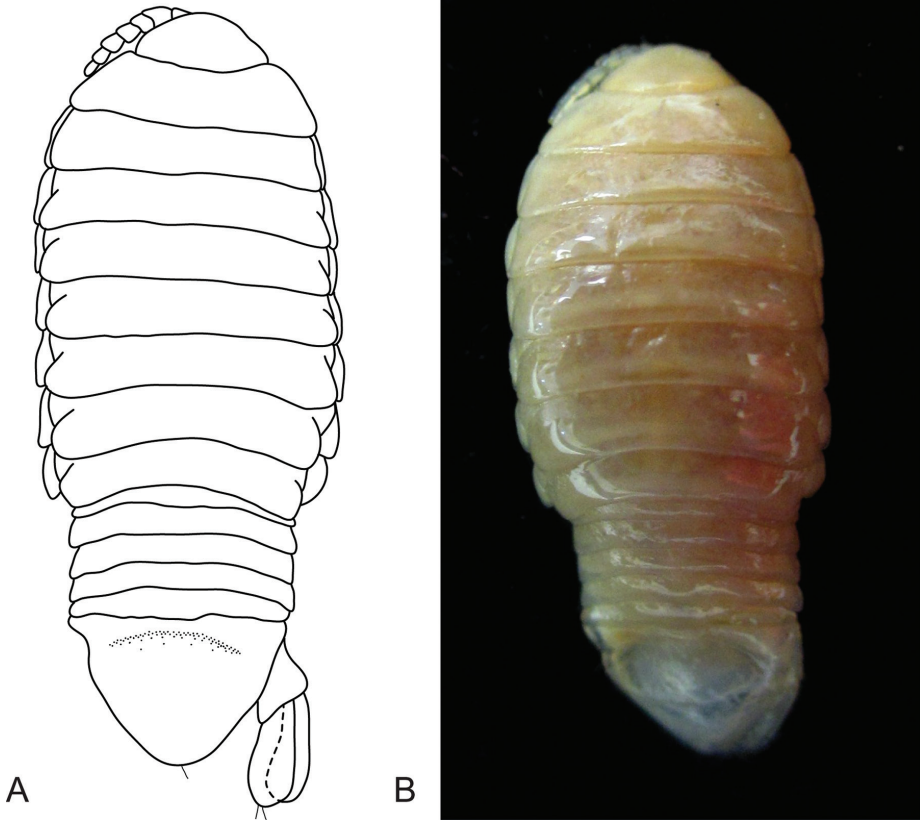### *Ceratothoa transversa* (Richardson, 1901), *species inquirenda*
Figure 11

Meinertia transversa Richardson, 1900: 221 [*nomen nudum*].
Meinertia transversa Richardson, 1901: 529–530, figs 12–13; 1905: 243, figs 250–252.—
    Menzies and Frankenberg 1966: 9.—Schultz 1969: 156, fig. 234.—Menzies and
    Kruczynski 1983: 39.
Ceratothoa transversa.—Brusca 1981: 178.—Trilles 1994: 128.

Figure 11. *Ceratothoa transversa* (Richardson, 1901), immature male holotype (17 mm) (USNM 9728). **A, B** dorsal view.

Material examined. *Holotype.* United States National Museum, USA (USNM 9728) – immature specimen (17 mm TL; 7 mm W) collected from the Gulf of Mexico, *Albatross* Station 2395, U.S.F.C., 347 fms (635 metres), host unknown.

Distribution. Between the Mississippi Delta and Cedar Keys, Florida (Richardson 1900, 1901, 1905, Menzies and Frankenberg 1966, Schultz 1969).

Hosts. Unknown.

Remarks. *Ceratothoa transversa* was originally noted as having a cephalon only slightly immersed in pereonite 1; long antennae extending past pereonite 1; uropods slightly longer than the pleotelson; and a sub-triangular pleotelson.

Richardson (1900) mentioned the name *Meinertia transversa* without differentiating characters or figures, without type locality, type deposition or type host, referring only to an "in press" paper and as such that name is a *nomen nudum*. Richardson (1901) later gave a short description of the species with figures of the cephalon and pleon were given. Schultz (1969) commented that this species is probably based on a young individual and Menzies and Kruczynski (1983) further stated that this species has never been adequately illustrated and probably also represents an *Aegathoa*-stage specimen as uropodal rami and pleotelson are shown with setae by Richardson (1900, 1901, 1905).

Examination of the holotype confirmed that it is an immature specimen. The antennae extend into the middle of pereonite 1, the pleon is almost as wide as the pereon, there are a few setae on the uropods and pleotelson, the pleopods overlap and the appendix masculina is absent. Without an adult female to characterise the species, and no known hosts to assist in directing the collection of a new specimen, the identity of this species is uncertain and therefore *C. transversa* is hereby placed into *species inquirenda*.

Ceratothoa triglae **Gourret, 1891,** *species inquirenda*

Ceratothoa triglae Gourret, 1891: 19–20, pl. 11, figs 14–19.

Remarks. *Ceratothoa triglae* (Gourret, 1891) was described from a male specimen measuring 7 mm TL. Gourret (1891) reported that it measured at least four times longer than wide and that it was found on the cheeks and belly of *Chelidonichthys lucerna* (previously *Trigla corax*). Second stage pulli were also found on the cheeks, probably newly released, along with the female; however, no other mention is made of the female after this statement. This species was subsequently placed into synonymy with *C. parallela* (see Radujković et al. 1984) and maintained there by Trilles (1994) and Horton (2000). As there is no known type material, and the description is based on a male specimen, this species can only be regarded as *species inquirenda*.

Elthusa parva **(Richardson, 1910), comb. n.**
Figure 12

*Meinertia parva* Richardson, 1910: 21, fig. 20.
Codonophilus parvus.—Nierstrasz 1931: 132.
Ceratothoa parva.—Trilles 1994: 127.

Material examined. *Holotype*. United States National Museum, USA (USNM 40938) – female (19 mm TL; 8.5 mm W), collected from Opol, Mindanao, Philippines, 4 August 1909, host unknown (Richardson 1910).
    Remarks. *Ceratothoa parva* was originally described as having distinct eyes; rounded anterolateral processes on pereonite 1 which extend half the length of the cephalon; and short uropods which do not reach the end of the pleotelson.
    Examination of the holotype revealed many characters not usually present in *Ceratothoa*. Pleonite 1 is as wide as the other pleonites (usually narrower), pleonite 4 is slightly wider than pleonite 5, slender and short antennae, and, most significantly, the bases of the antennae do not touch (a defining characteristic of *Ceratothoa*). These characters, together with the shape of the head and pereopod morphology all agree well with the generic characters for *Elthusa* Schioedte & Meinert, 1884 (see Bruce 1990), and the species is here placed in that genus.
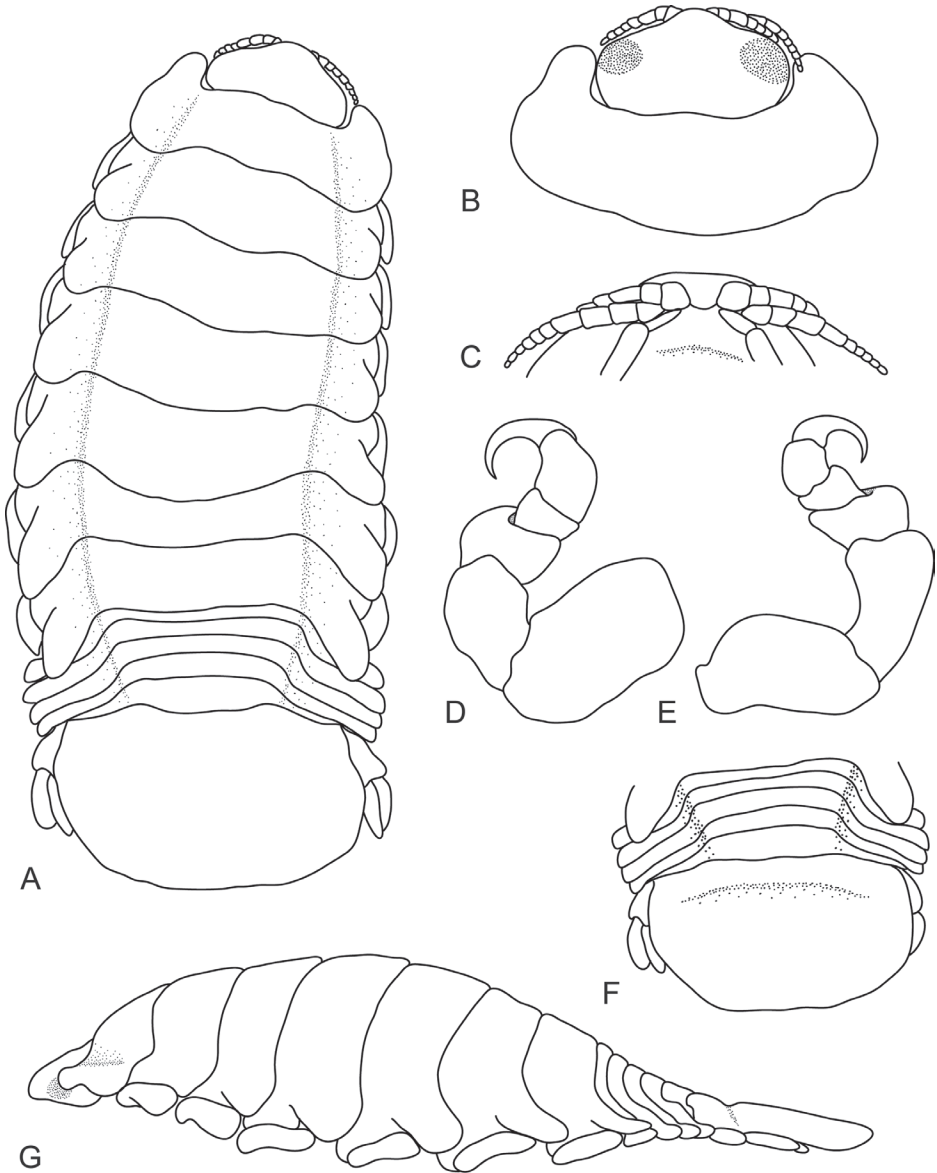
Figure 12. *Elthusa parva* (Richardson, 1910), comb. n., female holotype (19 mm) (USNM 40938). **A** dorsal view **B** dorsal view of pereonite 1 and cephalon **C** ventral view of cephalon **D** pereopod 1 **E** pereopod 7 **F** dorsal view of pleotelson **G** lateral view.

Ceratothoa species list

A number of recent papers revising *Ceratothoa* (Martin et al. 2013, 2015a, Hadfield et al. 2014a, 2014b), has resulted in a number of changes to the accepted species in the genus. Several species are no longer valid and some have been placed into synonymy

Table 1. Currently accepted species of *Ceratothoa*, their respective authorities and the most recent reference for each.

No.	Accepted name	Authority	Reference
1	*Ceratothoa africanae*	Hadfield, Bruce & Smit, 2014	Hadfield et al. 2014b
2	*Ceratothoa angulata*	(Richardson, 1910b)	Present study
3	*Ceratothoa arimae*	(Nunomura, 2001)	Martin et al. 2015b
4	*Ceratothoa banksii*	(Leach, 1818)	Martin et al. 2015a
5	*Ceratothoa barracuda*	Martin, Bruce & Nowak, 2015	Martin et al. 2015a
6	*Ceratothoa capri*	(Trilles, 1964c)	Present study
7	*Ceratothoa carinata*	(Bianconi, 1869)	Present study
8	*Ceratothoa collaris*	Schioedte & Meinert, 1883	Present study
9	*Ceratothoa famosa*	Hadfield, Bruce & Smit, 2014	Hadfield et al. 2014b
10	*Ceratothoa gilberti*	(Richardson, 1904)	Present study
11	*Ceratothoa globulus*	Martin, Bruce & Nowak, 2015	Martin et al. 2015a
12	*Ceratothoa gobii*	Schioedte & Meinert, 1883	Present study
13	*Ceratothoa guttata*	(Richardson, 1910b)	Present study
14	*Ceratothoa imbricata*	(Fabricius, 1775)	Hadfield et al. 2014
15	*Ceratothoa italica*	Schioedte & Meinert, 1883	Present study
16	*Ceratothoa marisrubri*	Trilles, Colorni & Golani, 1999	Present study
17	*Ceratothoa oestroides*	(Risso, 1826)	Present study
18	*Ceratothoa oxyrrhynchaena*	Koelbel, 1878	Present study
19	*Ceratothoa parallela*	(Otto, 1828)	Present study
20	*Ceratothoa retusa*	(Schioedte & Meinert, 1883)	Hadfield et al. 2014a
21	*Ceratothoa steindachneri*	Koelbel, 1878	Present study
22	*Ceratothoa trigonocephala*	(Leach, 1818)	Hadfield et al. 2014b
23	*Ceratothoa usacarangis*	(Avdeev, 1979a)	Present study
24	*Ceratothoa verrucosa*	(Schioedte & Meinert, 1883)	Present study

with other species. Currently accepted species of *Ceratothoa* are listed in Table 1; previous *Ceratothoa* combinations, showing the current status of the name as well as the earliest reference, are listed in Table 2.

Conclusion

A total of 50 *Ceratothoa* species names were found in the literature. Of these, 30 are regarded as valid (Bruce and Schotte 2016). Following recent work (Martin et al. 2013, 2015a, Hadfield et al. 2014b) and this present study, we accept 24 species of *Ceratothoa* as valid. This review of the poorly studied *Ceratothoa* resolves some of the uncertainties surrounding certain species and provides an updated list of valid *Ceratothoa* species.

Table 2. Species previously placed in combination with *Ceratothoa*, together with the current status and most recent reference. Full synonymies and nomenclatural details may be found in Trilles (1994).

No.	Former combination	Status	Reference
1	*Ceratothoa argus* (Haswell, 1881)	*nomen dubium* (no type, immature specimen)	Present study
2	*Ceratothoa atherinae* (Gourret, 1891)	= *Mothocya epimerica* (junior synonym)	Monod 1923c
3	*Ceratothoa brachyura* (White, 1847)	*nomen nudum* (no type, no description)	Present study
4	*Ceratothoa contracta* (Miers, 1880)	*species inquirenda* (type not located)	Martin et al. 2015a
5	*Ceratothoa crassa* Dana, 1853	*Glossobius crassa*	Stebbing 1893
6	*Ceratothoa curvicauda* Nunomura, 2006	= *Ceratothoa carinata* (junior synonym)	Martin et al. 2013
7	*Ceratothoa deplanata* Bovallius, 1885	= *Ceratothoa parallela* (junior synonym)	Horton 2000
8	*Ceratothoa directa* (Otto, 1821)	= *Ceratothoa parallela* (junior synonym)	Horton 2000
9	*Ceratothoa exocoeti* Cunningham, 1871	= *Glossobius impressus* (junior synonym)	Bruce and Bowman 1989
10	*Ceratothoa gaudichaudii* (Milne Edwards, 1840)	*species inquirenda* (no female type)	Martin et al. 2015
11	*Ceratothoa hemirhamphi* (Pillai, 1954)	= *Ceratothoa retusa* (junior synonym)	Bruce and Bowman 1989
12	*Ceratothoa huttoni* Filhol, 1885	= *Ceratothoa imbricata* (junior synonym)	Martin et al. 2015a
13	*Ceratothoa impressa* Say, 1818	= *Glossobius impressus* (junior synonym)	Bruce and Bowman 1989
14	*Ceratothoa laticauda* Milne Edwards, 1840	= *Glossobius auritus* (junior synonym)	Bruce and Bowman 1989
15	*Ceratothoa linearis* Dana, 1853	*Glossobius linearis*	Stebbing 1893
16	*Ceratothoa lineata* Miers, 1876	*Mothocya lineata*	Martin et al. 2015a
17	*Ceratothoa novae–zelandiae* Filhol, 1885	= *Ceratothoa trigonocephala* (junior synonym)	Trilles 1972b
18	*Ceratothoa parva* (Richardson, 1910b)	*incertae sedis* (not *Ceratothoa*, single specimen)	Present study
19	*Ceratothoa potassoniensis* (Penso, 1939)	*nomen dubium* (no type or description)	Present study
20	*Ceratothoa poutassouiensis* (Brian, 1939)	*nomen nudum* (no type or description)	Horton 2000
21	*Ceratothoa rapax* Heller, 1865	= *Ceratothoa gaudichaudii* (junior synonym)	Schioedte and Meinert 1883
22	*Ceratothoa salparum* Gourret, 1891	= *Emetha audouini* (junior synonym)	Trilles 1972a
23	*Ceratothoa sargorum* Gourret, 1891	= *Ceratothoa oestroïdes* (junior synonym)	Radujković et al. 1984
24	*Ceratothoa transversa* (Richardson, 1901)	*species inquirenda* (immature specimen)	Present study
25	*Ceratothoa triglae* Gourret, 1891	*species inquirenda* (no type, male specimen)	Present study
26	*Ceratothoa trillesi* (Avdeev, 1979a)	= *Ceratothoa imbricata* (junior synonym)	Martin et al. 2015a
27	*Ceratothoa venusta* Avdeev, 1978	= *Ceratothoa guttata* (junior synonym)	Bruce and Bowman 1989

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RESEARCH ARTICLE

A new species of *Acetalius* Sharp from eastern China (Coleoptera, Staphylinidae, Pselaphinae)

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Abstract

The genus *Acetalius* Sharp currently contains two species from Japan. In this paper, a third species, *A. grandis* Yin & Li, **sp. n.**, is described from eastern China. The foveal pattern of *Acetalius*, and polymorphism and major diagnostic features of *A. grandis* are figured. An updated key to *Acetalius* species is provided.

Keywords

Acetalius, new species, eastern China, Asia, key, taxonomy

Introduction

The genus *Acetalius* Sharp was originally described from a single species, *A. dubius* Sharp, based on a male collected from leaf litter in Kyushu (Suwa Shrine), Japan (Sharp 1883). The specific epithet (*dubius* means 'doubtful, dubious') reflected the author's uncertainty about the higher placement of this genus, by stating that the new species has a 'Pselaphini-like hind body' and an 'elongate *Euplectus* head'. Since the

original description, *Acetalius* has been historically placed in the tribe Brachyglutini (Raffray 1903, 1904a, 1904b, 1904c 1908, 1911), in its own tribe Acetaliini (Jeannel 1958), or together with *Philoscotus* Sawada in subtribe Acetaliina of Euplectini (Besuchet 1985, Nomura 1988a, Newton and Chandler 1989). Recently, Chandler (2001) restricted the concept of Euplectini, removed all taxa having visible tergites IX and sternite IX to the Trichonychini, and placed Acetaliina as a junior synonym of Panaphantina.

Besuchet (1985) redescribed *A. dubius* and added a second species, *A. pilosus* Besuchet, based on a single male from Shikoku. Thereafter, both species of *Acetalius* were treated by Nomura in a series of papers (Nomura 1988a, b, c) revising the subtribe Acetaliina. Thus *Acetalius* is so far represented by two species confined to Japan (Nomura 2013). Members of *Acetalius* lack distinct abdominal paratergites, which are reduced to pairs of marginal carinae, and lack the lateral metacoxal foveae that are often present in other genera of Panaphantina. During our investigation of the pselaphine fauna of eastern China, a third species of *Acetalius* was recognized among the material collected from Fengyangshan Nature Reserve, Zhejiang, which is described in this paper. The entire foveal pattern of *Acetalius* is investigated and described on the basis of a disarticulated male paratype on a slide preparation.

Material and methods

The type material treated in the present paper is deposited in the Insect Collection of the Shanghai Normal University (SNUC).

Dissected parts were preserved in Euparal mounting medium on a plastic slide that was placed on the same pin with the specimen. To investigate the foveal pattern, a male paratype was completely disarticulated and preserved on a slide preparation. Habitus images were taken using a Canon 5D Mark III camera in conjunction with a Canon MP-E 65mm f/2.8 1-5X Macro Lens and a Canon MT-24EX Macro Twin Lite Flash. Images of the morphological details were made using a Canon G9 camera mounted on an Olympus CX31 microscope. Line drawings were initially produced using an Olympus U-DA Drawing Tube, and then inked in Adobe Illustrator CS5. Zerene Stacker version 1.04 was used for image stacking. All images were modified and grouped in Adobe Photoshop CS5 Extended.

The collecting data of the material are quoted verbatim, information not included on the label is placed in parentheses. A slash is used to separate different labels.

The following abbreviations are applied: AL – length of the dorsally visible part of the abdomen (posterior to elytra) along the midline; AW – maximum width of the abdomen; EL – length of the elytra along the suture; EW – maximum width of the elytra; HL – length of the head from the anterior clypeal margin to the occipital constriction; HW – width of the head across the eyes; PL – length of the pronotum along the midline; PW – maximum width of the pronotum. Length of the body (BL) is a combination of HL + PL + EL + AL.

Systematics

Acetalius grandis Yin & Li, sp. n.
http://zoobank.org/2C9DA199-AEE1-4601-9446-E6470AB18F98

Type material. Holotype: macropterous ♂: 'China: S. Zhejiang, Longquan (龙泉 市), Fengyang Shan (凤阳山), creek valley nr. hotel, 27°54'42.85"N, 119°11'52"E, leaf litter, wood, sifted, 1190–1250 m, 28.iv.2014, Peng, Song, Yan, Yin, & Yu leg. / HOLOTYPE [red] ♂, *Acetalius grandis* sp. n., det. Z.-W. Yin, 2016' (SNUC). **Paratypes: 1 apterous** ♂, **1 apterous** ♀, same collecting data as the holotype (SNUC); **1 apterous** ♂, **3 apterous** ♀♀: 'China: S. Zhejiang, Longquan, Fengyang Shan, Da-Tian-Ping (大田坪), 27°54'36"N, 119°10'20"E, leaf litter, moss, ferns, sifted & beating, 1320 m, 27.iv.2014, Peng, Song, Yan, Yin, & Yu leg.' (SNUC). Each paratype bears a yellow type label similar to holotype except for 'PARATYPE, ♂ (or ♀)'.

Diagnosis. Body large-sized, 1.85–2.23 mm; frons with a Y-shaped carina extending anteriorly to reach clypeal anterior margin; each eye of macropterous male with about 55 facets, that of apterous male with about 25–30 facets, and apterous female about 12 facets; antennomeres III elongate, IV–X distinctly transverse; abdominal tergite IV with three pairs of marginal carinae, discal carinae parallel; sternite IV with long median and two shorter admesal carinae.

Description. Apterous male (Fig. 1B). Length 1.91–2.03 mm; head (Fig. 2A–C) roundly rectangular, HL 0.34–0.36 mm, HW 0.43–0.46 mm; with small, nude vertexal foveae (Figs 2A, 3A; *vf*) situated at dorsal level of midline of eyes, lacking sulcus connecting vertexal foveae; antennal tubercles moderately prominent, connected by Y-shaped frontal carina (Figs 2A, 3A; *fc*) that extends anteriorly to meet anterior margin of clypeus, area between antennal tubercles depressed; with pair of subantennal carinae (Fig. 3A; *sac*) just posterior of antennal bases; each eye composed of about 25–30 facets; mandibles exceptionally elongate; antennal clubs (Fig. 3B) formed by apical two antennomeres, antennae with large scapes and pedicles, antennomeres III elongate, IV–X strongly transverse, successively wider and larger, XI largest, nearly oval, apex forming thumb-like protuberance surrounded by ring of thick setae; ocular-mandibular carinae (Fig. 2B; *omc*) present; gular foveae (Fig. 2C; *gf*) in single pit, situated in strongly depressed area, median gular sulcus (Fig. 2C; *mgs*) thin; apicolateral genal projections (Fig. 2C; *agp*) present.

Pronotum (Fig. 2D–F) as long as wide, PL 0.35–0.37 mm, PW 0.35–0.36 mm, laterally rounded at apical half, sides of basal half successively constricted toward base; broad antebasal sulcus connecting nude lateral antebasal foveae (Fig. 2D–E; *laf*); lacking median antebasal fovea; disc moderately convex, with antebasal tubercles (Fig. 2D–E; *at*) in sulcus, mediobasal carina (Figs 2D, 3A; *mbc*) extending from anterior margin of antebasal sulcus to pronotal base, with pair of short basolateral carinae (Fig. 2D; *blc*); median longitudinal sulcus (Fig. 3A; *ms*) thin, lacking lateral longitudinal sulci; paranotal carinae (Fig. 2E–F; *pc*) sinuate, complete. Prosternite with distinct median carina (Fig. 2F; *mc*); lateral procoxal foveae small and close (Fig. 2F; *lpcf*).

Figure 1. Dorsal habitus of *Acetalius grandis*. **A** Macropterous male **B** Apterous male. Scale bars: 0.5 mm.

Elytra (Fig. 2G) rounded laterally, distinctly constricted at base, EL 0.62–0.64 mm, EW 0.66–0.71 mm; each elytron with two basal foveae (Fig. 2G; *bef*); with one pair of short discal striae extending from inner margins of outer basal foveae posteriorly to less than one-fourth of elytral length; sutural striae (Fig. 2G; *ss*) complete; lacking subhumeral foveae, marginal sulci, and apicolateral cleft. Metathoracic wings absent.

Mesoventrite with two widely separated median foveae (Fig. 2H; *mmsf*); lateral mesoventral foveae (Fig. 2H; *lmsf*) simple, slanted anteriorly; with lateral mesocoxal foveae (Fig. 2H; *lmcf*); metaventrite lacking lateral metacoxal foveae, posterior margin with narrow split at middle.
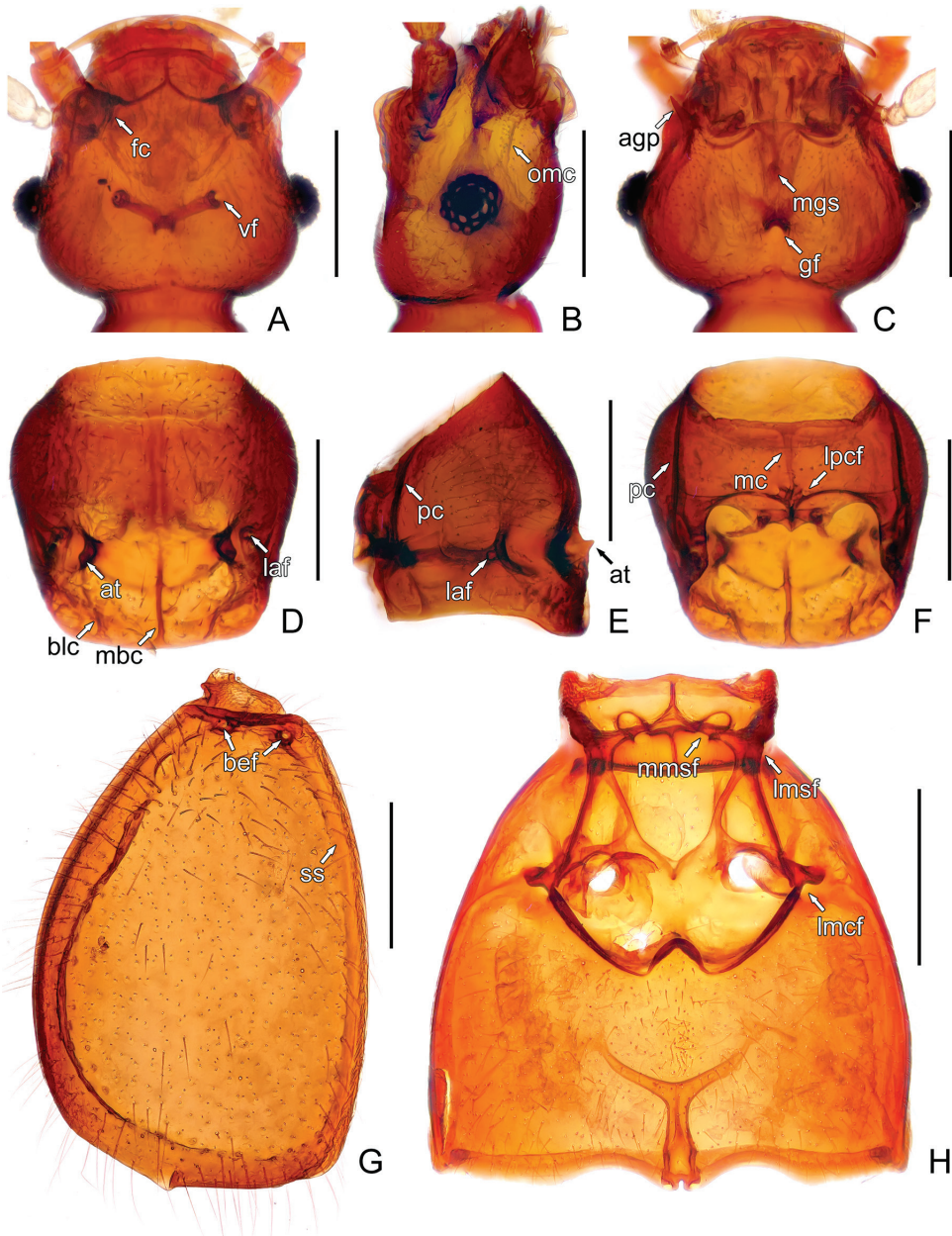
Figure 2. Morphology of *Acetalius grandis*. **A** Head, in dorsal view **B** Same, in lateral view **C** Same, in ventral view **D** Pronotum **E** Prothorax, in lateral view **F** Prosternite **G** Left elytron **H** Meso- and metaventrite. Abbreviations: agp – apicolateral genal projections, at – antebasal tubercles, bef – basal elytral foveae, blc – basolateral carinae, fc – frontal carina, gf – gular fovea, laf – lateral antebasal foveae, lmcf – lateral mesocoxal foveae, lmsf – lateral mesoventral foveae, lpcf – lateral procoxal foveae, mbc – mediobasal carina, mc – median carina, mgs – median gular sulcus, mmsf – median mesoventral foveae, omc – ocular-mandibular carinae, pc – paranotal carinae, ss – sutural striae, vf – vertexal foveae. Scale bars: 0.2 mm.
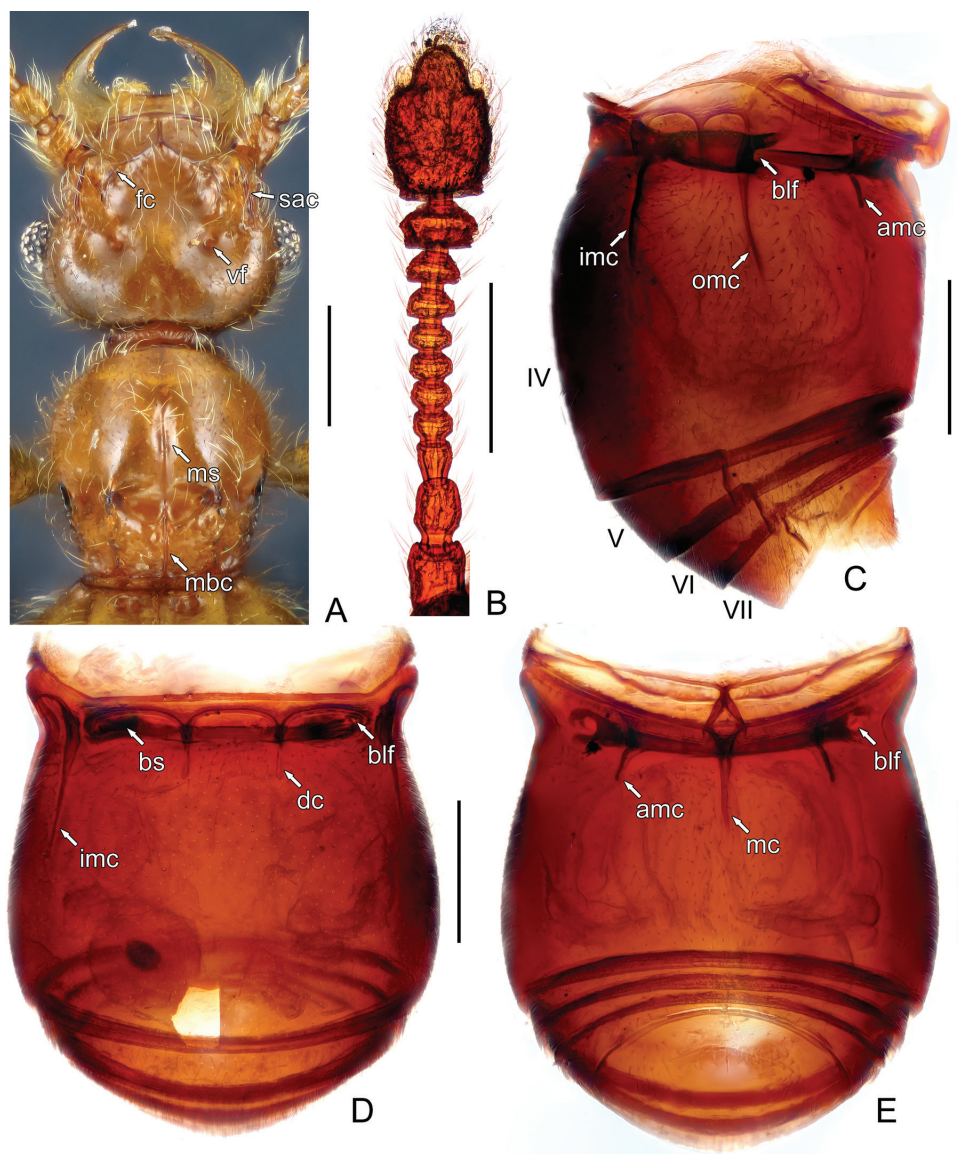
Figure 3. Morphology of *Acetalius grandis*. **A** Head dorsum and pronotum **B** Right antenna **C** Abdomen, in lateral view **D** Abdomen, in dorsal view **E** Abdomen, in ventral view. Abbreviations: amc – admesal carinae, blf – basolateral foveae, bs – basal carina, bs – basal sulcus, dc – discal carinae, fc – frontal carina, imc – inner marginal carinae, mbc – mediobasal carina, ms – median sulcus, omc – outer marginal carinae, sac – subantennal carinae, vf – vertexal foveae. Scale bars: 0.2 mm.

Legs with profemora simple ventrally, protibiae slightly expanded at apices.

Abdomen (Fig. 3C–E) widest at middle, AL 0.60–0.66 mm, AW 0.61–0.67 mm, with tergite IV (first visible tergite) longest, V–VIII successively shorter. Tergite IV
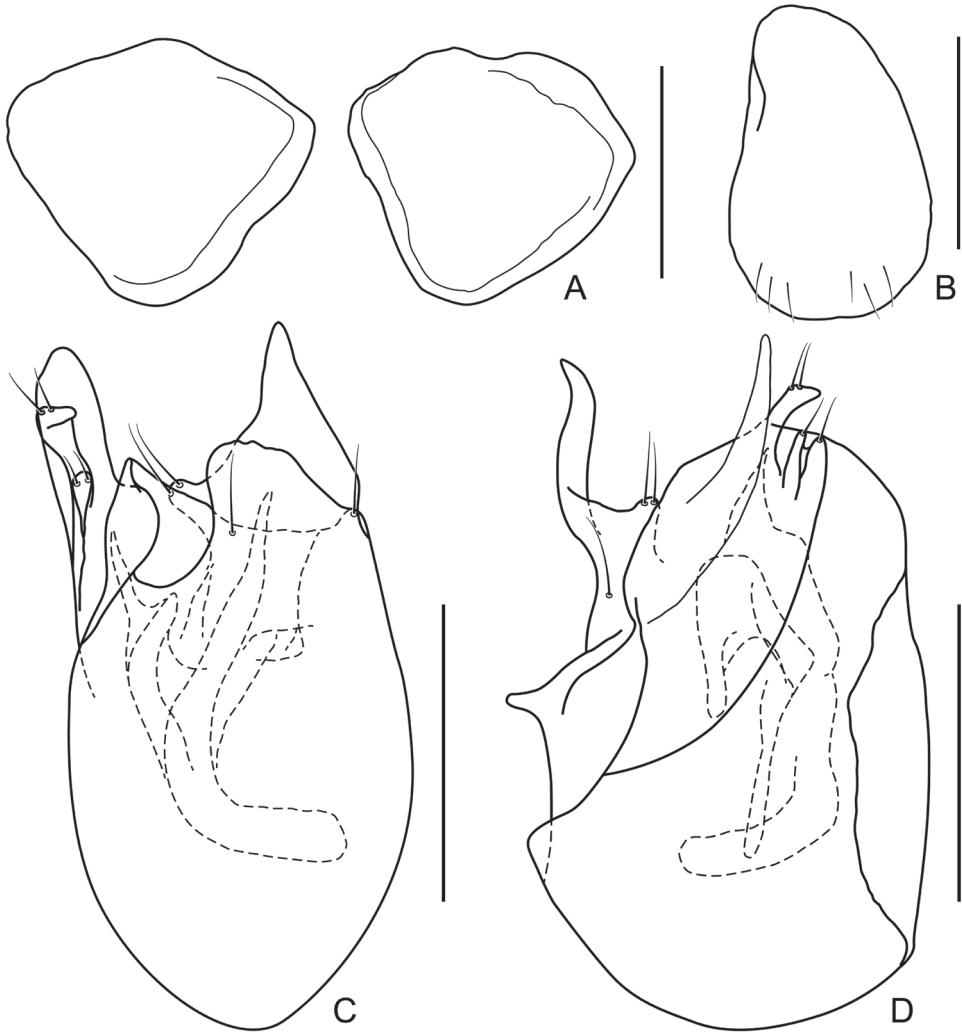
Figure 4. Morphology of *Acetalius grandis*. **A** Male tergites IX **B** Male sternite IX (penial plate) **C** Aedeagus, in dorsal view **D** Same, in lateral view. Scale bars: 0.1 mm.

with two small basolateral foveae (Fig. 3D; *blf*) in deep basal sulcus (Fig. 3D; *bs*) which is separated into three parts by pair of short, parallel discal carinae (Fig. 3D; *dc*), with short pair of short carinae between long inner and outer marginal carinae (Fig. 3C–D; *imc*, *omc*); V–VIII each with one pair of small basolateral foveae; tergites IX (Fig. 4A) paired, weakly sclerotized. Sternite III (first visible sternite) short, with arrow-like protuberance at middle; IV longest, with two large basolateral foveae (Fig. 3E; *blf*) in basal impression, with single long median carina (Fig. 3E; *mc*) and pair of shorter admesal carinae (Fig. 3E; *amc*); sternites V–III each short; sternite IX (Fig. 4B) nearly oval.

Length of aedeagus (Fig. 4C–D) 0.25–0.26 mm; aedeagus weakly sclerotized, parameres fused with median lobe; endophallus composed of one large, curved and several smaller sclerites.

Macropterous male (Fig. 1A). Measurements: BL 1.85 mm, HL 0.32 mm, HW 0.42 mm, PL 0.32 mm, PW 0.32 mm, EL 0.58 mm, EW 0.66 mm, AL 0.63 mm, AW 0.58 mm. Length of aedeagus 0.24 mm. Each eye composed of about 55 facets; base of elytra more expanded than that of apterous male, each elytron with two discal striae extending from lateral and mesal margin of outer basal foveae, respectively. Metathoracic wings fully developed.

Apterous female. Measurements: BL 2.19–2.23 mm, HL 0.40–0.42 mm, HW 0.48–0.50 mm, PL 0.39–0.42 mm, PW 0.36–0.38 mm, EL 0.66–0.72 mm, EW 0.76–0.80 mm, AL 0.70–0.71 mm, AW 0.70–0.75 mm. Each eye composed of about 12 facets. Elytral base constricted as that in apterous male. Metathoracic wings absent. Apices of protibiae not expanded.

Comparative notes. *Acetalius grandis* can be readily separated from both *A. dubius* (1.1–1.4 mm) and *A. pilosus* (1.4–1.6 mm) by the distinctly larger body size (1.85–2.23 mm), and presence of a frontal carina on the head, which is lacking in the other species. The new species shares with *A. pilosus* the presence of three pairs of marginal carinae on tergite IV, and similar structure of the aedeagus, but differs also by the elongate antennomeres III, which are as long as wide in *A. pilosus*. *Acetalius dubius* has the smallest body size, and there are only two pairs of marginal carinae on tergite IV.

Distribution. Eastern China: Zhejiang.

Etymology. The specific epithet implies that *Acetalius grandis* is the largest species in the genus.

Key to *Acetalius* species (modified from Nomura 1988a)

1   Body size 1.85–2.23 mm; frons with a Y-shaped carina extending anteriorly to reach anterior margin of the clypeus. (Eastern China: Zhejiang)..............
       .... ***A. grandis* Yin & Li, sp. n.**
–   Body size 1.1–1.6 mm; frons lacking carina...**2**
2   Body size relatively smaller, 1.1–1.4 mm; tergite IV with two pairs of marginal carinae, discal carinae slightly divergent; female has eyes each with 2–3 facets. (Japan: Honshû, Shikoku, Kyûshû, Tsushima) ....***A. dubius* Sharp**
–   Body size relatively larger, 1.4–1.6 mm; tergite IV with three pairs of marginal carinae, discal carinae strictly parallel; female has eyes each with about 20 facets. (Japan: Shikoku, Kyûshû)..............................***A. pilosus* Besuchet**

Acknowledgments

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RESEARCH ARTICLE

Possible living fossil in Bolivia: A new genus of flea beetles with modified hind legs (Coleoptera, Chrysomelidae, Galerucinae, Alticini)

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Abstract

A new genus (*Chanealtica*) with three new species (*C. cuevas*, *C. ellimon*, and *C. maxi*) from Bolivia is described and illustrated. It is compared with *Aphthonoides* Jacoby, 1885, *Argopistes* Motschulsky, 1860, *Metroserrapha* Bechyne, 1958, *Psylliodes* Berthold, 1827 and *Psyllototus* Nadein, 2010. Remarkably, based on the available characters, among all the flea beetles, *Chanealtica* is mostly similar to an extinct genus *Psyllototus*. A discussion of diversity and function of the hind leg in flea beetles is provided.

Keywords

Introduction

As currently understood, flea beetles (Coleoptera: Chrysomelidae: Galerucinae: Alticini) constitute a polyphyletic group (about 9900 valid species assigned to 577 valid genera) most members of which are characterized by an enlarged metafemora with metafemoral spring inside and an ability to leap (Konstantinov 1994). In beetles and other small insects the leaping ability is understood as high-speed escape reflex (Brackenbury and Wang 1995). It is reasonable to assume that enlarged metafemora with

metafemoral spring and associated leaping ability, at least in part, are responsible for extraordinary flea beetle radiation (they are the most species rich family level taxon among about 36,000 named leaf beetle species). The associated diversification resulted in a remarkable variety of forms and shapes of the flea beetle bodies, but particularly in the structure of the hind legs. Flea beetle hind legs differ not only in proportions of metafemora, tibiae and tarsi, but also in shapes, armaments and sites of attachment of hind leg structures, particularly that of the metatarsi.

The current representatives of the vast majority of flea beetle genera have their first metatarsomere attached to the apex of the metatibia (just like in much of all beetles). In Monoplatina flea beetles (47 valid genera and 583 species), the first metatarsomere is attached slightly away from the metatibial apex. Until now, leaf beetles from only four extant genera (*Aphthonoides* Jacoby, 1885, *Argopistes* Motschulsky, 1860, *Metroserrapha* Bechyne, 1958, and *Psylliodes* Berthold, 1827) have their first metatarsomere attached to the metatibia at a significant distance from its apex.

Chanealtica, a new genus discovered in Bolivia (described in this paper) possesses the same character state. The overall structure of the hind leg in *Chanealtica* is mostly similar to that of *Psylliodes*. However all 204 recent *Psylliodes* species have 10 antennomeres in their antennae, a condition rarely observed in flea beetles (e.g. *Deciplatus* Linzmeier & Konstantinov, 2009 and *Monotalla* Bechyne, Konstantinov et al. 2015), while *Chanealtica* has 11.

Remarkably, based on the characters that are available for observation, among all the flea beetles, the only one with combination of 11 antennomeres and the first metatarsomere attached to the metatibia at a significant distance from its apex is a recently described from Baltic amber, extinct flea beetle genus *Psyllototus* Nadein (in Nadein and Perkovsky 2010). Although, details of the *Psyllototus* head and ventral side of the body are not available for study. In total, 14 species in 11 genera of fossil flea beetle are known so far (Bukejs and Konstantinov 2013, Bukejs et al. 2015). Morphologically they are close to most typical flea beetles, e.g. *Altica* Geoffroy, with antebasal transverse impression on pronotum and the first metatarsomere attached to the apex of the metatibia. Overall *Psyllototus* is as different from fossil flea beetles as *Chanealtica* from recent.

Material and methods

Dissecting techniques, measurements, and terminology follow Konstantinov (1998). Observations were made with a Zeiss Discovery V20 microscope and digital images were taken with an AxioCam HRC digital camera attached to it. Habitus illustration was produced after a technique described in Litwak and Harel (2013). Specimens are deposited in the Museo de Historia Natural Noel Kempff Mercado, Universidad Autonoma "Gabriel Rene Moreno", Santa Cruz, Bolivia (MNKB) and National Museum of Natural History, Smithsonian Institution, Washington DC, USA (USNM).

Results

Chanealtica gen. n.
http://zoobank.org/40F47B5F-3D6F-4711-8D1F-F98681353855
Figs 1–36

Description. Body length: 2.59–3.29 mm; body width (widest point of elytra): 1.35–1.67 mm. Pronotum width to length ratio: 1.66–1.77. Width of elytra at base (in middle of humeral calli) to width of pronotum at base ratio: 1.20–1.21.

Body light ochre with last eight antennomeres, elytral apices (in *C. cuevas*) and bases of metatibia dark brown.

Head with midcranial and frontal sutures absent. Supraorbital sulcus deep. Orbital sulcus visible, situated close to eye. Supracallinal sulcus absent. Supraantennal sulcus shallow, poorly developed. Midfrontal sulcus developed only dorsally, absent ventrally, antennal calli completely separated only dorsally, connected ventrally. Suprafrontal sulcus poorly developed. Frontolateral sulcus well developed. Antennal callus long, oblique, nearly triangular, entering interantennal space. Surface of antennal callus covered with fine, long, transverse wrinkles, situated slightly above surface of vertex. Vertex densely and evenly covered with deep, but poorly delineated punctures. Frontal ridge and vertex separated by antennal calli. Width of frontal ridge to width of antennal socket (including surrounding ridges) ratio 0.85 - 0.88. Frontal ridge short, in lateral view almost straight. Area below antennal socket concave. Orbit normally wide, nearly as wide as transverse diameter of antennal socket. Distance between eyes above antennal sockets to transverse diameter of eye in frontal view ratio 3.25 - 3.32. Sides of head below eyes converging ventrally. Labrum flat, with 2 pairs of long setae; anterior margin complete, with slight indentation on upper surface. Apical maxillary palpomere conical. Preapical maxillary palpomere wider than apical palpomere. Antennal sockets situated below middle of eye. Antenna filiform, with 11 antennomeres. Length of antenna over pronotum reaching beyond middle of elytron.

Pronotum wider than long, with sides slightly convex to nearly straight. Pronotal base slanted from posterolateral callosities, straight in middle. Lateral margin narrowly explanate, without setae. Anterolateral callosity relatively short, nearly perpendicular to lateral margin. Posterolateral callosity short, not protruding beyond lateral margin. Pronotal punctures relatively dense, shallow. Procoxal cavities open. Intercoxal prosternal process short, narrowing posteriorly, does not extend beyond procoxae, lateral sides straight, posterior end rounded.

Scutellum present. Elytron with punctation confused and few irregular poorly defined longitudinal ridges. Elytra at base wider than base of pronotum. Humeral calli well developed. Basal calli present, poorly separated from elytral disc. Epipleura slightly oblique outwardly, gradually narrowing from base to apex, reaching end of elytron side, but not apex. Mesosternum without elevated projection in middle, flat, in shape similar to intercoxal prosternal process. Metasternum anteriorly without elevated projection in middle and not projecting forward hiding mesosternum.

First adominal ventrite free. Abdominal ventrites about equally long. First abdominal ventrite between coxae without longitudinal ridges, with apex truncate. Last visible tergite without longitudinal groove in middle. Male last abdominal sternite with transverse ridge. In female ridge absent, instead last abdominal sternite forms posteriorly directed lobe with slightly converging sides.

Pro- and mesotibiae caniculate dorsally. Protibial and mesotibial spurs absent. Metatibia strongly curved. Metatibia in cross section around its middle more or less triangular. Dorsal surface flat to concave. Bristles present on lateral and mesal sides of metatibiae. Metatarsomere 1 attached away from metatibial apex, distance between metatarsal attachment and metatibial apex about 0.3 of metatibial length. Apical spur of metatibia simple, wide, ending in one tooth, situated in middle, but directed medially. Metatarsomere 1 nearly round, longer than rest of metarsomeres together. Claws appendiculate.

Median lobe of aedeagus in cross section somewhat flat, with shallow impressions near apex.

Spermatheca with distinct border between receptacle and pump. Receptacle slightly longer than wide, much wider but about as long as pump, ovoid. Spermathecal duct very wide at base, curved, without coils. Vaginal palpi not fused medially, gradually narrowing posteriorly with a few bristles at apex. Tignum with narrow and relatively short base and wide and long sclerotization posterior.

Etymology. I named the genus after the Chané, a native ethnic group of people, whose traditional lands are in the plains and valleys between the Gran Chaco and the Andes in Bolivia and also in northern Argentina and Paraguay. The name is feminine.

Type species. *Chanealtica cuevas* Konstantinov, sp. n.

Host plant. *Tecoma stans* (L.) Juss. (Bignoniaceae) (Figs 11, 12).

Remarks. *Chanealtica* is markedly different from most known flea beetle genera. However, based on the general structure of the hind tibia and tarsi, *Chanealtica* is similar to extant *Aphthonoides*, *Argopistes*, *Metroserrapha*, *Psylliodes*, and extinct *Psyllototus*. Representatives of all these genera have their metatarsi attached not at the apex of the metatibia but before the apex (Figs 37–42). Many flea beetles of the subtribe Monoplatina have their metatarsi attached to the metatibia before its apex, but the distance from the place of the attachment to the apex is very short in Monoplatina compared to that of *Aphthonoides*, *Argopistes*, *Chanealtica*, *Metroserrapha*, *Psylliodes*, and *Psyllototus*. In addition Monoplatina are clearly different based on a number of phylogenetically important characters (such as globose fourth meso- and metatarsomere, densely setose dorsal surface of the body etc.).

Chanealtica can be easily separated from *Aphthonoides* based on a much larger size, metatibia much longer than metatibial spur, confused elytral punctures and numerous features of the head and thorax (*Aphthonoides* has much smaller body, metatibia much shorter than metatibial spur, elytral punctures arranged into striae). *Chanealtica* clearly differs from *Argopistes* in having an elongate and flat body in lateral view, prognathous head, short frontal ridge, narrow and oblique outwardly elytral epipleura (in *Argopistes* body round and convex in lateral view, head opistognathous, frontal ridge long, elytral epipleura wide and oblique inwardly).

In the site of the metatarsal attachment and general shape, metatibia of *Chanealtica* is more similar to that of *Metroserrapha* and *Psylliodes*. In these genera, the metatarsi are attached at about basal 2/3 of the metatibia; the dorsal surface of the metatibia before the metatarsal attachment is flat or canaliculate with lateral margins lacking denticles; the dorsal surface posterior to the metatarsus is deeply channeled and lateral margins are covered with denticles or a row of stiff and short bristles; the metatibial apex is armed with a large, acute spur. *Chanealtica* can be easily distinguished from *Metroserrapha* and *Psylliodes* based on a large body flat in lateral view, confused elytral punctuation, short frontal ridge, anterolateral callosity relatively short, nearly perpendicular to lateral margin of pronotum. In addition, *Chanealtica* and *Metroserrapha* species have 11 antennomeres while all *Psylliodes* species have 10.

The last abdominal sternite contains the unique feature of *Chanealtica*. In male the apex of the sternite is swollen into a transverse ridge. In female the ridge is absent, instead the last abdominal sternite forms a posteriorly directed lobe, which is common in males of many other flea beetle genera (e.g. *Longitarsus* Berthold).

Based on the characters that are available for observation in amber preserved specimens, among all the flea beetles, *Chanealtica* is mostly similar to an extinct genus *Psyllototus*. They share two most vivid character states: structure of the hind leg and antennae with 11 antennomeres. Details of *Psyllototus* head and ventral side of the body are not available for study.

Chanealtica cuevas sp. n.

http://zoobank.org/421BC2E0-630A-4C73-A0FC-73E5DA33C2AF
Figs 1–12

Description. Body length 2.75–3.24 mm. Width 1.35–1.67 mm. Color light ochre with last eight antennomeres, elytral apices and bases of metatibia dark brown. Metatibial apices black.

Proportions of male antennomeres 1–6 as follows: 13:6:7:11:13:13.

Pronotum with lateral margins slightly and evenly convex, at base almost as wide as at apex. Length to width ratio of first protarsomere of male 1.78.

Median lobe of aedeagus in ventral view relatively narrow, widening relatively abruptly (Fig. 5). Apex in ventral view with low, slightly channeled ridge separating two wide and shallow impressions lateral of it. Apex in lateral view with distinct knob facing ventrally. Spermathecal receptacle with basal part significantly smaller than apical (Fig. 8). Narrow, anterior part of tignum shorter than posterior part (Fig. 9).

Host plant. *Tecoma stans* (L.) Juss. (Bignoniaceae) (Figs 11, 12).

**Etymology.** This species is named after the type locality.

Material examined. Holotype, male. Labels: 1) BOLIVIA: Santa Cruz Dept. Florida Prov., 7 km SE of Cuevas WP-407, 1332m, 18°12.414'S, 63°40.808'W, 27.XI.2013, leg. A. Konstantinov; 2) Holotype *Chanealtica cuevas* n. sp. des. A. Konstantinov, 2016 (will be deposited at MNKB, currently at USNM). Paratypes
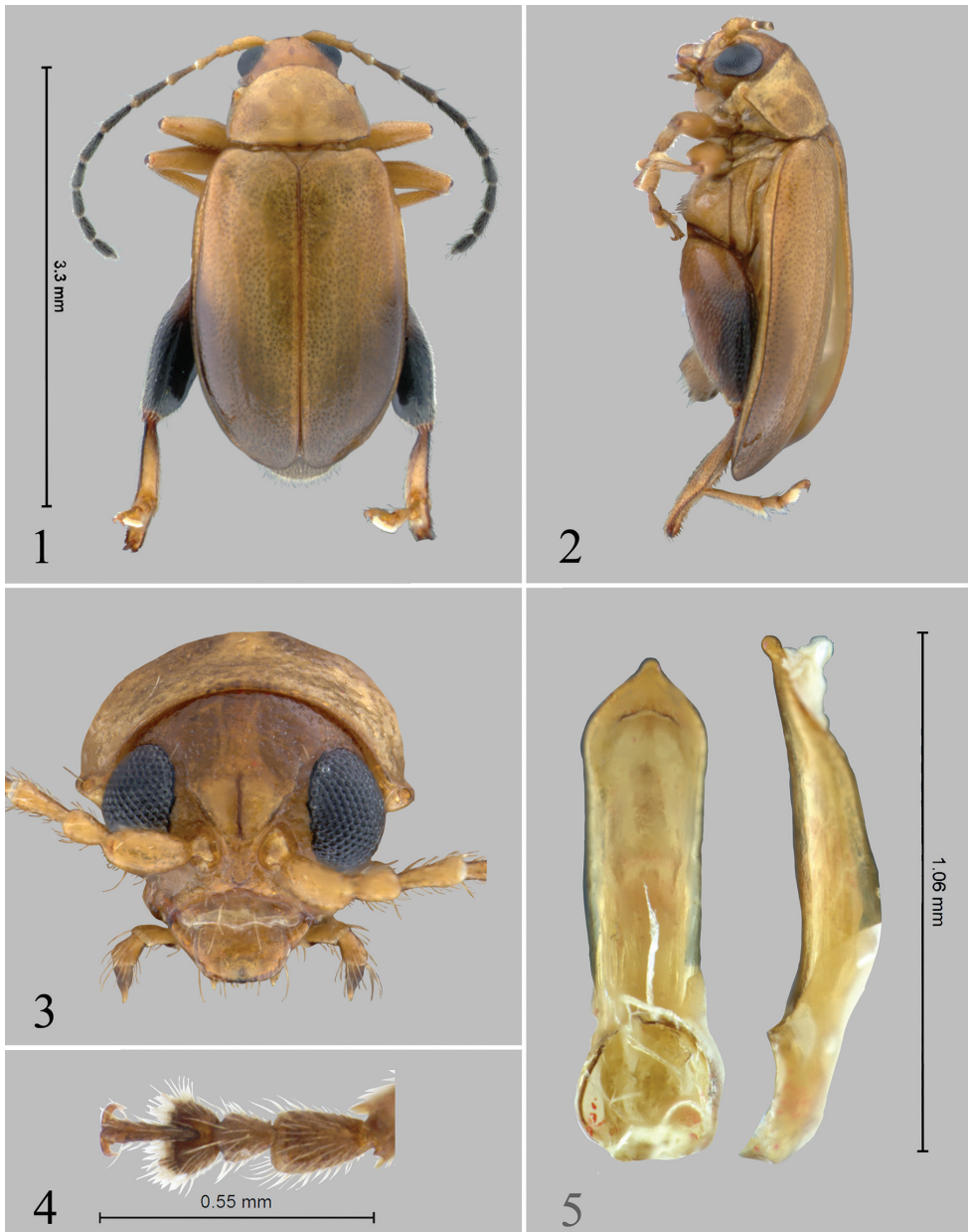
Figures 1–5. *Chanealtica cuevas.* **1** Habitus dorsal **2** Habitus lateral **3** Head, frontal view **4** Front tarsi, male **5** Aedeagus, ventral and lateral views.

38 specimens. Same labels as holotype (19 USNM, 2 MNKB). Paratypes. Labels: 1) BOLIVIA: Santa Cruz Dept. Florida Prov., 7 km SE of Cuevas WP-408, 1350m, 18°12.734'S, 63°40.776'W, 28.XI.2013, leg. A. Konstantinov; 2) Paratype *Chanealtica cuevas* n. sp. des. A. Konstantinov, 2016 (15 USNM, 2 MNKB).
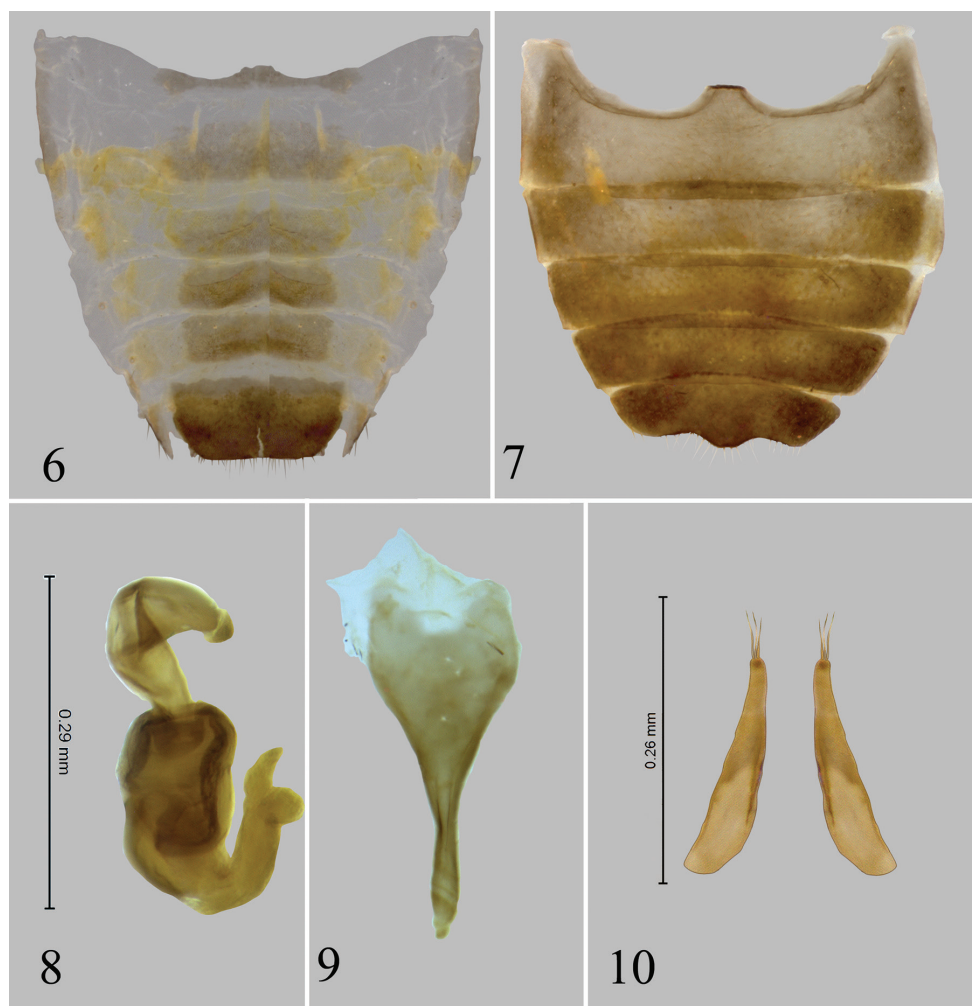
Figures 6–10. *Chanealtica cuevas.* **6** Abdominal tergites, female **7** Abdominal sternites, female **8** Spermatheca **9** Tignum **10** Vaginal palpi.

Chanealtica ellimon sp. n.

http://zoobank.org/AA7F0AC0-8871-4049-9361-FFB101C2E1EB
Figs 13–25

Description. Body length 2.64–3.08 mm. Width 1.51–1.62 mm. Color light ochre with last eight antennomeres and apices of metatibia dark brown.

Proportions of male antennomeres 1–6 as follows: 13:6:7:10:13:13.

Pronotum with lateral margins slightly convex, at base more so than at apex, also narrower at apex than at base. Length to width ratio of first protarsomere of male 1.98.
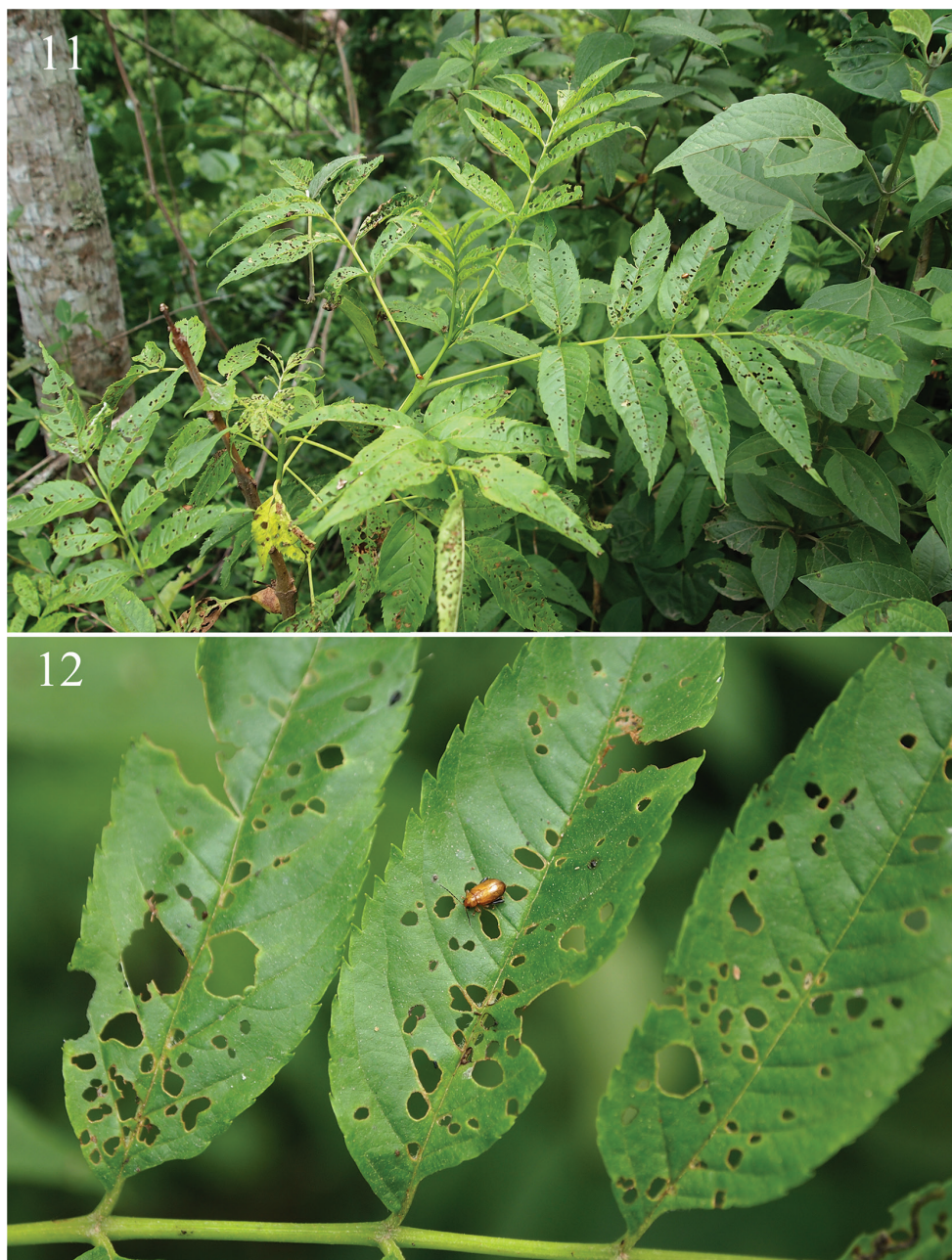
Figures 11–12. *Chanealtica cuevas.* Host plant, *Tecoma stans* (L.) Juss. (Bignoniaceae).
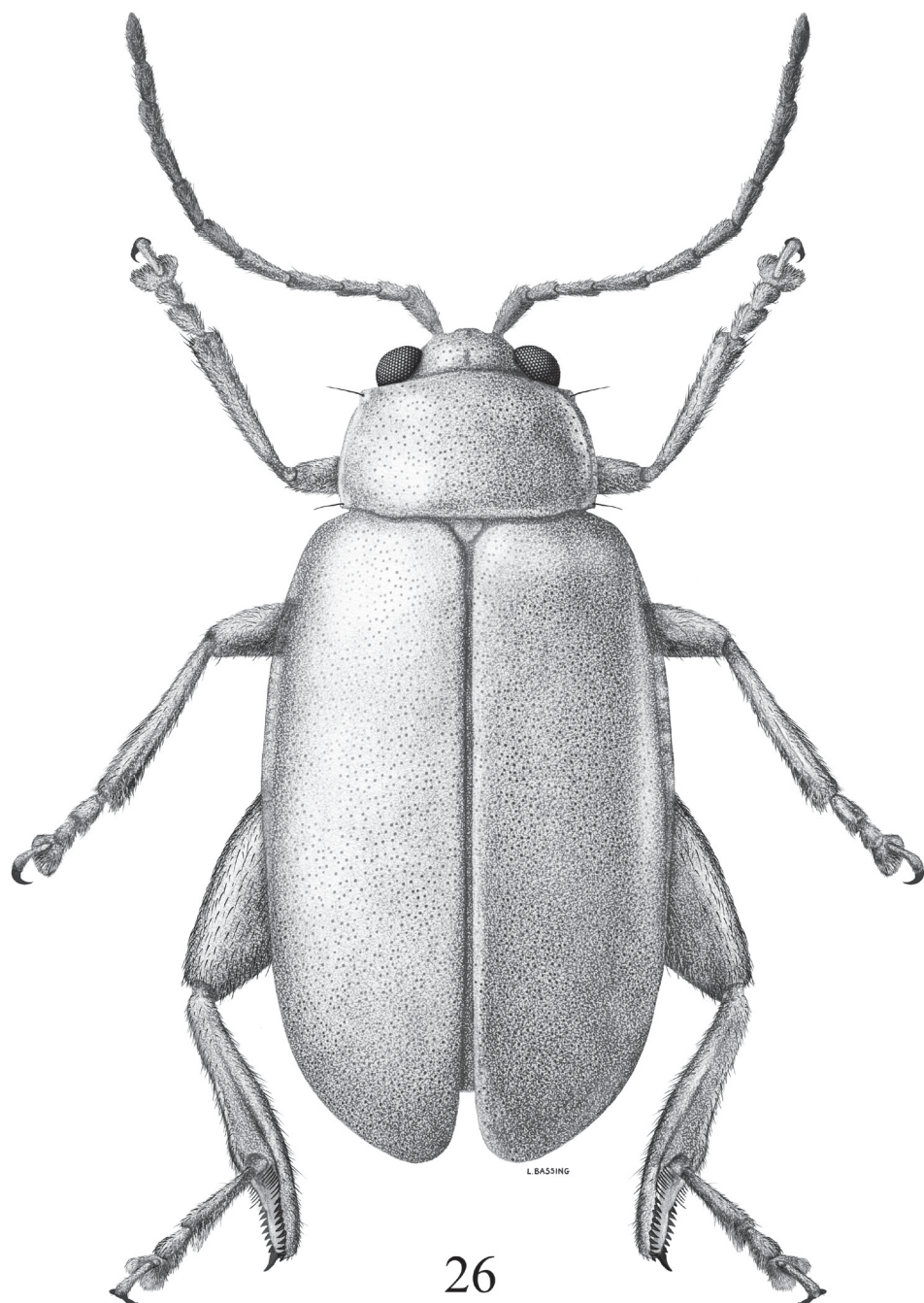
Median lobe of aedeagus in ventral view relatively narrow, widening relatively gradually (Fig. 19). Apex in ventral view with low, not channeled ridge ending into shallow impression lateral and behind it. Two elongate and shallow impressions situated on

Figures 13–18. *Chanealtica ellimon*. **13** Habitus dorsal **14** Habitus lateral **15** Head, frontal view **16** Pronotum **17** Front tarsi, male **18** Hind leg, male.

sides of apical part of median lobe. Apex in lateral view without distinct sphere, bend ventrally. Spermathecal receptacle with basal part significantly smaller than apical (Fig. 21). Narrow, anterior part of tignum about as long as posterior part (Fig. 24).

Figures 19–25. *Chanealtica ellimon*. **19** Aedeagus, ventral and lateral views **20** Abdominal tergites, female **21** Spermatheca **22** Vaginal palpi **23** abdominal sternites, female **24** Tignum **25** 8th abdominal tergite.

Etymology. This species is named after the type locality.

Material examined. Holotype male. Labels: 1) Bolivia: Santa Cruz Dept. Cordillera Prov., Mirador, El Limón 845 meters, 19°04'S, 63°28'W 8.XI.2007, leg. A.

Konstantinov; 2) Holotype *Chanealtica ellimon* sp. n. des. A. Konstantinov 2016 (will be deposited at MNKB, currently at USNM). Paratypes with same labels as holotype (3 USNM, 1 MNKB).

Chanealtica maxi sp. n.
http://zoobank.org/856EF8FF-617D-4555-ABB2-9C8098C962A1
Figs 26–36

Description. Body length 2.59–3.29 mm. Width 1.51–1.72 mm. Color light ochre with last eight antennomeres and apices of metatibia dark brown.

Proportions of male antennomeres 1–6 as follows: 14:6:8:10:14:13.

Pronotum with lateral margins nearly straight, at base wider than at apex. Length to width ratio of first protarsomere of male 1.43.

Median lobe of aedeagus in ventral view relatively wide, widening relatively gradually (Fig. 29). Apex in ventral view without ridge, but with wide and shallow impression. Apex in lateral view with indistinct knob slightly bent ventrally. Spermathecal receptacle with basal part significantly smaller than apical (Fig. 34). Narrow, anterior part of tignum shorter than posterior part (Fig. 35).

Etymology. This species is named after Max Joseph Miles, the "newest" member of the Konstantinov/Miles family.

Material examined. Holotype female. Labels: 1) Bolivia: Santa Cruz Dept. Florida Prov., Refugio Los Volcanes, 4 km N of Bermejo 1000–1300 m 29.X.2007 18°06'S, 63°36'W leg. S. Lingafelter & N. Woodley; 2) Holotype *Chanealtica maxi* sp. n. des. A. Konstantinov 2016 (will be deposited at MNKB, currently at USNM). Paratypes: 1) Bolivia: Santa Cruz Dept. Florida Prov., Refugio Los Volcanes, 4 km N of Bermejo, 1000–1300 m 28.X.2007, 18°06'S, 63°36'W leg. A. Konstantinov; 2) Paratype *Chanealtica maxi* sp. n. des. A. Konstantinov 2016 (2 USNM). 1) Bolivia: Santa Cruz Dept. Florida Prov., Refugio Los Volcanes, 4 km N of Bermejo, 1000-1300 m 4.XI.2007, 18°06'S, 63°36'W Laurel trail leg. A. Konstantinov; 2) Paratype *Chanealtica maxi* sp. n. des. A. Konstantinov 2016 (2 USNM).

Key to *Chanealtica* species

1   Elytral apices dark brown, darker than rest of elytra. Apex of median lobe of aedeagus in ventral view with low, slightly channeled ridge separating two wide and shallow impressions lateral of it. Apex in lateral view with distinct knob facing ventrally ....***C. cuevas***

–   Elytral apices light ochre, as light as rest of elytra. Apex of median lobe of aedeagus in ventral view without low ridge, or if ridge present, its is not chanelled **2**

Figure 26. *Chanealtica maxi*. Dorsal habitus.
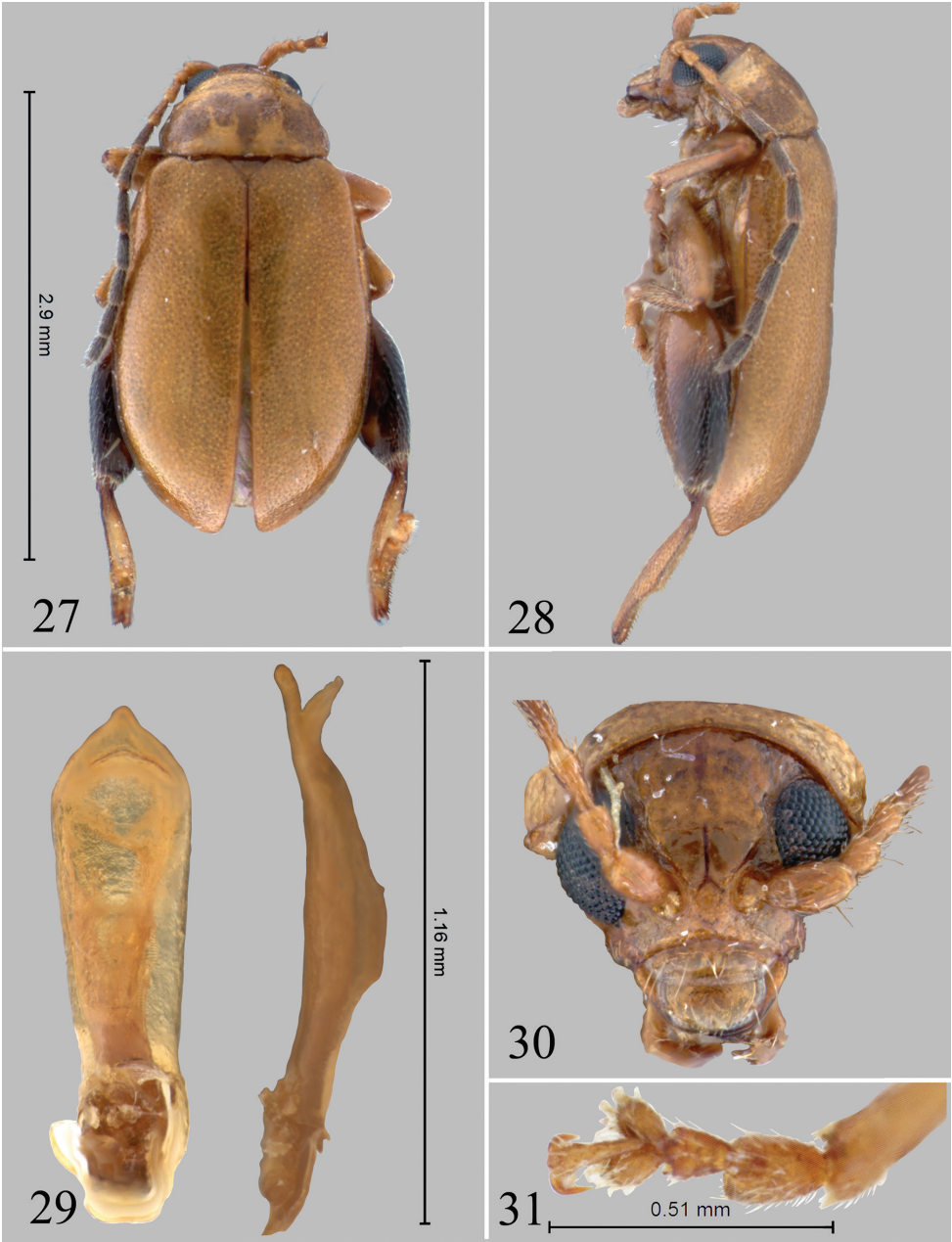
Figures 27–31. *Chanealtica maxi*. **27** Habitus dorsal **28** Habitus lateral **29** Aedeagus, ventral and lateral views **30** Head, frontal view **31** Front tarsi, male.

Figures 32–36. *Chanealtica maxi*. **32** Abdominal tergites, female **33** Abdominal sternites, female **34** Spermatheca **35** Tignum **36** Vaginal palpi.

2(1)   Pronotum with lateral margins slightly convex, at base more so than at apex. Median lobe of aedeagus in ventral view relatively narrow. Apex of median lobe of aedeagus in ventral view with low, not channeled ridge ending into shallow impression lateral and behind it. Two elongate and shallow impressions situated on sides of apical part of median lobe (Fig. 19) ...... *C. ellimon*

–   Pronotum with lateral margins nearly straight, at base wider than at apex. Median lobe of aedeagus in ventral view relatively wide. Apex of median lobe of aedeagus in ventral view without ridge, but with wide and shallow impression (Fig. 29) .... *C. maxi*

Discussion on flea beetle hind leg diversity and jumping

It seems reasonable to assume that the diversity of the hind legs in flea beetles (Figs 37–42) (exceeding that of many other much more species rich groups of beetles that are not in habit of leaping) is somehow influenced by their leaping. Indeed, the hind leg modifications in beetles are associated with their particular functions (Crowson 1981).

A study of the kinematics of the flea beetle jump (Brackenbury and Wang 1995) suggested that there is a difference in the jump parameters between species with different hind leg structure. Schmitt (2004) argued that three variables affect the flea beetle jumping performance: 1) metafemoral spring structure (a more slender spring with a
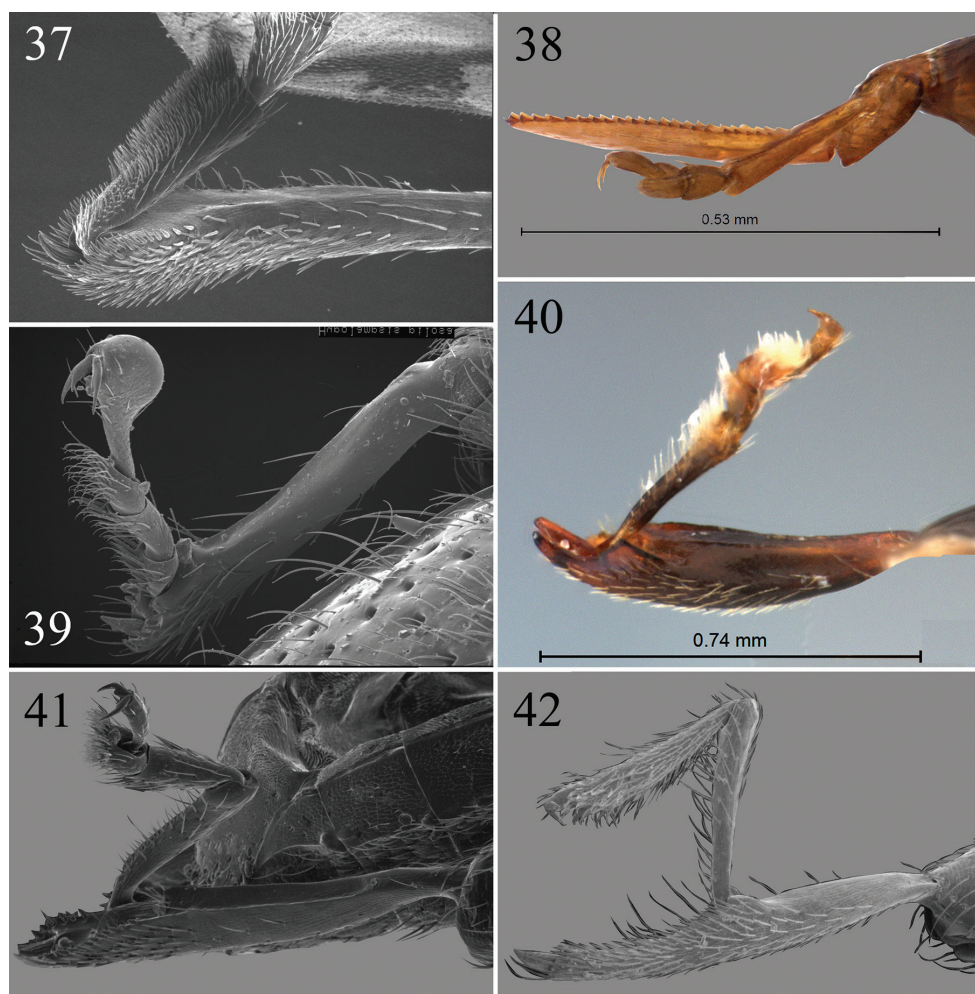
Figures 37–42. Examples of the metatibiae and metatarsi in flea beetles. **37** *Aphthona nonstriata* (Goeze) **38** *Aphthonoides* sp. **39** *Hypolampsis pilosa* (Illiger) **40** *Argopistes* sp. **41** *Psylliodes luteola* (Muller) **42** *Metroserrapha* sp.

seemingly solid ventral lobe, as in *Longitarsus* species, seem to result in more efficient jump); 2) muscle volume (the greater it is, the more effective the jump); and 3) length of the hind leg including tarsi (as in *Longitarsus* species with a highly elongate first metatarsomere). This contradicts a bit to an observation by Alexander (1995) who, based on mathematical models, investigated the effect of the muscle properties, leg design and jumping techniques on the jump height in humans, other vertebrates and insects. For animals without catapult jumping mechanism "… longer legs makes a higher jumps possible and additional leg segments, such as the elongated tarsi of bush-babies and frogs, increase jump heights …" (Alexander 1995). However, according to Alexander (1995), for insects with a catapult jumping mechanism (which all flea beetle posses) "the advantage of long legs might be small".

Schmitt (2004) also pointed out that *Psylliodes* hind leg structure with metatarsus attached away from the metatibial apex is most likely another adaptation for an effective jump. In both Schmitt (2004) and Brackenbury and Wang (1995) studies, *Longitarsus* and *Psylliodes* species exhibit the greatest take-off acceleration, velocity and translational energy (Table 1, Brackenbury and Wang 1995). Brackenbury and Wang (1995) call them "high-speed jumpers" compared to "low-speed jumpers" all of which have a more common position of relatively short metatarsi, right at the apex of the metatibia. According to Brackenbury and Wang (1995) and based on unpublished but available to me recordings of the flea beetle jumps, beetles push themselves from the substratum with the metatibial apex. Metatarsi do not participate in the initial push and sometime are lifted from the substratum. They may function to prevent slippage (Brackenbury and Wang 1995). It may be possible that high-speed jump with tarsi right at the end of the metatibia may not be as efficient or result in tarsal injury, while position of the tarsi away and at a distance from the metatibial apex may allow for a more efficient jump or be safer for a more powerful jump without a possibility of an injury.

A discovery of *Chanealtica*, another flea beetle genus with metatarsi attached away from the metatibial apex, provides a new example of this remarkable jumping leg adaptation.

Acknowledgements

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RESEARCH ARTICLE

A DNA barcode library for ground beetles (Insecta, Coleoptera, Carabidae) of Germany: The genus *Bembidion* Latreille, 1802 and allied taxa

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Abstract

As molecular identification method, DNA barcoding based on partial cytochrome *c* oxidase subunit 1 (COI) sequences has been proven to be a useful tool for species determination in many insect taxa including ground beetles. In this study we tested the effectiveness of DNA barcodes to discriminate species of the ground beetle genus *Bembidion* and some closely related taxa of Germany. DNA barcodes were obtained from 819 individuals and 78 species, including sequences from previous studies as well as more than 300 new generated DNA barcodes. We found a 1:1 correspondence between BIN and traditionally recognized species for 69 species (89%). Low interspecific distances with maximum pairwise K2P values below 2.2% were found for three species pairs, including two species pairs with haplotype sharing (*Bembidion atrocaeruleum*/*Bembidion varicolor* and *Bembidion guttula*/*Bembidion mannerheimii*). In contrast to this, deep intraspecific sequence divergences with distinct lineages were revealed for two species (*Bembidion geniculatum*/*Ocys harpaloides*). Our study emphasizes the use of DNA barcodes for the identification of the analyzed ground beetles species and represents an important step in building-up a comprehensive barcode library for the Carabidae in Germany and Central Europe as well.

Keywords

Introduction

The Carabidae (ground beetles) is a large cosmopolitan family of the Coleoptera, with an estimated number of 40,000 species world-wide, about 2,700 in Europe and 567 in Germany (Arnett et al. 2000, Arndt et al. 2005, Luff 2007, Trautner et al. 2014). Their body is usually rather flattened, especially in species living in crevices in soil such as some species of *Bembidion* Latreille, 1802, *Pterostichus* Bonelli, 1810, and *Polistichus* Bonelli, 1810, or under bark as in some *Dromius* Bonelli, 1810 species (Luff 2007). Although the majority of ground beetles are dark-colored and often black, there are many exceptions to this general rule, for example various colorful species of the genera *Anchomenus* Bonelli, 1810, *Carabus* Linnaeus, 1758, *Ceroglossus* Solier, 1848, or *Lebia* Latreille, 1802. Most ground beetles are active terrestrial beetles which forage on the ground surface and prey on other small invertebrates. Carabid beetles show, however, different levels of habitat selectivity, ranging from generalists to specialists. As consequence, carabid assemblages can be used as highly valuable bioindicators for characterizing disturbances in various habitats such as forests, meadows or fens (Lövei and Sunderland 1996, Rainio and Nie-melä 2003, Pearce and Venier 2004, Koivula 2011, Kotze et al. 2011).

Within the Carabidae, the genus *Bembidion* Latreille, 1802 is the largest in this family, with more than 1,200 described species mostly in the temperate regions of the world (Maddison 2012), including about 220 in Europe (Luff 2007) and more than 80 in Germany (Trautner et al. 2014). Species of *Bembidion* are typically small preda-tors that inhabit shores of running or standing waters including coastlines in temper-ate regions. Most adults have a body length between 2 and 9 mm (Lindroth 1985). Typically, species of this genus vary in the form of the prothorax and elytra, micro-sculpture, color pattern, mouthparts, male genitalia, and other characters (Maddison 2012) (Fig. 1). However, a study of males is often indispensable for the identification of morphologically similar species. Moreover, the identification of larvae is even more difficult due to a lack of documentation as well as missing experts with relevant skills and reference material.

During the last years the analysis of DNA sequence data, in particular the use of an approx. 650 base pair (bp) fragment of the mitochondrial cytochrome *c* oxidase subu-nit 1 (COI) known as DNA barcode, was proposed as marker of choice for specimen identification (Hebert et al. 2003a, Hebert et al. 2003b). The idea of DNA barcoding is based on the assumption that the observed interspecific genetic variation exceeds the intraspecific variation to such proportion that a clear gap exists, allowing the assign-ment of unidentified individuals to their species (Hebert et al. 2003a, Hebert et al. 2003b). Thus, the compilation of comprehensive DNA barcode libraries represents an essential step for subsequent studies, e.g. biodiversity assessment studies via metabar-coding using high-throughput sequencing technologies in the near future (e.g. Zhou et al. 2013, Cristescu 2014, Brandon-Mong et al. 2015).

However, the application of COI (and other mitochondrial markers in general) for species identification is not without problems. Recent speciation and hybridization events (e.g. Kubota and Sota 1998, Sota et al. 2000), heteroplasmy (e.g. Boyce et al.
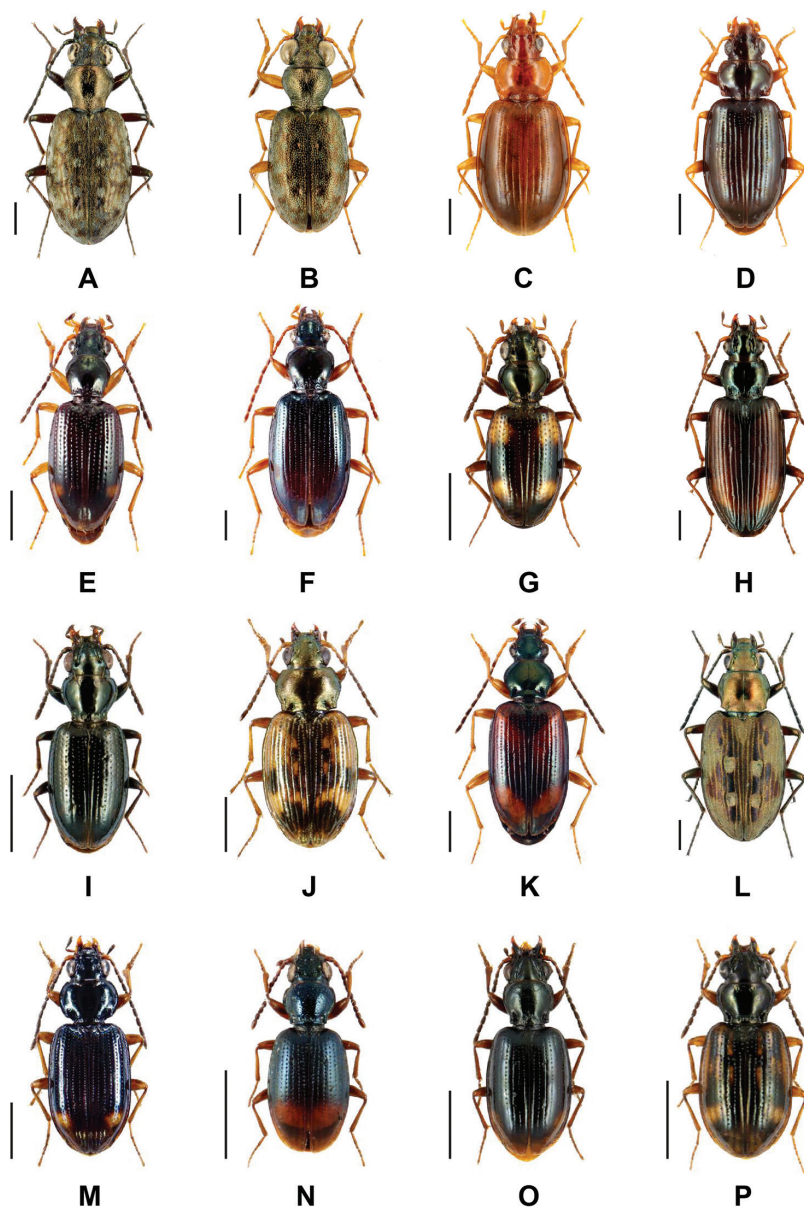
Figure 1. Representative images of analyzed beetle species. **A** *Asaphidion caraboides* (Schrank, 1781) **B** *Asaphidion flavipes* (Linnaeus, 1761) **C** *Ocys harpaloides* (Audinet-Serville, 1821) **D** *Ocys quinquestriatus* (Gyllenhal, 1810) **E** *Sinechostictus elongatus* (Dejean, 1831) **F** *Sinechostictus ruficornis* (Sturm, 1825) **G** *Bembidion* (*Bembidion*) *quadrimaculatum* (Linnaeus, 1761) **H** *Bembidion* (*Bembidionetolitzkya*) *fasciolatum* (Duftschmid, 1812) **I** *Bembidion* (*Emphanes*) *azurescens* Dalla Torre, 1877 **J** *Bembidion* (*Notaphus*) *semipunctatum* (Donovan, 1806) **K** *Bembidion* (*Ocydromus*) *testaceum* (Duftschmid, 1812) **L** *Bembidion* (*Bracteon*) *litorale* (Olivier, 1790) **M** *Bembidion* (*Philochthus*) *biguttatum* (Fabricius, 1779) **N** *Bembidion* (*Talanes*) *aspericolle* (Germar, 1829) **O** *Bembidion* (*Trepanedoris*) *doris* (Panzer, 1796), and **P** *Bembidion* (*Trepanes*) *octomaculatum* (Goeze, 1777). Scale bars = 1 mm. All images were obtained from www.eurocarabidae.de.

1989), the presence of mitochondrial pseudogenes (numts; e.g. Hazakani-Covo et al. 2010, Maddison 2012), or incomplete lineage sorting as consequence of occasional complex phylogeographic processes (e.g. Petit and Excoffier 2009) can influence the mitochondrial variability of the barcode fragment. In the case of terrestrial arthropods and in particular insects, maternally inherited α-proteobacteriae as *Wolbachia* Hertig, 1936 can limit the application of DNA barcodes for valid species identification also (e.g. Dobson 2004, Duron et al. 2008, Werren et al. 2008). It is also possible to generate *Wolbachia* COI sequences using standard insect primers (Smith et al. 2012). Finally, other studies highlight methodological problems of the analysis of DNA barcodes, for example an inappropriate use of neighbor-joining trees or of fixed distance thresholds (e.g. Will and Rubinoff 2004, Goldstein and DeSalle 2010, Collins and Cruickshank 2013). Nevertheless, numerous studies clearly demonstrate the usefulness of DNA barcoding for vertebrates (e.g. Lijtmaer et al. 2011, Ivanova et al. 2012, Knebelsberger et al. 2014) as well as invertebrates (e.g. Costa et al. 2007, Lobo et al. 2015, Barco et al. 2016). Not surprisingly, most DNA barcoding studies of arthropods focus on insects (Raupach and Radulovici 2015). In this context, numerous sequence libraries have been build-up for a broad range of insect taxa, including Heteroptera (Jung et al. 2011, Park et al. 2011, Grebennikov and Heiss 2014, Raupach et al. 2014), Neuroptera (Morinière et al. 2014), Hymenoptera (e.g. Smith and Fisher 2009, Quicke et al. 2012, Schmidt et al. 2015), Trichoptera (Zhou et al. 2009, Zhou et al. 2011, Ruiter et al. 2013), and in particular Lepidoptera (e.g. deWaard et al. 2009, Dincă et al. 2011, Hausmann et al. 2011, Hebert et al. 2013, Rajaei Sh et al. 2013, Kekkonen et al. 2015). Beside various other articles analyzing Coleoptera by the means of DNA barcoding (e.g. Hendrich et al. 2010, Greenstone et al. 2011, Jusoh et al. 2014, Pentinsaari et al. 2014, Oba et al. 2015, Rougerie et al. 2015), an amazingly large DNA barcode library of beetles has been published just recently (Hendrich et al. 2015). However, the number of barcoding studies focusing specifically on ground beetles is still low (e.g. Greenstone et al. 2005, Maddison 2008, Raupach et al. 2010, Raupach et al. 2011, Woodcock et al. 2013).

In this study we present as part of the German Barcode of Life project a comprehensive DNA barcode library of a variety of Central European species of the genus *Bembidion* and associated taxa. Our new barcode library includes 65 species of the genus *Bembidion* as well as five species of the closely related genera *Asaphidion* Des Gozis, 1886, two species of the genus *Ocys* Stephens, 1828 and six species of the genus *Sinechostictus* Motschulsky, 1864. In total, our library comprised 819 sequences of 78 species.

Material and methods

Sampling of specimens

All analyzed ground beetles were collected between 1997 and 2015 using various sampling methods (i.e. hand collecting, pitfall traps). All specimens were stored in ethanol

(96%). The analyzed beetles were identified by one of the authors (KH) using the keys in Müller-Motzfeld (2006). For our analysis we also included 481 DNA barcodes of three previous studies (Raupach et al. 2010: 63 specimens, 11 species; Raupach et al. 2011: 26 specimens, 7 species; Hendrich et al. 2015: 392 specimens, 68 species). In total, 338 new barcodes of 57 species were generated.

Most specimens were collected in Germany (n = 617, 75%), but for comparison some specimens were also included from Austria (n = 107, 13%), Belgium (n = 3, 0.04%), Czech Republic (n = 1, 0.01%), Italy (n = 41, 0.5%), France (n = 34, 0.4%), Slovenia (n = 15, 0.2%) and Sweden (n = 1, 0.01%). The number of analyzed specimens per species ranged from one (8 species, 10.3%) to a maximum of 38 in the case of *Bembidion tetracolum* Say, 1823.

DNA barcode amplification, sequencing and data depository

Laboratory operations were carried out either at the Canadian Center for DNA Barcoding (CCDB), University of Guelph, following standardized high-throughput protocols for COI amplification and sequencing (Ivanova et al. 2006, deWaard et al. 2008), the molecular labs of the Zoologisches Forschungsmuseum Alexander Koenig in Bonn, Germany, or the German Center of Marine Biodiversity Research, Senckenberg am Meer, in Wilhelmshaven, Germany. Photographs were taken for each studied beetle before molecular work was performed. For very small specimens with a body length <3 mm, complete specimens were used for DNA extraction, whereas tissue samples (legs) were used for beetles >3 mm. In the case of own molecular studies, DNA was extracted using the QIAmp© Tissue Kit (Qiagen GmbH, Hilden, Germany) or NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany), following the extraction protocol.

Polymerase chain reaction (PCR) has been used for amplifying the COI barcode fragment using the primer pair LCO1480 and HCO2198 (Folmer et al. 1994) or LCO1480 and NANCY (Simon et al. 1994, Simon et al. 2006). The PCR mix contained 4 µl Q-Solution, 2 µl 10x Qiagen PCR buffer, 2 µl dinucleotide triphosphates (dNTPs, 2 mmol/µl), 0.1 µl of each primer (both 25 pmol/µl), 1 µl of DNA template with of between 2 and 150 ng/µl, 0.2 µl Qiagen Taq polymerase (5 U/µl), and was filled up to 20 µl with sterile H_2O. All PCR amplification reactions were conducted in Thermal Cycler GeneAmp PCR System 2700/2720 (Applied Biosystems, Darmstadt, Germany) or the Eppendorf Mastercycler Pro system (Hamburg, Germany). Thermocycling conditions of the PCR included an initial denaturation at 94 °C (5 min), followed by 38 cycles at 94 °C (denaturation, 45 s), 48 °C (annealing, 45 s), 72 °C (extension, 80 s), and a final extension step at 72 °C (7 min). For each round of reactions negative and positive controls were included. Two µl of the amplified products were verified for size conformity by electrophoresis in a 1% agarose gel stained with GelRed™ and using commercial DNA size standards. The remaining PCR product was purified with the QIAquick© PCR Purification Kit (Qiagen GmbH, Hilden, Germany) or the NucleoSpin Gel and PCR Clean-up

(Macherey-Nagel, Düren, Germany). Purified PCR products were cycle-sequenced and sequenced in both directions at contract sequencing facilities (Macrogen, Seoul, Korea, or GATC, Konstanz, Germany), using the same primers as used in PCR. Double stranded sequences were assembled and checked for mitochondrial pseudogenes (numts) analysing the presence of stop codons, frameshifts as well as double peaks in chromatograms with the Geneious version 7.0.4 program package (Biomatters, Auckland, New Zealand) (Kearse et al. 2012). For verification, BLAST searches (nBLAST, search set: others, program selection: megablast) were performed to confirm the identity of all new sequences as beetle sequences based on already published sequences (high identity values, very low E-values) (Zhang et al. 2000, Morgulis et al. 2008). All analyzed sequences had a length of at least 352 base pairs (bp). Relevant voucher information, taxonomic classifications, photos, DNA barcode sequences, used primer pairs and trace files (including their quality) are publicly accessible through the public data set "DS-BABEM" (Dataset ID: dx.doi.org/10.5883/DS-BABEM) on the Barcode of Life Data Systems (BOLD; www.boldsystems.org) (Ratnasingham and Hebert 2007). New barcode data were also deposited in GenBank (accession numbers KU876564 to KU876786).

DNA Barcode analysis

Intra- and interspecific distances of the analyzed ground beetle species were based on the Kimura 2-parameter (K2P; Kimura 1980), using the analytical tools of the BOLD workbench (align sequences: BOLD aligner; ambiguous base/gap handling: pairwise deletion). Beside this, all analyzed COI sequences were subject to the Barcode Index Number (BIN) system implemented in BOLD. This approach clusters DNA barcodes in order to produce operational taxonomic units that closely correspond to species (Ratnasingham and Hebert 2013). Such BINs are unique in that clusters are indexed in a regimented way so genetically identical taxa encountered in different studies reside under shared identifiers (Ratnasingham and Hebert 2013). Using default settings, a recommended threshold of 2.2% was used for a rough differentiation of low and high intraspecific as well as interspecific K2P distances (Ratnasingham and Hebert 2013).

A neighbor joining cluster analysis (NJ; Saitou and Nei 1987) was performed for a graphical representation of the genetic differences between sequences and clusters of sequences in the dataset based on K2P distances using MEGA6.4 (Tamura et al. 2013). Sequences were aligned using MUSCLE (Edgar 2004), implemented in MEGA. Non-parametric bootstrap support values were obtained by resampling and analyzing 1,000 replicates (Felsenstein 1985). Finally, we constructed statistical maximum parsimony networks for species that shared identical haplotypes with TCS 1.21 with a fix connection limit at 50 mutational steps (Clement et al. 2000). Such networks allow the identification of haplotype sharing between species as a consequence of recent speciation or on-going hybridization processes.

Results

Overall, 819 DNA barcode sequences of 78 carabid beetle species were analyzed. A full list of the analyzed species is presented in the supporting information (Suppl. material 1). For the genus *Bembidion* we analyzed 63 species which represent 77% of the recorded species (*n* = 82) of this genus for Germany. Furthermore, our sampling covered five species (71%) of the genus *Asaphidion* (recorded species for Germany: *n* = 7), two species (100%) of the genus *Ocys* (*n* = 2), and six species (86%) of the genus *Sinechostictus* (*n* = 7). Two analyzed species, *Bembidion dalmatinum* Dejean, 1831 and *Bembidion italicum* De Monte 1943, are actually not recorded from Germany.
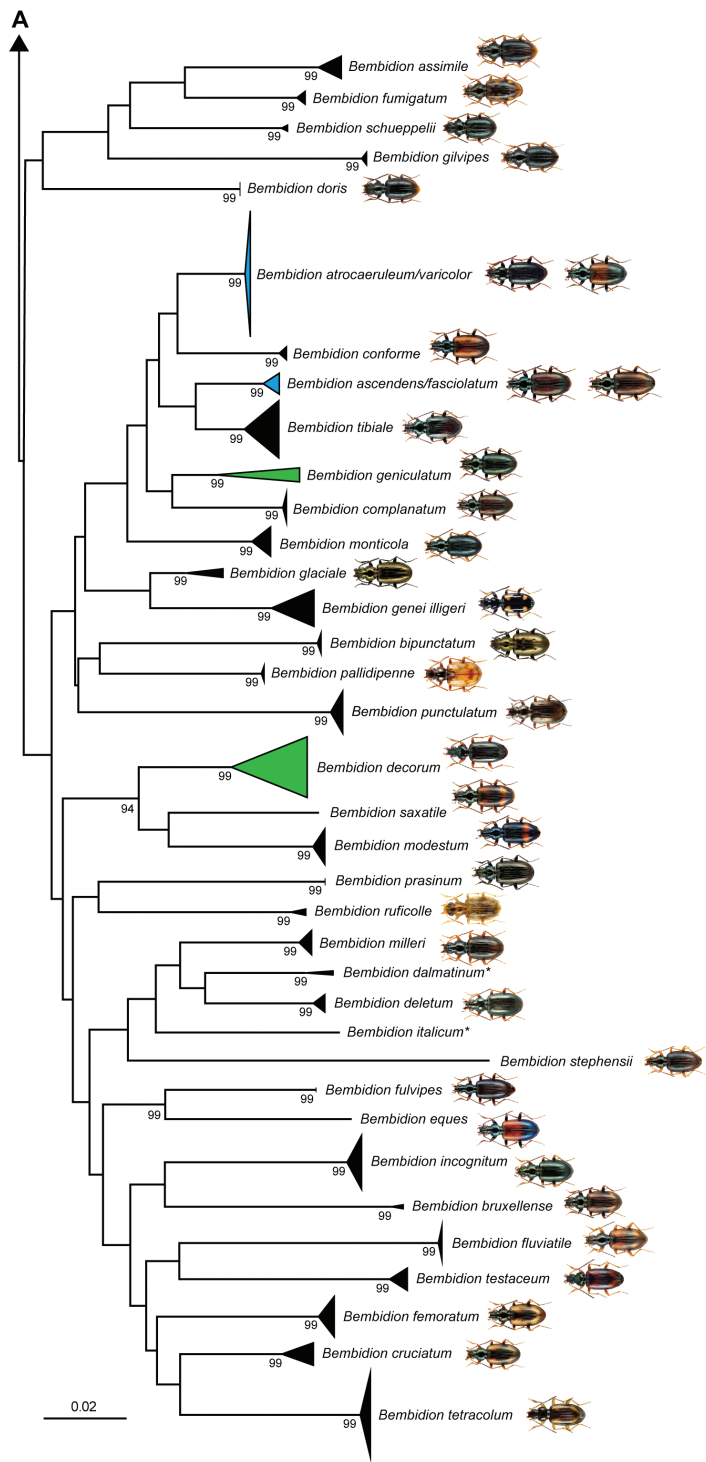
Fragment lengths of the analyzed DNA barcode fragments ranged from 352 to 657 bp. As it is typically known for arthropods, our sequence data also revealed a high AT-content for the DNA barcode region: the mean sequence compositions were A=16.6%, C=15.9%, G=29.8% and T=37.7%. Intraspecific K2P distances within a genus ranged from zero to 9.62% (*Ocys harpaloides* (Audinet-Serville, 1821)) whereas interspecific distances within the analyzed genera had values between zero and 14.72%. In this context, the lowest interspecific distances of distinct barcode clusters were revealed for *Bembidion ascendens* K. Daniel, 1902 and *Bembidion fasciolatum* (Duftschmid, 1812) with values ranging from 0.49% to 0.82%. Both species had the same BIN (ACJ7842).

In total, unique BINs were revealed for 69 species (89%), two BINs for 3 species (4%), and one BIN for two species for 6 species (7%). Interspecific distances of zero were found for two species pairs (5.1%): *Bembidion atrocaeruleum* Stephens, 1828 vs. *Bembidion varicolor* Fabricius, 1803 and *Bembidion guttula* (Fabricius, 1792) vs. *Bembidion mannerheimii* C.R. Sahlberg, 1827. In contrast to this, maximum intraspecific pairwise distances >2.2% were found for three species (3.8%): *Bembidion decorum* (Panzer, 1799) (2.56%), *Bembidion geniculatum* Heer, 1837 (4.49%), and *Ocys harpaloides* (Audient-Serville, 1821) (9.62%). The NJ analyses based on K2P distances revealed non-overlapping clusters with bootstrap support values >95% for 63 species (81%) with more than one analyzed specimen (Fig. 2). A detailed topology is presented in the supporting information (Suppl. material 2).

Our statistical maximum parsimony analysis showed multiple sharing of haplotypes for two species pairs: *Bembidion atrocaeruleum* (*n* = 32) and *Bembidion varicolor* (*n* = 22) (Fig. 3A) as well as *Bembidion guttula* (*n* = 7) and *Bembidion mannerheimii* (*n* = 14) (Fig. 3B). For *Bembidion atrocaeruleum* and *Bembidion varicolor* we identified 15 different haplotypes with one dominant haplotype (h1) that was shared by 19 specimens of *Bembidion atrocaeruleum* and two specimens of *Bembidion varicolor*. Interestingly, this haplotype was separated by only one additional mutation step from haplotype h2 which was exclusively composed of specimens of *Bembidion varicolor* (*n* = 15). Whereas a number of haplotypes (h3-h7, h10) was located close to these both major ones, seven haplotypes that were found only within one specimen (*Bembidion atrocaeruleum*: h9, h14, h15; *Bembidion varicolor*: h8, h11, h12, h13) were separated from this core network by high numbers of mutational steps.
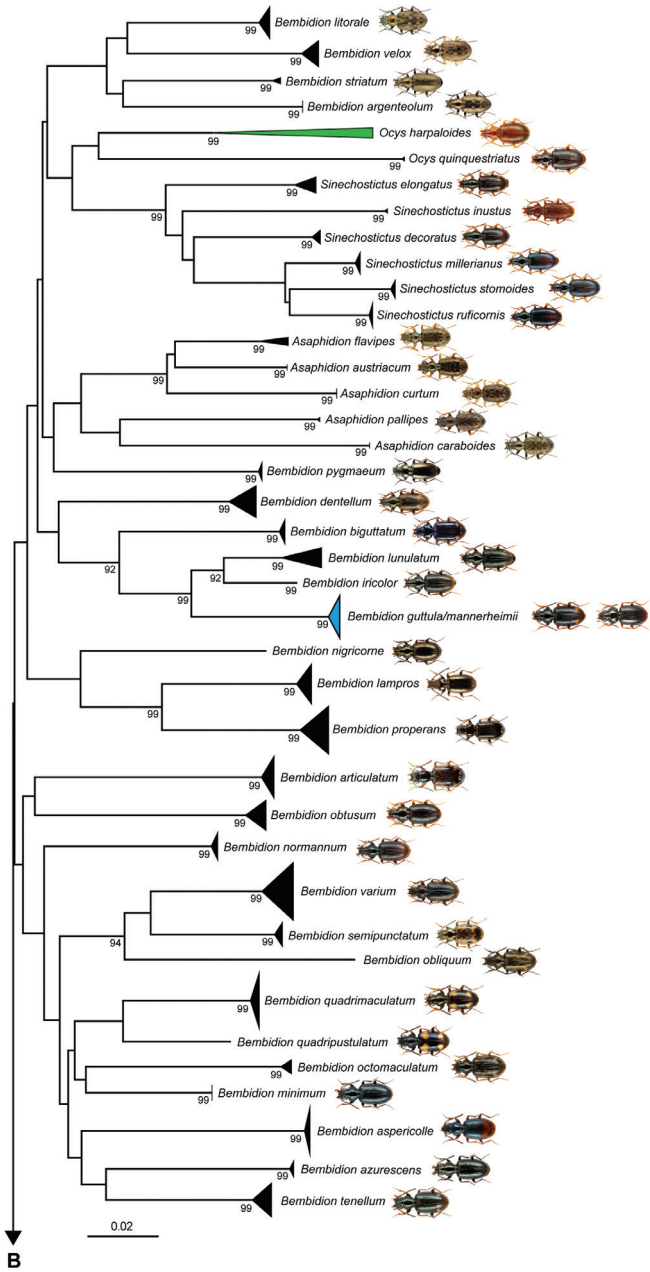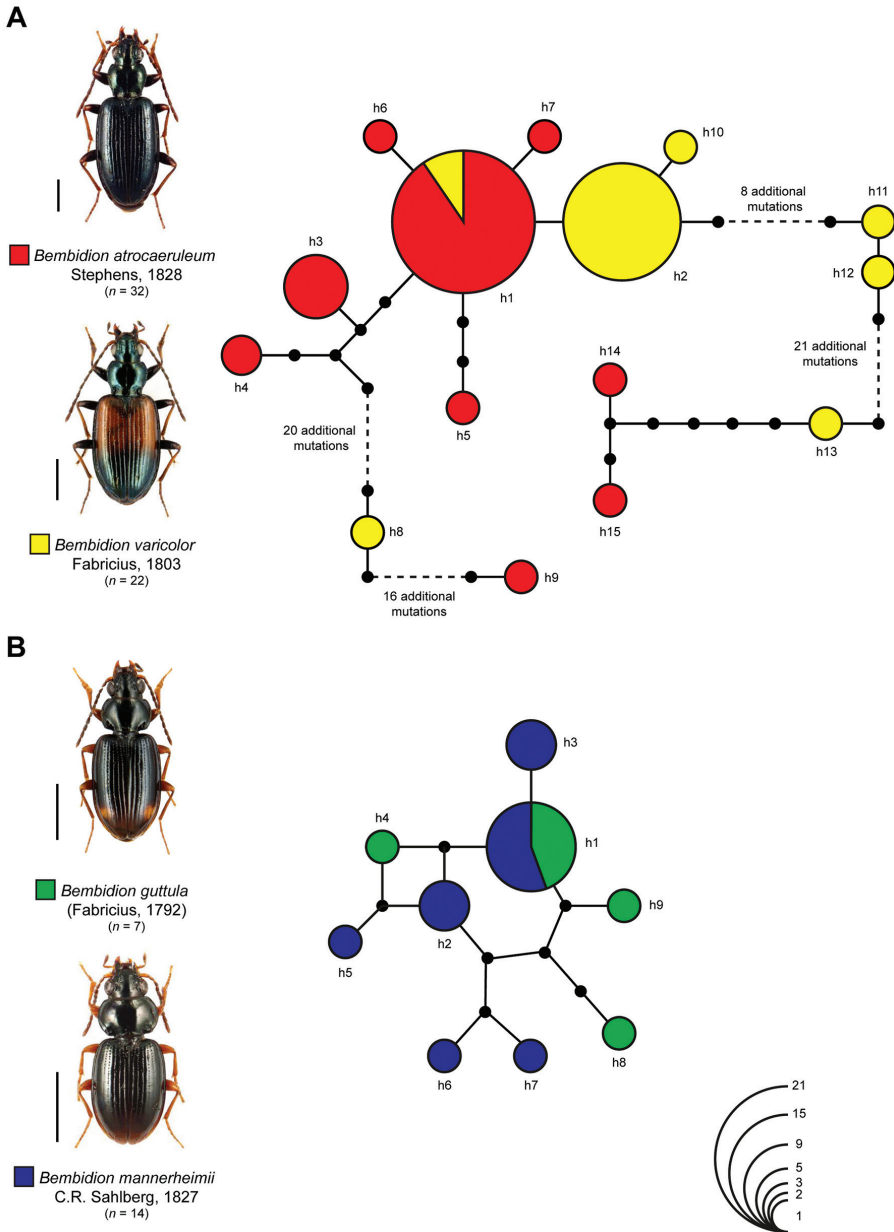
Figure 2. Neighbor joining topology of the analyzed ground beetle species based on Kimura 2-parameter distances. Triangles indicate the relative number of individual's sampled (height) and sequence divergence (width). Green triangles indicate species with intraspecific maximum pairwise distances >2.2%, blue triangles species pairs with interspecific distances <2.2%. Numbers next to nodes represent non-parametric bootstrap values >90% (1,000 replicates). Asterisks indicate species not recorded in Germany. All images were obtained from www.eurocarabidae.de.

Figure 2. Continue.

Figure 3. Maximum statistical parsimony network of *Bembidion* species sharing COI haplotypes: **A** *Bembidion atrocaeruleum* Stephens, 1828 and *Bembidion varicolor* Fabricius, 1803 **B** *Bembidion guttula* (Fabricius, 1792) and *Bembidion mannerheimii* C.R. Sahlberg, 1827. Used settings included a user specified maximum of connection steps at 50, gaps were treated as fifth state. Each line represents a single mutational change whereas small black dots indicate missing haplotypes. The numbers of analyzed specimens (*n*) are listed, whereas the diameter of the circles is proportional to the number of haplotypes sampled (see given open half circles with numbers). Scale bars = 1 mm. Beetle images were obtained from www.eurocarabidae.de.

In the case of *Bembidion guttula* and *Bembidion mannerheimii*, our analysis revealed nine haplotypes. The most dominant haplotype h1 was shared by five specimens of *Bembidion mannerheimii* and four specimens of *Bembidion guttula*. All others were connected to this haplotype by a maximum of five mutational steps, generating a compact network.

Discussion

Our study clearly confirms the usefulness of DNA barcodes for the identification of species of the genera *Asaphidion*, *Bembidion*, *Ocys*, and *Sinechostictus* of Central Europe, in particular Germany. Unique BINs were found for 69 species (89%) of the analyzed 78 beetle species, coinciding with high rates of successful species identification of previous barcoding studies of ground beetles (Raupach et al. 2010, Raupach et al. 2011, Pentinsaari et al. 2014, Hendrich et al. 2015). Nevertheless, our data also highlights species pairs that share haplotypes as well as species with high genetic diversity and distinct lineages. We will discuss these cases in the following more in detail.

Species pairs that share haplotypes

Haplotype sharing of COI sequences was found for two species pairs. In the case of *Bembidion guttula* and *Bembidion mannerheimii* identical COI sequences are not surprising (Fig. 3B). A close relationship of both species has been already documented in a previous study (Maddison 2012). In this context our results give some evidence for ongoing hybridization between both species.

A somewhat similar situation was revealed for *Bembidion atrocaeruleum* and *Bembidion varicolor*. Nevertheless, the statistical maximum parsimony network revealed a more complex structure (Fig. 3A). Both species are part of the subgenus *Bembidionetolitzkya* Strand, 1929 and can be easily distinguished by coloration, but morphological differences are subtle, e.g. variations of the male genitalia (Müller-Motzfeld 2006). Both species are also riparian specialists but are found in different regions. In Germany, specimens of *Bembidion atrocaeruleum* are documented in the low mountain ranges whereas beetles of *Bembidion varicolor* are inhabitants of the foothills of the Alps (Müller-Motzfeld 2006, Trautner et al. 2014). A similar situation is given in Switzerland (Luka et al. 2009). These two species are also found in other European countries, e.g. France, Italy, or Slovakia and Slovenia (see www.carabidae.org), but detailed distribution information are not available. However, such a close relationship of both species was not discussed before. Only detailed analysis of a) more specimens sampled from additional localities, b) other faster evolving nuclear markers, e.g. microsatellites or RAD-Seqs, c) ecological parameters, and d) comprehensive morphological and morphometric studies will give us more insights if we face two species or morphotypes of only one species.

Species with high intraspecific variability

Maximum intraspecific pairwise distances >2.2% were observed for three species. Whereas *Bembidion decorum* showed no conspicuous substructure for the analyzed COI sequences (see Suppl. material 2), two distinct monophyletic lineages were revealed within *Bembidion geniculatum* as well as *Ocys harpaloides* (Fig. 4). In both cases, specimens sampled from Germany form monophyletic clusters (*Bembidion geniculatum*: A, *Ocys harpaloides*: B) that are separated from all other specimens (*Bembidion geniculatum*: four specimens from Austria and France, *Ocys harpaloides*: three specimens from France). Whereas distances between cluster A and B for *Bembidion geniculatum* range from 3.7 to 4.4%, distances from 8.8% to 9.6% are documented for *Ocys harpaloides*.

Based on the given data we are unable to clarify the reasons of the observed distinct lineages which can be caused by various effects, including phylogeographic events (e.g. Zhang et al. 2005, Zhang et al. 2006, Schmidt et al. 2012), infections of maternally inherited endosymbionts as *Wolbachia* (e.g. Roehrdanz and Levitan 2007, Werren et al. 2008, Gerth et al. 2011), or the presence of nuclear copies of mitochondrial DNA (numts) (Bensasson et al. 2001, Hazakani-Covo et al. 2010). Interestingly, numts have been shown for various *Bembidion* species (Maddison 2008, Maddison 2012, Maddison and Swanson 2010), but we found no evidence for any numts within our dataset. Finally, the observed variability may be also caused by the
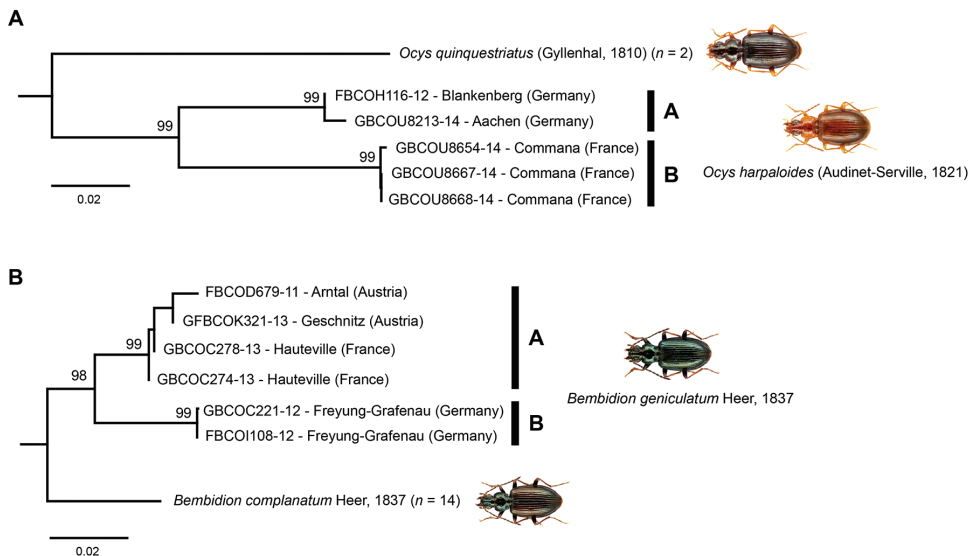
Figure 4. Subtrees of the neighbor joining topology based on Kimura 2-parameter distances of all analyzed specimens of **A** *Ocys harpaloides* (Audinet-Serville, 1821) and nearest neighbor, and **B** *Bembidion geniculatum* Heer, 1837 and nearest neighbor. Branches with specimen ID-number from BOLD, species names and sample localities. Numbers next to internal nodes are non-parametric bootstrap values (in %).

existence of overseen or cryptic species. It is obvious that more specimens of all four species have to be analyzed using morphological as well as molecular data to answer these questions in detail.

Conclusions

Carabid beetles are one of the best-known taxa in entomology that have been studied intensively by numerous generations of coleopterists, clarifying their taxonomy and phylogeny, biogeography, habitat associations and ecological requirements, life history and adaptations, especially in Central Europe (see review in Kotze et al. 2011). Our analysis revealed some interesting results that should motivate carabidologists to check the species status of various "well known" species more in detail. Due to the fact that specimens of a number of species were collected at the same or close localities (e.g. *Bembidion octomaculatum* (Goeze, 1777)), or only a low number of specimens have been analyzed (e.g. *Bembidion striatum* (Fabricius, 1792)), the intraspecific variability of such species may be underestimated (e.g. Linares et al. 2009, Bergsten et al. 2012, but see Huemer et al. 2014). Nevertheless, our study clearly emphasizes the use of DNA barcodes for the identification of the analyzed ground beetles species of the genera *Asaphidion*, *Bembidion*, *Sinechostictus* and *Ocys*. Therefore, this data set represents an important step in building-up a comprehensive barcode library for the Carabidae in Germany which will be used in modern molecular biodiversity assessment studies.

Acknowledgments

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Supplementary material 1

Barcode analysis using the BOLD workbench

Authors: Michael J. Raupach, Karsten Hannig, Jérome Morinière, Lars Hendrich

Data type: Data table

Explanation note: Molecular distances based on the Kimura 2-parameter model of the analyzed specimens of the analyzed species of the genera *Asaphidion*, *Bembidion*, *Ocys* and *Sinechostictus*. Divergence values were calculated for all studied sequences, using the Nearest Neighbour Summary implemented in the Barcode Gap Analysis tool provided by the Barcode of Life Data System (BOLD). Align sequencing option: BOLD aligner (amino acid based HMM), ambiguous base/gap handling: pairwise deletion. ISD = intraspecific distance. BINs are based on the barcode analysis from 01-02-2016. Asterisks indicate species not recorded from Germany. Species with intraspecific maximum pairwise distances >2.2% and species pairs with interspecific distances <2.2% are marked in bold. Country codes: A = Austria, B = Belgium, CZ = Czech Republic, D = Germany, F = France, I = Italy, S = Sweden and SLO = Slovenia.

Supplementary material 2

Neighbor joining topology

Authors: Michael J. Raupach, Karsten Hannig, Jérome Morinière, Lars Hendrich

Data type: Neighbor joining topology

Explanation note: Neighbor joining topology of all analyzed carabid beetles based on Kimura 2-parameter distances. Specimens are classified using ID numbers from BOLD and species name. Numbers next to nodes represent non-parametric bootstrap values (1,000 replicates, in %).

RESEARCH ARTICLE

Reconstructing the historical distribution of the Amur Leopard (*Panthera pardus orientalis*) in Northeast China based on historical records

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Abstract

The range of the Amur leopard (*Panthera pardus orientalis*) has decreased dramatically over the last 100 years. This species is still under extreme risk of extinction and conservation efforts are rigorous. Understanding the long-term dynamics of the population decline would be helpful to offer insight into the mechanism behind the decline and endangerment and improve conservation perspectives and strategies. Historical data collection has been the challenge for reconstructing the historical distribution. In China, new gazetteers having systematic compilation and considerable local ecological data can be considered as an important complementary for reconstruction. Therefore, we have set up a data set (mainly based on the new gazetteers) in order to identify the historical range of the Amur Leopard from the 1950s to 2014. The result shows that the Amur leopard was historically widely distributed with large populations in Northeastern China, but it presented a sharp decline after the 1970s. The decline appeared from the plains to the mountains and northeast to southwest since the 1950s. Long-term historical data, mainly from new gazetteers, demonstrates that such resources are capable of tracking species change through time and offers an opportunity to reduce data shortage and enhance understanding in conservation.

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Introduction

Long-term historical data would offer insight into understanding the ecological and biogeographic characteristics of population decline, and help develop predictive power for conservation management (Boakes et al. 2010; Chapron et al. 2014; Rondinini and Visconti 2015; Turvey et al. 2015). However, despite recognition of the considerable potential of long-term datasets for conservation research, policy and practice, applying long-term ecology data is still restricted by practical and conceptual barriers, including data accessibility, spatially and temporally variable and non-standardized sampling (Hortal et al. 2008; McClenachan et al. 2012; Davies et al. 2014). Therefore, recent studies always address time-scales of less than a decade (Davies et al. 2014). Only a few researchers use records over periods longer than 20 years (Vandel and Stahl 2005; Prigioni et al. 2007; Rick et al. 2012; Turvey et al. 2015). There is an increasing awareness of the need, not only to integrate historical data into conservation and environment management, but also to assess the usefulness and potential limitation of this data for developing our understanding of long-term species change.

Local gazetteers, called "*difangzhi*" (地方志), also translated as local records or annals, contain abundant information on environmental conditions and resources in China (Wen et al. 2006; Looney 2008). New gazetteers typically record considerable local ecological data from the 1950s, including animal records, as well as economic, political and demographic information (Looney 2008; Xue 2010). Most of them are compiled following a specific "scientific" natural history tradition and this makes it easy to identify the species records. In addition, compilation of gazetteers is systematized and provides a geographical coverage across most of China. For the records from field surveys, museums and papers, new gazetteers may have been neglected for a long time. This may be seen as reasonable, because new gazetteers are only concerned with species which are economic, unique or common. Recent research, however, highlights that this resource can provide effective records for reconstructing long-term population dynamics (Turvey et al. 2015; Zhang et al. 2016). In this case, we suggest that new gazetteers can be an irreplaceable source for conservation biology resources.

The Amur leopard, or Far Eastern leopard (*Panthera pardus orientalis*) is considered to be one of the most endangered subspecies in the world and has been listed as critically endangered on the IUCN red list since 1996 (Uphyrkina et al. 2002). Compared to the distribution in the late 19th century, the range of the Amur leopard has decreased dramatically over the last 100 years. Recent research shows that there are only 14–20 adults and 5–6 cubs in the southwestern Primorye region of Russia (Henschel et al. 2008; Spitzen et al. 2012); Perhaps some may still occur in North Korea, although their status there is uncertain (Uphyrkina et al. 2002); Fewer than 10 leopards likely existed in Jilin and Heilongjiang Provinces of northeast China in the later 1990s, but the population seems to have increased in recent years (42 leopards were photographed, including 40 adults (21 males, 17 females, and two of unknown sex) and two cubs) (Yang et al. 1999; Wang et al. 2015). The tiny population that

survives today is under extreme risk of extinction because of poaching, deforestation, inbreeding, and anthropogenic pressure (Ma 1989; Yang et al. 1999; Yang et al. 2000; Piao et al. 2011; Hebblewhite et al. 2011; Kelly et al. 2013; Cat Specialist Group 2014). Therefore, a basic understanding of their distribution over decades is necessary to design conservation strategies on a regional scale. To address this problem, we suggest that new gazetteers can be a useful resource. Combining new gazetteers, which regularly have Amur leopard records, with historical information from literature, news and scientific surveys, we identified the range of the Amur leopard in Northeast China from the 1950s to the 1990s. The historical dynamic for the Amur leopard in Northeast China was then reconstructed primitively by integrating historical records within a geographic information system (GIS).

Materials and methods

Area

The study area comprises Heilongjiang province, Jilin province and northeast of Inner Mongolia covered by temperate forest and boreal forest (N40°5'~53°17', E115°30'~135°06', about 938000 km² with a forested area of 402,000 km²), which is the most important forestry and agricultural production base in China. The climate is a continental monsoon climate with a negative water balance. The annual precipitation is 400–1000mm, and the annual average temperature is 1–4°C, with the north - south temperature gradient of 25. The region includes coniferous forest, broad-leaved mixed forest, secondary forest, woodland shrub and marshy grass areas, and contains more than 2500 plant species (Zhou 1997; Hou 2001). Coniferous forest is mainly located in the Greater Khingan Mountains where it is characterized by *Larix gmelinii*. The area of broad-leaved mixed forest includes the Lesser Khingan Mountains, Changbai Mountains and Wanda Mountains dominated by *Larix gmelinii*, *Pinus koraiensis* and *Betula platyphylla* (Cheng & Yan, 2008). Forest ecosystems in Northeast China support a number of forest-dependent carnivores and herbivores, for instance the Amur leopard and its potential prey – the Siberian roe deer (*Capreolus pygargus*), wild boar (*Sus scrofa*) and sika deer (*Cervus nippon*).

Data

Amur leopard historical records were obtained in the following five ways:

(1) New gazetteers. Due to being an important cultural symbol, economic species and having an ecological niche in human history and culture, large carnivores like the Amur leopard are recorded regularly in new gazetteers. The data from new gazetteers can be considered as the hard fact, because they came from the fur trade

records, hunter records, sightings, wild life surveys and conflicts. Systematic compilation made it possible to divide the records into different periods and to cover most of China. Also utilizing fauna and nature reserve scientific surveys, papers, scientific research and news, it is possible to compile substantial records for a long period and covering a large geographical area. In this paper, more than 90% of historical records came from new gazetteers. They were obtained from the National Library of China (http://www.nlc.gov.cn/), Duxiu Search Engine (http://www.duxiu.com/), and the Wanfang database (http://www.wanfangdata.com.cn/). Relevant information was found with the keywords – "Amur leopard", "Far Eastern Leopard" and "*Panthera pardus orientalis*".

(2) Fauna surveys. Fauna surveys recorded the specimen data with geographic information. We collected the distribution data from 9 Fauna surveys (Shou. 1962; Ma et al. 1986; Gao et al. 1987; Ma et al. 1989; Wu. 1993; Wang. 1998; Wang et al. 1998; Sheng et al. 1999; Zhao et al. 1999; State Forestry Administration of the People's Republic of China 2009).

(3) Nature reserve scientific surveys. The relevant data was collected from the National Library of China and the School of Nature Conservation, Beijing Forestry University.

(4) Papers and scientific research. The keyword "Amur leopard", "Far Eastern Leopard" and "*Panthera pardus orientalis*" were used to search several online databases such as the China National Knowledge Internet, Wanfang Database, Duxiu Search Engine, Google scholar (https://scholar.google.com/) and Biodiversity Heritage Library (http://www.biodiversitylibrary.org/). Records from 1998 mainly came from surveys (WCS program) in Heilongjiang and Jilin provinces.

(5) News. The keywords "Amur leopard", "Far Eastern Leopard" and "*Panthera pardus orientalis*" were used to search several online databases such as the China National Knowledge Internet, Wanfang Database, Duxiu Search Engine, Science Daily and Google. Records with detail location associated with photograph or video were effective.

Data analysis

Filters are essential for further analysis, because historical records may contain potential errors or uncertainties. Records from four of the ways above (2-5) were integrated into a dataset with brief information on the area of occupancy, and then this is compared with the gazetteer records. Records that conflicted with the dataset without any hard facts, including relevant or detailed descriptions, were excluded from analysis. All Chinese-language records were translated directly by the authors.

Maps of Northeast China (1:1,000,000) were acquired from the National Geographic Information Bureau, and spatial coordinates of all occurrence records were assigned using Google Earth. Multiple occurrences at matching sites, such as repetitions of the same record or when the distance between locations was less than 5km were

excluded. Records from killed animals, attacks on humans, prey remains, claw traces, footprints and photographs were excluded if they were repeated. Valid records were assigned with coordinates from public resources such as Google Earth v 7.1.2, classified into different decades (1950s, 1960s, 1970s, 1980s, 1990s and 2000–2014).

Amur leopard occurrence positions were then uploaded into ArcGIS 10.2, and overlaid with layers representing altitude (http://srtm.csi.cgiar.org/) and the borders of administrative regions. Using the number and intensity of records, it is possible to reconstruct and evaluate the distribution change in different decades of the Amur leopard population.

Results

A total of 84 documents of the 2235 new gazetteers examined presented information on the Amur leopard. These provided evidence regarding 294 Amur leopard records, while another 169 Amur leopard records were retrieved from the other four sources (as mentioned in the data sources section (2)-(5), giving a total of 463 Amur leopard records). Some records were excluded (as mentioned in the methods section) and finally 341 Amur leopard records were mapped and used for further analysis (Table S1).

New gazetteers were considered as the main resource before the 1990s. In the 1950s, 96.4% of total records came from new gazetteers. 36.2% of the new gazetteers records were confirmed by fauna and papers. 97.5%, 96.3% and 93.9% of total records came from new gazetteers in the 1960s, 1970s and 1980s respectively. 42.4%, 41.2%, and 54.0% of the new gazetteers records were confirmed by fauna, papers and nature reserve scientific surveys in the 1960s, 1970s and 1980s respectively. New gazetteers were still an important resource in the 1990s (44.7% of total in the 1990s) (Table S1). Scientific research for Amur leopard provided 17 records. Nature reserve scientific surveys and news dominated after 2000 and only one record came from new gazetteers.

Our result implied that the Amur leopard was widely distributed with a large population in Northeast China - Southern Lesser Khingan Mountains, Changbai Mountain and Wanda mountains (Fig. 1). Before 1970, the Amur leopard occurrence points didn't present obvious alterations (Fig. 1A, B). In the 1970s, the situation deteriorated continually. Compared with the distribution in the 1950s, there was a reduction of 19.6% of records in the 1970s. From the 1980s onwards the Amur leopard became limited to the core area of the Wanda Mountains and Changbai Mountains. There was a decline of 56.1% of records in the 1980s (Fig. 1C, D). In the 1990s, there were only a few records limited to several places, for example Hunchun, Wanqing, Yanji and Helong. Afterwards, the distribution became concentrated in the Hunchun and Wanqing in 2000–2014 (Fig. 1E, F). In addition, records in areas higher than 200m continuously increased before 2000, but a slight decrease presented in 2000–2014 (1950s: 79%; 1960s: 81%; 1970s: 84%; 1980s: 89%; 1990s: 92%; 2000–2014: 88%).
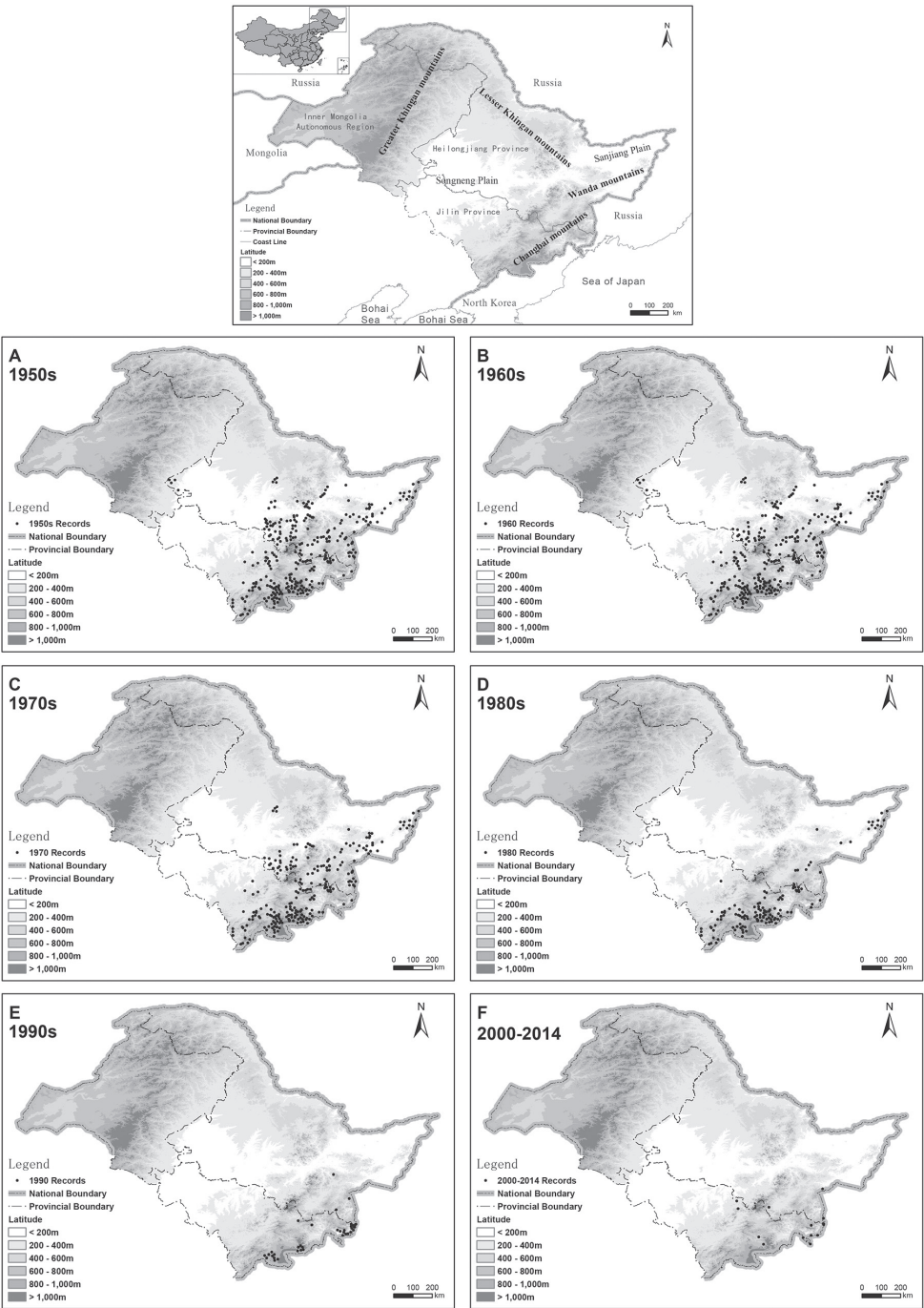
Figure 1. Amur leopard distribution in different periods.

Discussion

Our investigation of the potential of using long-term historical records to reconstruct long-term population dynamics shows that new gazetteers along with multiple effective resources can contribute novel insights for tracking the target species dynamics across longer timescales than is usually addressed in ecology or conservation biology. However, it is inevitable that the new gazetteer data compiled by non-scientific observers cannot provide a complete species records at the standard typically expected by modern ecologists, even if combined with multiple resources (2-5 resources). For example, hunt archives are the major component in new gazetteer records (like "Amur leopard was killed by snare in Liushuihe Forest Farm in 1964, winter"). Since hunting was banned in the 1980s, such records decreased sharply and can increase omission error after 1990. Spatial and temporal bias in new gazetteer records also generates deviation. For this reason, our investigation highlights some ways to reduce the commission or omission errors in new gazetteer records, including choosing identifiable and economic species, no generic species absent in the same area, and integrating with scientific data. Indeed, it is possible that the Amur leopard still exists in an area or specific period with no record, or be present in contrary circumstances. However, such historical dynamics for the Amur leopard in Northeast China still reveals some aspects of the pattern and process of population decline across more than 50 years that cannot be fully understood by the present-day population: The Amur leopard population presents a sharp decline after the 1970s; and the decline is likely to appear from the plain to the mountain and northeast to southwest, which implies negative impact from anthropogenic pressure.

Some questions still need to be clarified. For example, the Amur leopard population in the Greater Khingan Mountains is elusive (the range is unknown, but they are present). Only one fauna researcher (Gao et al. 1987) provides one record in Horqin Right Front Banner, Inner Mongolia autonomous region in the southwestern Greater Khingan Mountains. Four other fauna researchers describe the distribution briefly (Ma et al. 1986, 1989; Wu 1993; Zhao et al. 1999), and two of four suggest that local population have been extinct in the 1970s with none appearing in hunting or witness records after 1970 (Ma et al. 1989; Wu 1993). An extract taken from the Longjiang xianzhi says" the leopard was bountiful at the time of the foundation of the PR of China". Longjiang town is about 80km away from the north of the Horqin Right Front Banner. It is safe to say that the Amur leopard was distributed in the Greater Khingan Mountains in the 1950s, but the range change is still unclear. Moreover, higher latitude records increased before 2000 and this implies that the negative impacts under anthropogenic pressure for the Amur leopard and the decline in 2000-2014 can be either a promising sign on the effectiveness of the conservation strategy, or such a phenomenon may just be associated with data shortage.

Our reconstruction of the dynamics of the Amur leopard population decline enlightens a new case study with new perspectives shows that the historical records have considerable potential to contribute to ecological baselines for informing conserva-

tionists. We recommend further investigation to not only detect feasibility for reconstructing the dynamics of other species in China by Chinese gazetteers, or quantifying the human pressure by the response of wildlife over time in modern China, but also to evaluate the possibility of revealing the potential distribution in the past with the historical records and distribution models. Furthermore, we encourage conservation researchers and practitioners to consider this data type (not only the gazetteers, but also other data resource considered as local historical record around the world) as complementary, in order to reduce data shortage and enhance understanding in conservation. Sufficient data would provide greater opportunities for conservationists and environmental planners to put plans in place that would reduce the danger of endangered species going extinct in China and elsewhere.

Acknowledgments

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Supplementary material 1

Distribution information from new gazetteers records and from other resources

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Data type: PDF file

Supplementary material 2

Records in different periods from new gazetteers records

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Data type: PDF file