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Article



Three new species of copepods (Copepoda: Calanoida and Cyclopoida) from anchialine habitats in Indonesia

GEOFF A. BOXSHALL¹ & DAMIÀ JAUME²

¹Department of Zoology, The Natural History Museum, Cromwell Road, London SW7 5BD, United Kingdom E-mail: g.boxshall@nhm.ac.uk ²IMEDEA (CSIC - UIB), Instituto Mediterráneo de Estudios Avanzados, c/ Miquel Marquès, 21, 07190-Esporles (Illes Balears), Spain. E-mail: damiajaume@imedea.uib-csic.es

Abstract

Three new species of copepod crustaceans are described from material collected from anchialine and brackish habitats in and around the village of Walengkabola on the coast of Muna Island, to the southeast of Sulawesi. A new species of cyclopoid, *Paracyclopina sacklerae* **n. sp.**, was described from material collected from the tidal inflow entering into the bottom of sinkholes a few metres inland from the shoreline. Detailed comparisons are made with *Paracyclopina orientalis* (Lindberg, 1941), **n. comb.**, a closely related congener here transferred from its original genus *Cyclopetta* Sars, 1913. The assignment of *Paracyclopina* Smirnov, 1935 to the family Cyclopettidae is followed here despite uncertainty over the validity of some of the families created by the break up of the former Cyclopinidae. Two new species of *Boholina* Fosshagen & Iliffe, 1989 are described, based on material from the same sinkholes and from caves located up to 700m inland from the coast and exhibiting further reduced salinity down to 1.8 ppt. One species, *B. parapurgata* **n. sp.**, is very closely related to *B. purgata* Fosshagen & Iliffe, 1989 from Bohol island in the Philippines, the other *B. munaensis* **n. sp.**, is very closely related to *B. crassicephala* Fosshagen & Iliffe, 1989 also from Bohol island, but a number of fine scale differences in the leg 5 of both sexes are recognised in each case. Keys to valid species of both genera are provided.

Key words: taxonomy, anchialine caves, Paracyclopina, Boholina, Muna Island, Sulawesi

Introduction

Exploration of anchialine habitats around the coastal margins of tropical and subtropical islands and around the coast of Central America (especially on the Yucatan Peninsula) has revealed a rich assemblage of new copepods belonging to the orders Platycopioida (Fosshagen & Iliffe 1985), Calanoida (e.g. Fosshagen & Iliffe 1989; Fosshagen *et al.* 2001), Harpacticoida (Huys 1988, 1996), Misophrioida (e.g. Boxshall & Iliffe 1986, 1990; Boxshall & Jaume 2000) and Cyclopoida (e.g. Rocha & Iliffe 1991; Jaume & Boxshall 1997). These anchialine copepods often represent relatively basal lineages: the Platycopioida, for example, is the sister-taxon to the lineage comprising all other copepod orders (Huys & Boxshall 1991). In the Calanoida, typical anchialine families such as the Epacteriscidae and Ridgewayiidae, were both recovered as basal offshoots in a recent phylogenetic analysis (Bradford Grieve *et al.* 2010). Similarly, the Speleophriidae, within the order Misophrioida – itself an early offshoot of the Podoplea – retains a high proportion of plesiomorphic character states (Boxshall & Jaume 2000).

To date, representatives of 17 different families of copepods have been reported from anchialine habitats world wide (Boxshall & Halsey 2004), but the Indo-Pacific anchialine fauna has proven to be less diverse than that of the Central Atlantic including the Caribbean and Mediterranean Seas. Known Pacific sites for anchialine copepods include the Galapagos Islands, Fiji, Palau and Western Australia (Boxshall & Iliffe 1990; Fosshagen *et al.* 2001; Jaume *et al.* 2001; Figueroa & Hoefel 2008) but collecting effort has been patchy. A preliminary reconnaissance trip to Muna island (south of Sulawesi) in 2001 had collected malacostracan crustaceans from several caves around the island, some of which have already been described, such as the anthurid isopod *Stygocyathura munae* Botosaneanu, 2003 (Botosaneanu 2003) and the brachyuran *Sulaplax ensifer* Naruse, Ng & Guinot, 2008 (Naruse *et al.*

2008). Joining an expedition, led by Prof Louis Deharveng of the Museum National d'Histoire Naturelle, Paris, to the Maros karst on Sulawesi and to Muna Island provided an opportunity to sample intensively for copepods in suitable coastal habitats with the aim of filling some of the gaps in our knowledge of the Indo-Pacific anchialine copepod fauna.

Material and methods

On Muna, our collecting effort was focused around the village of Walengkabola on the southwestern side of the island. There was no piped water in the village so caves and springs in the Walengkabola district were used by the villagers for domestic water. One site, Mata Air Kawaouwou, was restricted for use as a drinking water source, but the other four cave "springs" in the village were used for bathing and washing clothes. Salinity varied from 1.8 ppt at Lawou, about 700 m from the coast, and ranged up to 6.0 ppt at La Ode Panu, about 200 m from the coast. Right at the coast, the limestone platform was riddled with sinkholes, which showed a marked tidal influence: with many appearing dry or with little water at low tide but with water 50 to 60 cm deep at high tide. The cave springs and sinkholes were sampled for copepods and other crustaceans.

The copepods were collected using a hand-held plankton net of mesh size 50 µm. The net was swept through the water in the caves while wading up to waist high. In sinkholes samples were taken at high tide, using the nets after climbing down into the hole. Specimens were sorted live, then fixed and stored in 80% ethanol or 10% formalin in seawater. They were studied whole and as dissected temporary preparations in lactophenol. Drawings were prepared using a drawing tube on a Leitz Diaplan microscope equipped with differential interference contrast. All drawings are made from dissected paratypes unless otherwise stated in the figure legend. Body length measurements include caudal rami, but not the caudal setae; setal measurements were taken from dissected paratypes. The terminology used in the descriptions follows Huys & Boxshall (1991).

Type material is deposited in the collections of the Museum Zoologicum Bogoriense (MZB) in Bogor, Indonesia, and of the Natural History Museum, London (BMNH).

Systematics

Order Cyclopoida Burmeister, 1834

Family Cyclopettidae Martínez Arbizu, 2000

Genus Paracyclopina Smirnov, 1935

Paracyclopina sacklerae n. sp. (Figs. 1–4)

Type material. Holotype female, 7 paratype females, 5 paratype males collected from sinkholes at Walengkabola village (5° 11.052' S 122° 35.159' E), Muna Island, Indonesia on 18 September 2007 by G.A. Boxshall and D. Jaume. Registration numbers: holotype [MZB.Cru Cop.103], 4 paratype females and 3 paratype males [MZB.Cru Cop.104] in Museum Zoologicum Bogoriense, 3 paratype females and 2 paratype males in Natural History Museum, London [BMNH 2011.1167-1171].

Etymology. The species is named in honour of Mrs Theresa Sackler in recognition of her long term philanthropic support of the research and education work of the Natural History Museum, London.

Description of adult female. Body cyclopiform (Fig. 1A), divided into anterior prosome and posterior urosome with prosome–urosome boundary well defined at podoplean position. Prosome comprising cephalothorax and 4 free pedigerous somites; first pedigerous somite free, but partly concealed by posterior extension of dorsal cephalic shield. Epimeral angles of free second to fourth pedigerous somites rounded. Rostrum rounded, well developed. Nauplius eye not observed. Ratio of prosome to urosome length (including caudal rami) about 1.5:1. Urosome 5-segmented (Fig. 1B), comprising fifth pedigerous somite, genital double-somite formed by fusion of genital and first abdominal somites, and 3 free abdominal somites. Genital apparatus comprising paired copulatory pores located within dorsolateral gonopores: seminal receptacles paired (Fig. 1B). Egg sacs paired (Fig. 1A), containing 7 to 9 eggs. Mean body length 0.549 mm, range 0.524 to 0.573 mm (based on 5 specimens).

Caudal rami (Fig. 1B) 56 μ m long by 43 μ m wide, about 1.3 times longer than wide and bearing 6 caudal setae (seta I absent). Seta II plumose, 60 – 65 μ m in length; outer distal angle seta III plumose, ca. 71–80 μ m, but shorter than inner distal angle plumose seta VI (85–97 μ m); inner apical seta V (268–274 μ m) longer than outer apical seta IV (189–227 μ m); dorsal seta VII plumose and 61–73 μ m long.

Antennule 17-segmented (Fig. 1C); probable segmental homologies as follows: segment 1 (I–II) double, segment 2 (III–V) compound, segment 3 (VI–IX) compound, segment 4 (X) free, segment 5 (XI) free, segment 6 (XII–XIV) compound, segment 7 (XV–XVI) double, segments 8 (XVII) to 16 (XXV) all free, apical segment 17 (XXVI–XXVIII) compound. Setal armature comprising: 3, 5, 7, 2, 2, 4, 2, 1, 1, 0, 1, 1 + aesthetasc, 1, 1, 1 + 1, 1 + 1, 6 + aesthetasc. Apical aesthetasc and adjacent seta arising from common base.

Antenna (Fig. 2A) uniramous; with coxa and basis fused and separated from first endopodal segment by nonfunctional articulation; coxa-basis armed with long plumose inner seta and short outer seta representing exopod; endopod 3-segmented; first endopodal segment with 1 inner margin seta, second segment with 5 setae, the shortest and most proximal separated by gap from other 4, third endopodal segment with 7 setae; first endopodal segment ornamented with spinules on inner surface; second and third endopodal segments each ornamented with row of spinules adjacent to outer margin, second segment with additional transverse spinule row.

Labrum (Fig. 2B) ovoid, with entire posterior margin; surface ornamentation of spinules on raised anterior zone.

Mandible (Fig. 2C–D) comprising coxa with well developed gnathobase bearing numerous blades along oblique margin, and biramous palp: palp consisting of large basis bearing single plumose seta, 2-segmented endopod with 3 + 5 setae, and 4-segmented exopod bearing total of 5 sparsely plumose setae [figured specimen with only 4 exopodal setae, missing seta indicated by arrow in Fig. 2C].

Maxillule (Fig. 2E) with large praecoxal arthrite bearing 8 marginal setal elements; coxal endite bearing single setae; coxal epipodite represented by long plumose seta; basis produced into 2 endites medially bearing 3 (proximal) and 2 (distal) spinulose setae; endopod comprising single expressed segment bearing 1, 1, 5 spinulose setae; exopod 1-segmented with 4 long plumose setae.

Maxilla well developed, 5-segmented (Fig. 2F); praecoxa and coxa incompletely separated, bearing 4 inner margin endites, armed with 3, 1, 3, 2 spinulose setae (from proximal to distal); basis bearing powerful claw-like element and spinulose seta, ornamented with spinule rows; endopod 3-segmented, bearing 4, 1, 3 setal elements (from proximal to distal).

Maxilliped (Fig. 2G) smaller than maxilla; 4-segmented; first segment (syncoxa) produced into two endites, proximal endite with 3 spinulose setae, distal endite armed with 2 setae and ornamented with row of spinules; second segment (basis) with 2 spinulose setae and row of spinules; endopod 2-segmented, first endopodal segment with 2 unequal spinulose setae and second with 4 spinulose setae.

Legs 1 to 4 biramous, with 3-segmented rami (Fig. 3A–C) and with intercoxal sclerites present; free posterior margins of sclerites smoothly rounded, lacking any ornamentation on either surface in legs 1 to 3; intercoxal sclerite of leg 4 with 2 spinule rows on posterior surface. Spine and seta formula as follows:

	coxa	basis	exopodal segments	endopodal segments
leg 1	0-1	1-I	I-1; I-1; III,I,4	0-1; 0-1; 1,2,3
leg 2	0-1	1-0	I-1; I-1; III,I,5	0-1; 0-2; 1,2,3
leg 3	0-1	1-0	I-1; I-1; III,I,5	0-1; 0-2; 1,2,3
leg 4	0-1	1-0	I-1; I-1; II,I,5	0-1; 0-2; 1,2,2

Coxa with spinule rows near outer distal angle in all legs (transversely orientated in leg 1 only); additional spinule rows present on coxa and basis of leg 4 only (Fig. 3C). Outer margin spines on all exopods and inner spine on basis of leg 1 bilaterally ornamented with strips of serrated membrane. Inner margin of basis of legs 1 to 4 and outer margins of all endopodal segments ornamented with row of setules; outer margins of all exopodal segments ornamented proximally with row of spinules. Spinule rows located at articulation between all endopodal and exopodal segments. Terminal spine on exopod of leg 4 very powerfully developed (Fig. 3C); spine 39µm in length with base 13µm in width, and 1.15 times longer than segment (length 34µm).

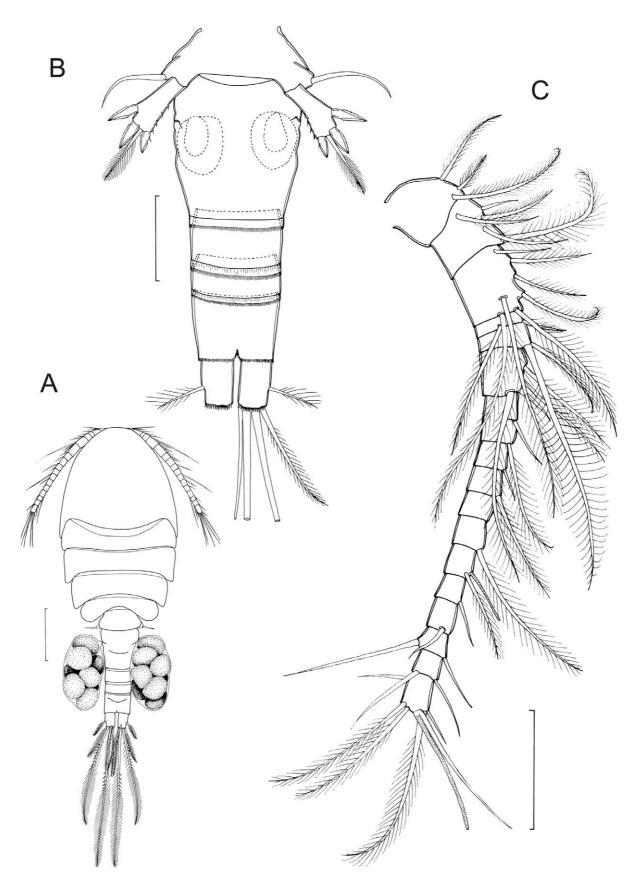


FIGURE 1. *Paracyclopina sacklerae* **n. sp.**, adult female. A, habitus of ovigerous holotype female, dorsal; B, urosome (with dorsal caudal seta omitted), ventral; C, antennule. Scale bars: $A = 100 \mu m$, B, C = 50 μm .

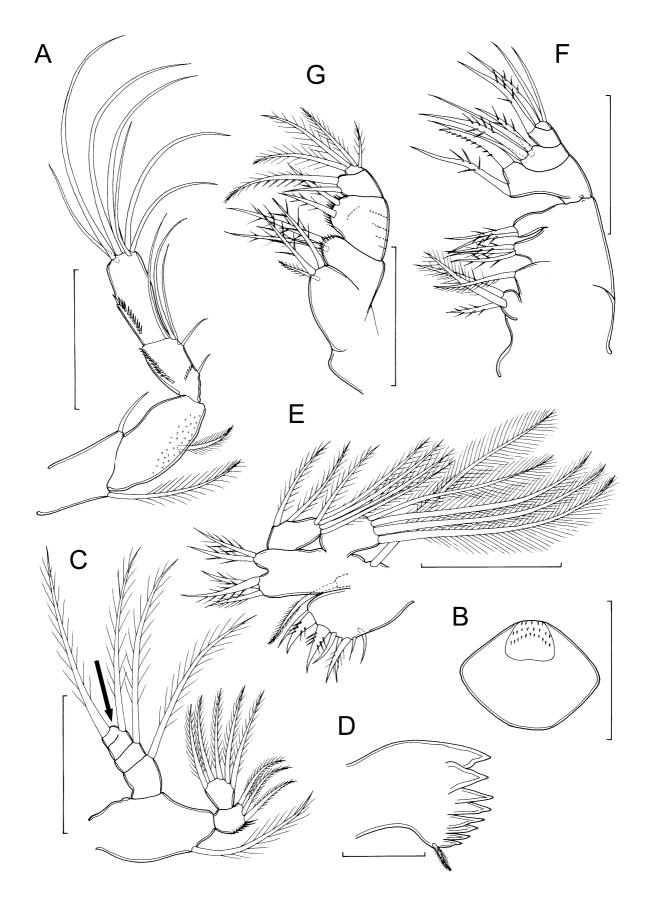


FIGURE 2. *Paracyclopina sacklerae* **n. sp.**, adult female. A, antenna; B, labrum, ventral; C, mandibular palp, with position of missing seta shown by arrow; D, biting edge of mandibular gnathobase; E, maxillule; F, maxilla; G, maxilliped. All scale bars = $50 \mu m$.

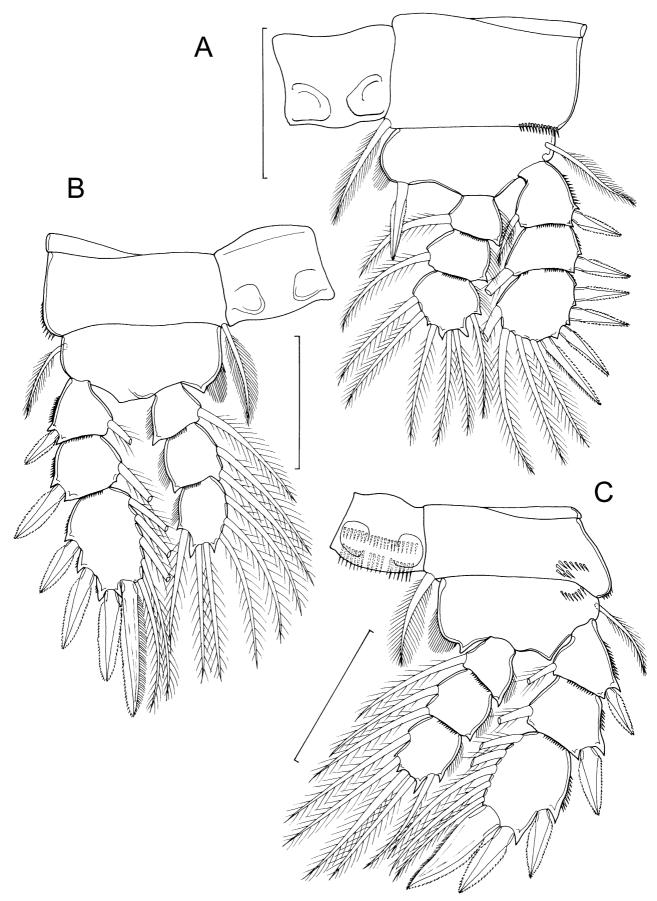


FIGURE 3. Paracyclopina sacklerae n. sp., adult female. A, leg 1, anterior; B, leg 2, anterior; C, leg 4, anterior. All scale bars = $50 \mu m$.

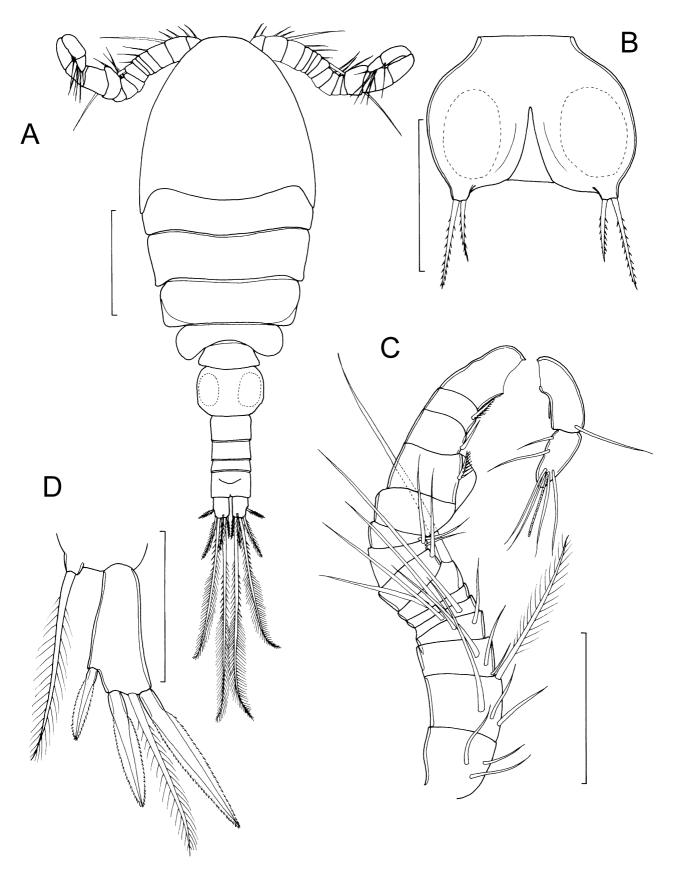


FIGURE 4. *Paracyclopina sacklerae* **n. sp.**, adult male. A, habitus, dorsal; B, genital somite, ventral; C, antennule, with 2 segments distal to geniculation shown disarticulated; D, leg 5, ventral. Scale bars: $A = 100 \mu m$, B, $C = 50 \mu m$, $D = 25 \mu m$.

Fifth legs located ventro-laterally, comprising basal part incorporated into pedigerous somite armed with outer seta on small papilla and free exopodal segment (Fig. 1B). Outer basal seta visible in dorsal view (Fig.1A). Free exopodal segment 35μ m long by 15μ m in maximum width (measured at level of base of lateral spine), about 2.3 times longer than wide; bearing lateral margin spine (21μ m long), outer distal spine (17μ m), plumose apical seta (39μ m) and inner distal spine (12μ m); exopodal segment ornamented with sparse row of spinules along inner margin.

Description of adult male. Body cyclopiform (Fig. 4A), divided into anterior prosome and posterior urosome as in female. Epimeral angles of free third pedigerous somites slightly produced. Ratio of prosome to urosome length (including caudal rami) about 1.7:1. Urosome 6-segmented, comprising fifth pedigerous somite, genital somite and 4 free abdominal somites. Genital somite (Fig. 4B) bearing paired genital openings ventrally. Caudal rami (Fig. 4A) 20µm long, by 16µm wide, about 1.25 times longer than wide and bearing 6 caudal setae (seta I absent), relative lengths of setae as for female. Mean body length 0.430 mm, range 0.408 to 0.461 mm (based on 3 specimens).

Antennae to maxillipeds, and legs 1 to 4, as in female.

Fifth legs (Fig. 4D) as in female except free exopodal segment 22 μ m long by 9 μ m in maximum width (measured at level of base of lateral spine), about 2.4 times longer than wide; bearing lateral margin spine (13 μ m long), outer distal spine (20 μ m), plumose apical seta (29 μ m) and inner distal spine (28 μ m); inner margin of segment smooth.

Remarks. The genus *Paracyclopina* was established in 1935 by Smirnov (1935) who designated his new species *P. nana* Smirnov, 1935 as the type species. At that time, the genus was included within the Cyclopinidae Sars, 1913, a heterogeneous and probably paraphyletic family deliberately retained by Boxshall & Halsey (2004) even though proposals had already been made by Martínez Arbizu (2000a, b, 2001a, b) to break it up to form four other families, the Cyclopettidae Martínez Arbizu, 2000, Giselinidae Martínez Arbizu, 2000, Hemicyclopinidae Martínez Arbizu, 2001 and Psammocyclopinidae Martínez Arbizu, 2001. Subsequently Martínez Arbizu (2006) proposed a fifth new family, the Schminkepinellidae, to include some other former members of the Cyclopinidae bringing the total number of "cyclopinid" families to seven (including the Cyclopinidae sensu stricto and the Pterinopsyllidae). No comprehensive parsimony based test of the validity of the new families derived from the break up of the Cyclopinidae has yet been carried out. Indeed, no other publications have addressed this issue except Karanovic (2008) who considered that characters exhibited by the new cyclopinidae *sensu lato* as unreliable and he recognised only the family Cyclopinidae. Clearly, uncertainty remains over the validity of some of these lineages as family level taxa.

Although its relationships with the family Oithonidae Dana, 1853 remain to be resolved, the Cyclopettidae is reasonably well supported and is recognised here. When he established the Cyclopettidae, Martínez Arbizu (2000a) tentatively included *Paracyclopina* as *incertae sedis* in his new family. Such caution was justified since all four of the species attributed to this genus were inadequately described. The four species recognised by Martínez Arbizu (2000a) as belonging to *Paracyclopina* were: *P. nana*, *P. intermedia* (Sewell, 1924), *P. longifurca* (Sewell, 1924) and *P. minuta* (Sewell, 1934). The key characters used to support placement of *Paracyclopina* in a lineage with *Cyclopetta* Sars, 1913 were the 2-segmented endopod of the maxilliped and the lateral location of the leg 5 which comprises, in both sexes, one free exopodal segment carried on a slight pedestal representing the protopodal part of the limb that is incorporated into the somite. Martínez Arbizu (2000a) pointed out that the transformation of the inner apical and two outer margin spines on the exopod of leg 5 into setae was a synapomorphy of the core group of genera *Cyclopetta*, *Paracyclopetta* Wells, 1967 and *Arctocyclopina* Mohamed & Neuhof, 1985. This transformation is not shared by *Paracyclopina* species, but the other synapomorphies support its placement in the Cyclopetti-dae (Martínez Arbizu 2000a).

Cyclopetta orientalis Lindberg, 1941 was not mentioned by Martínez Arbizu (2000a): it was neither treated as a species of Cyclopetta, nor transferred to any other genus within the Cyclopinidae sensu lato. Although it was

described as having a 1-segmented rather than a 2-segmented endopod on the maxilliped, it shares this form of laterally-located leg 5 and, in addition, has a 17-segmented antennule in the female, as in *P. nana* and *P. minuta*. Accordingly *Cyclopetta orientalis* is here transferred to *Paracyclopina* as *Paracyclopina orientalis* (Lindberg, 1941) **n. comb.** It is assumed here that the description of the maxilliped is mistaken and that *P. orientalis* has a 2segmented endopod as found in the new species.

The new species, *P. sacklerae* **n. sp.**, is closely related to *P. orientalis*. Both species possess a strikingly robust terminal spine on the exopod of leg 4 in both sexes and have three robust spines on the free exopodal segment of the female leg 5. No other *Paracyclopina* species share both of these features. There are several significant differences between these two species. Most obviously, the caudal rami are much shorter in *P. sacklerae*, only about 70% as long as the anal somite, whereas in *P. orientalis* the caudal rami are longer (125%) than the anal somite. The caudal rami themselves are more slender in *P. orientalis*, 3.5 times longer than wide compared to only 1.3 times longer than wide in *P. sacklerae*. Other differences include the shape of the antenna: in *P. sacklerae* the distal part of the antenna (second and third endopodal segments combined) is about equal in length to the proximal part (coxa-basis and first endopodal segment combined), whereas in *P. orientalis* the distal part is significantly shorter than the proximal part. The eggs sacs contained between 25 and 31 small eggs per sac in *P. orientalis* compared to 7 to 9 larger eggs per sac in the new species.

As highlighted by Martínez Arbizu (2000a), all four species he ascribed to *Paracyclopina* were found in brackish to fresh waters. *Paracyclopina orientalis*, transferred to *Paracyclopina* here, was collected in shallow salty waters near to the coast at Pondichery ("des mares saumâtres peu profondes, près du bord de la mer à Pondichéry") and in a lagoon at Oupalom (Lindberg 1941). It seems likely that this species also inhabits brackish waters. Finally, the new species was found in small sink holes, about 2 m deep, located in the coastal limestone platform only 5 to 10 m inland from the shoreline on Muna Island. During low tide many of the sinkholes appeared dry, but at high tide, water was clearly visible in most of them. The copepods were caught using a long-handled plankton net in a water column that was up to 50 or 60 cm deep. Salinity measurements were not taken, but given the tidal influence and the proximity to the coast, it is highly likely that the water in the sinkholes was brackish but close to fully marine. The species of *Paracyclopina* appear to prefer low salinity and brackish water habitats, although *P. minuta* was reported from "absolutely fresh" water in the Hooghly River, according to Sewell (1934).

The genus is known only from the Indo-Pacific. Four *Paracyclopina* species were described from the coast of India: *P. longifurca* and *P. intermedia* were originally described from Chilka Lake (Sewell 1924) and the former is also known from the South India coast and from the canal system of the salt lakes at Chingrighatta (Sewell 1934); *P. minuta* is known from the Hooghly River which bounds the salt lakes and associated canal system near Chingrighatta (Sewell 1934); *P. orientalis* was collected at Pondichery and Oupalom (Lindberg 1941). The new species, *P. sacklerae*, is from Indonesia. The type species, *P. nana*, was described from the mouth of the Suifun river near Vladivostok (Smirnov 1935) and is also known from the coasts of Japan, South Korea and China (Tai & Chen 1979; Chang 2009; Ueda *et al.* 2001).

Key to species of Paracyclopina

Including the new species, *Paracyclopina* now comprises six species, the largest of which attains a body length of up to 0.73 mm, while the smallest can be as short as 0.43 mm (Table 1). Males are unknown for two of these six species. The females can be separated using the following key:

1.	Caudal rami 3.5 to 4.0 times longer than wide
-	Caudal rami 1.0 to 2.0 times longer than wide
2.	Antenna with second and third endopodal segments subequal P. longifurca
-	Antenna with third endopodal segment longer than second P. orientalis
3.	Caudal rami about 2.0 times longer than wide
-	Caudal rami 1.3 times longer than wide
4.	Body slender, with prosome about 1.2 times longer than urosome P. nana
-	Body with prosome about 1.5 times longer than urosome
5.	Inner apical spine of leg 5 robust, longer than lateral margin spine and outer apical spine
-	Inner apical spine of leg 5 markedly shorter than both lateral margin spine and outer apical spine

TABLE 1. Morphometrics of known Paracyclopina species, based on data from Smirnov (1935), Sewell (1924, 1934), Lind-
berg (1941) and this account. [BL = body length, Pr:Ur ratio = prosome:usorome length ratio, C. ramus = caudal ramus, L:W
ratio – length:width ratio]

Species	\bigcirc BL (mm)	∂BL (mm)	Pr:Ur ratio	C. ramus L:W ratio	Eggs/sac
P. nana	0.47-0.50	0.41	1.2:1.0	2.0:1.0	?
P. intermedia	0.51	0.40	1.5:1.0	2.0:1.0	3–4
P. longifurca	0.59	?	1.3:1.0	3.8:1.0	11–12
P. minuta	0.43-0.50	0.47	1.5:1.0	2.0:1.0	6–7
P. orientalis	0.63-0.73	?	?	3.5:1.0	25-31
P. sacklerae n.sp.	0.52-0.57	0.41-0.46	1.5:1.0	1.3:1.0	7–9

Order Calanoida G.O. Sars, 1903

Family Boholinidae Fosshagen & Iliffe, 1989

Genus Boholina Fosshagen & Iliffe, 1989

Boholina parapurgata n. sp.

(Figs. 5–10)

Type material. Holotype female, 7 paratype females, 5 paratype males collected from sinkholes on the coast at the edge of Walengkabola village (5° 11.052' S 122° 35.159' E), Muna Island, Indonesia, on 18 September 2007 by G.A. Boxshall and D. Jaume. Registration numbers: holotype female [MZB.Cru Cop.105], 3 paratype females and 3 paratype males [MZB.Cru Cop.106] in Museum Zoologicum Bogoriense, 4 paratype females and 2 paratype males in Natural History Museum, London [BMNH 2011.1172-1177].

Etymology. The name of the new species alludes to its close resemblance to *Boholina purgata* Fosshagen & Iliffe, 1989.

Description of adult female. Body (Fig. 5A) with a mean length of 0.78 mm (range 0.74 to 0.83 mm, based on 7 specimens). Prosome 5-segmented, comprising cephalosome, first to third free pedigerous somites and double somite consisting of completely fused fourth and fifth pedigerous somites; prosome reaching maximum width at rear of cephalosome; postero-lateral corners of second and third pedigerous somites forming acute posteriorlydirected points (Fig. 9B); postero-lateral corners of posterior double-somite rounded, symmetrical. Nauplius eye lacking. Ratio of prosome to urosome length about 3:1. Urosome with only 3 obvious segments (Fig. 5B) but 4segmented, comprising genital double-somite and 2 free abdominal somites plus hoop-like anal somite (arrowed in Fig. 5D) largely telescoped within preceding second free abdominal somite and entirely concealed beneath extensive somitic hyaline membrane. Genital double-somite (Fig. 5C) symmetrical, widest about at mid-length; posterior border ornamented with smooth hyaline membrane dorsally and strongly dentate hyaline membrane ventrally (Fig. 5C); genital openings paired, located close together on mid-ventral surface; seminal receptacles paired, located lateral to and posterior to genital openings; 2 pairs of sensillae present, one pair positioned adjacent to medial margin of gonopores, second pair located ventro-laterally near posterior margin of double-somite. First and second free abdominal somites subequal in length; first with smooth hyaline membrane all around posterior margin, second with posterior margin hyaline membrane expanded mid-dorsally to form double-pointed flap functioning as pseudoperculum concealing anal opening. Anal somite extremely short, concealed within posterior rim and hyaline membrane of second free abdominal somite.

Caudal rami (Fig. 5A–B) about 1.5 times longer than wide (measured from base to level of insertion of distal seta IV), with pointed dorsal process in middle of distal margin (Fig. 5B, D); caudal setae II to VII present (seta I lacking); seta II spiniform, seta III about half length of seta VI, seta V longer than seta IV, both plumose; dorsal seta VII short, plumose; slight asymmetry noted with short row of tiny setules present distally on inner margin of left ramus.

Rostrum weakly developed (Fig. 10A) as triangular area of cuticle tapering back from broad base at junction with frontal margin of dorsal cephalic shield to narrow rounded tip between bases of antennules; pair of long sensillae in middle third of rostrum.

Antennules (Fig. 6A) symmetrical, extending almost to end of prosome; 24-segmented with all articulations expressed, except between segments II–IV and XXVI–XXVIII; segment 8 double comprising ancestral segments X and XI, but with articulation incompletely expressed. Armature formula as follows: segment 1 (ancestral segment I) 1 seta + 1 aesthetasc; segment 2 (ancestral segments II–IV) 6 setae + ae; segment 3 (V) 2 + ae; segment 4 (VI) 2; segment 5 (VII) 2 + ae; segment 6 (VIII) 2; segment 7 (IX) 2 + ae; segment 8 (X–XI) 3 + 2 ae; segment 9 (XII) 1 seta; segment 10 (XIII) 1 + ae; segment 11 (XIV) 1 + ae; segment 12 (XV) 1 + ae; segment 13 (XVI) 1 + ae; segment 14 (XVII) 1 seta; segment 15 (XVIII) 1 + ae; segment 16 (XIX) 1 seta; segment 17 (XX) 1 seta; segment 18 (XXI) 1 + ae; segment 19 (XXII) 1 seta; segment 20 (XXIII) 1 seta; segment 21 (XXIV) 1 + 1; segment 22 (XXV) 1 + 1 + ae; segment 23 (XXVI) 1 + 1; apical segment 24 (compound XXVII–XXVIII) 5 + ae.

Antenna (Fig. 6B) biramous. Coxa short, bearing plumose seta at distomedial angle. Basis with 2 subequal plumose setae on distomedial angle. Exopod 9-segmented, articulation between fourth and fifth segments incomplete, apical segment small: setal formula as follows: 1, 1, 1, 1, 1, 1, 1, 3: all setae plumose; articulations between two to five with spinule rows. Endopod 2-segmented: proximal segment with 2 unequal, naked setae, ornamented with oblique spinule row; compound distal segment expanded into medial lobe bearing 8 setae distally and on outer distal margin, and with distal portion crowned with 6 setae; segment ornamented with small serrated process subdistally on medial margin and adjacent spinule row.

Mandible (Fig. 6C) with cutting edge of coxal gnathobase comprising about 10 short, cuspidate or simple teeth plus small dorsal spinulose seta; ventral-most teeth larger. Palp biramous; basis with 4 unequal setae on inner margin, and with spinule row proximally on outer surface. Exopod indistinctly 5-segmented, setal formula 0, 1, 1, 1, 2. Proximal endopodal segment with 4 setae at distomedial angle; distal segment with 10 distal margin setae.

Maxillule (Fig. 7A) with praecoxal arthrite carrying 10 marginal spines plus 4 stiff setae on posterior surface. Coxal epipodite with 9 plumose setae; coxal endite with 4 spinulose setae. Basis fused to both rami: armed with 4 spinulose setae on proximal endite; distal basal endite incorporated into segment, with 5 setae; basal exite represented by single vestigial seta. Exopod bearing 10 marginal setae. Endopod with first segment fused to basis, represented by 4 distal margin setae; 2 free segments armed with 4 and 7 setae respectively.

Maxilla (Fig. 7B) 6-segmented, comprising praecoxa and coxa, allobasis and 3-segmented free endopod. Armature of praecoxal and coxal endites 5, 3, 3, 3; all setae bilaterally spinulate; distal coxal endite with spinule row. Allobasis with 6 setal elements in total, 4 on produced basal endite, 1 strong and claw-like, plus 2 setae derived from fused first endopodal segment; ornamented with spinule row. Free endopod setal formula: 2, 1, 2; long setae on allobasis and on endopodal segments sparsely spinulate bilaterally.

Maxilliped (Fig. 7C) 8-segmented with syncoxa, basis and free 6-segmented endopod. Syncoxa with 1, 2, 2, 3 setae on medial margin lobes; first lobe seta bilaterally spinulate; one seta on second lobe very long and naked, other seta plus setae on third lobe sparsely bilaterally spinulate; fourth lobe setae naked; small patch of denticles present on medial surface of fourth lobe. Basis about as long as syncoxa, armed with 3 plumose setae and carrying row of strong spinules along medial margin of segment. Free endopod setal formula: 2, 4, 4, 3, 3 + 1, 4.

Legs 1–5 (Fig. 8A–E) biramous, with 3-segmented rami except endopod 2-segmented in leg 5. Legs with smooth, unornamented intercoxal sclerites, except short spinule rows present on anterior surface of leg 2. Basis of leg 1 with rounded digitiform process distally, arising posteriorly near base of endopod. Armature of legs 1 to 5 as follows:

	coxa	basis	exopodal segments	endopodal segments
Leg 1	0-1	I-I	I-0; I-1; II,I,4	0-1; 0-1; 0,2,3
Leg 2	0-1	0-0	I-1; I-1; II,I,5	0-1; 0-2; 2,2,4
Leg 3	0-1	0-0	I-1; I-1; III,I,5	0-1; 0-2; 2,2,4
Leg 4	0-1	1-0	I-1; I-1; III,I,5	0-1; 0-2; 2,2,3
Leg 5	0-1	1-0	I-0; I-1; II,II,3	0-1; 2,2,3

Outer distal angles of first and second endopodal segments of legs 1 to 4 each drawn out into acute process, particularly marked in legs 1 and 2. Endopod segment 3 with large, acute process at outer distal angle in leg 1 only. Second exopodal segment of leg 1 (Fig. 8A) with conspicuous spinulate process distally, in axil of outer spine; third exopodal segment with marginal spinule row on anterior surface. Articulations between endopodal and

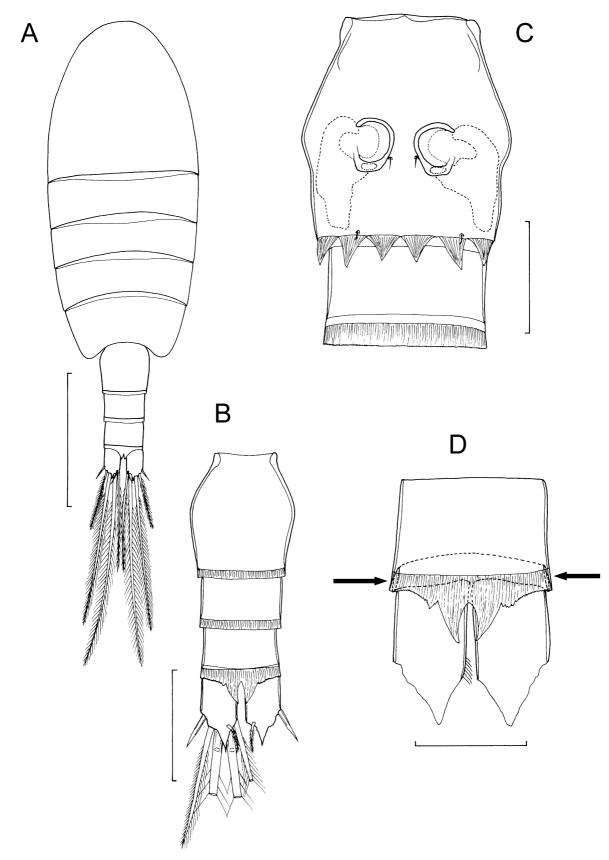


FIGURE 5. *Boholina parapurgata* **n. sp.**, adult female. A, habitus, dorsal; B, urosome, dorsal; C, genital double-somite and first free abdominal somite, ventral view showing paired gonopores and internal paired seminal receptacles (dotted lines); D, posterior end of urosome, showing reduced, hoop-like anal somite (arrowed) entirely telescoped within preceding somite and concealed by extensive intersomitic membrane, dorsal. Scale bars: $A = 250 \mu m$, $B = 100 \mu m$, $C, D = 50 \mu m$.

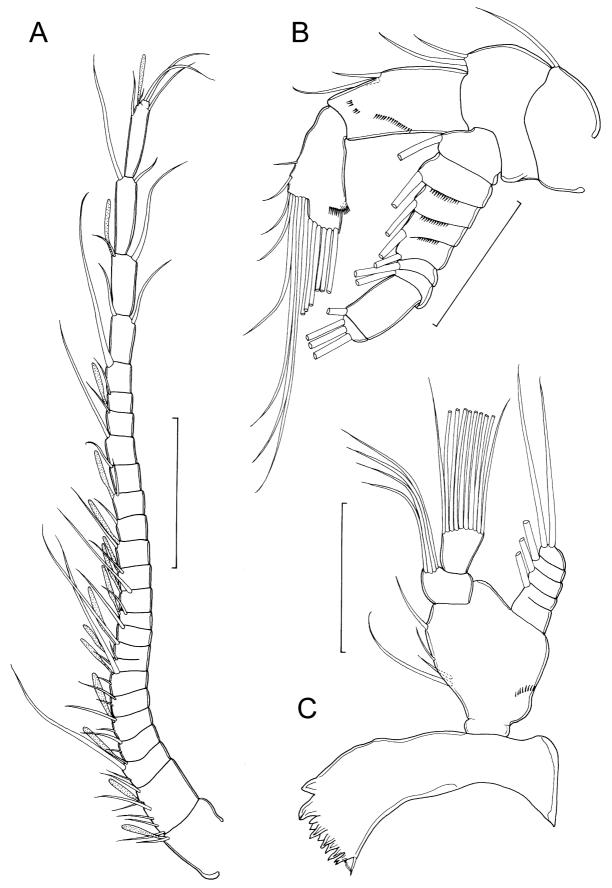


FIGURE 6. *Boholina parapurgata* **n. sp.**, adult female. A, antennule; B, antenna; C, mandible. Scale bars: $A = 100 \mu m$, B, C = 50 μm .

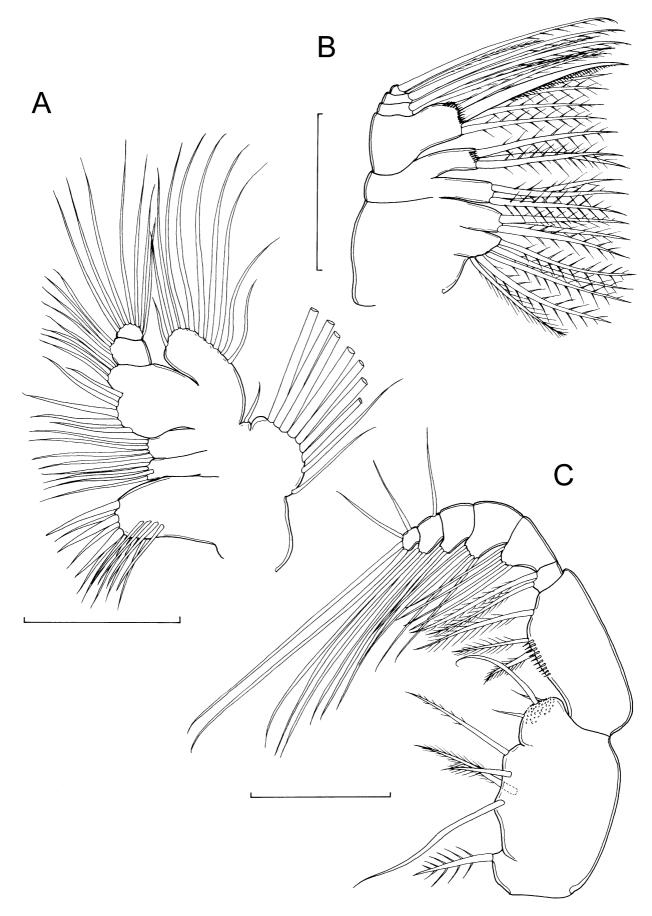


FIGURE 7. Boholina parapurgata n. sp., adult female. A, maxillule; B, maxilla; C, maxilliped. All scale bars = 50 µm.

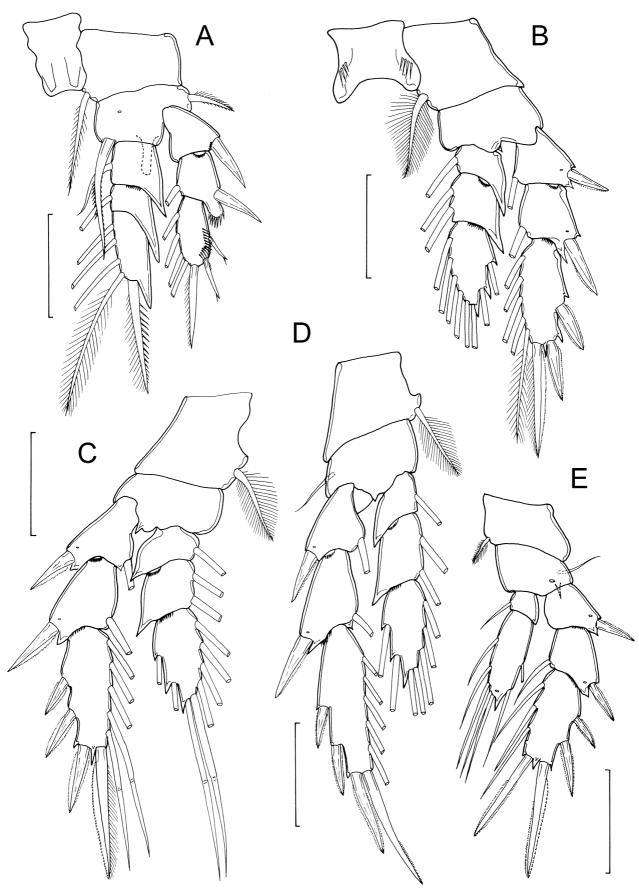


FIGURE 8. *Boholina parapurgata* **n. sp.**, adult female. A, leg 1, anterior; B, leg 2, anterior; C, leg 3, anterior; D, leg 4, anterior; E, leg 5, anterior. All scale bars = 50 µm.

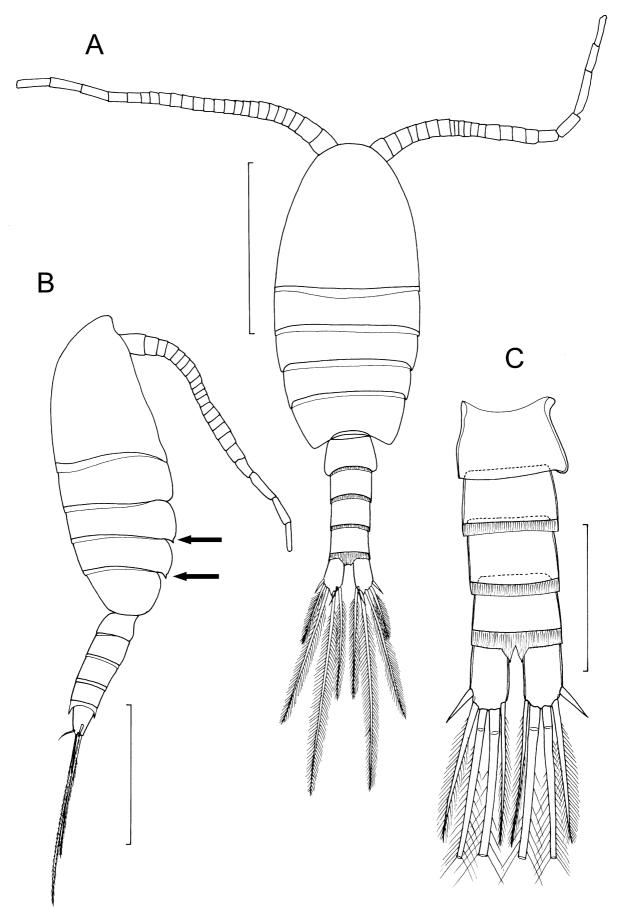


FIGURE 9. *Boholina parapurgata* **n. sp.**, adult male. A, habitus, dorsal; B, habitus lateral, showing pointed angles to tergites of pedigerous somites (arrowed); C, urosome, ventral: Scale bars: A, B = $250 \mu m$, C = $100 \mu m$.

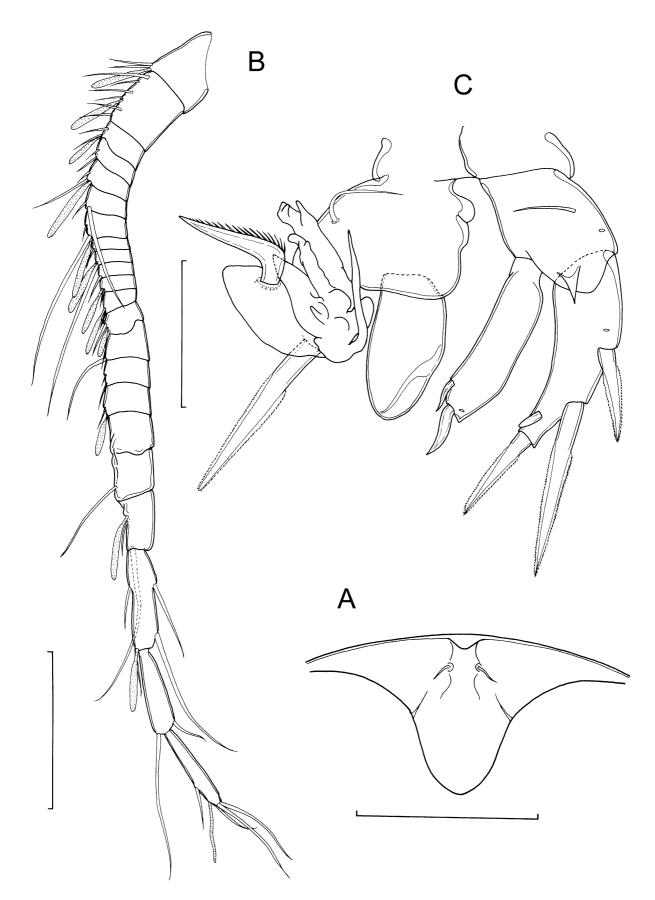


FIGURE 10. *Boholina parapurgata* **n. sp.**, adult female. A, rostrum, ventral; adult male. B, geniculate right antennule; C, leg 5, posterior: All scale bars = $50 \mu m$.

exopodal segments ornamented with spinule rows. Inner basal seta on leg 1 long, bilaterally spinulate, reaching to base of middle seta on third endopodal segment. Setae on both rami plumose; outer spines on distal exopodal segment of leg 1 flagellate; outer spines on legs 2 to 4 with serrate marginal membrane(s) as figured. Terminal spine on exopod of legs 1 to 3 ornamented with serrate membrane externally and plumose internally, that on leg 4 bilaterally serrate. Leg 4 with inner distal setal element on third exopodal segment 61 μ m long, about 1.5 times longer than adjacent terminal spine (41 μ m) and shorter than segment (= 70 μ m long).

Leg 5 (Fig. 8E) biramous, with 3-segmented exopod and 2-segmented endopod, intercoxal sclerite smooth and unornamented. Basis of leg 5 with small, acute process located on posterior surface near base of exopod and with large pore on anterior surface. Exopod longer than endopod: tip of endopod reaching beyond level of proximal outer spine on third exopodal segment; distal endopodal segment 2.6 times longer (42 μ m) than wide (16 μ m). Outer spines on exopod each with serrate membrane bilaterally. Terminal spine on exopod with serrate membrane externally and finely serrate membrane internally, terminal spine (53–57 μ m) about 1.2 times longer than inner distal spine (41–47 μ m), and longer than segment (44–48 μ m). First and second exopodal segments each ornamented with pore on anterior surface at origin of outer spine.

Description of adult male. Body (Fig. 9A, B) length ranging from 0.66 to 0.71 mm, with a mean of 0.69 mm (based on 4 specimens). Prosome 5-segmented as in female: maximum width at level of first pedigerous somite; postero-lateral corners of second and third pedigerous somites (arrowed in Fig. 9B) forming acute posteriorlydirected points, as in female. Ratio of prosome to urosome length about 2.4:1. Urosome 5-segmented (Fig. 9C); comprising genital somite and 3 free abdominal somites plus hoop-like anal somite largely telescoped within preceding second free abdominal somite and entirely concealed beneath somitic hyaline membrane, as in female. Genital somite slightly asymmetrical, with single gonopore opening posterolaterally on left side; genital and first to third free abdominal somites similar in length, although often variably telescoped within preceding somite; each with hyaline membrane around posterior border, except membrane lacking ventrally on genital somite. Anus opening terminal, located between caudal rami, concealed beneath pseudoperculum formed by hyaline membrane on third free abdominal somite.

Caudal rami (Fig. 9A, C) symmetrical, about 1.7 times longer than wide and bearing distal spinous process dorsally, as in female. Caudal seta I absent; caudal setae II–VII, as in female.

Antennules short, asymmetrical. Left antennule non-geniculate, 24-segmented as in female and extending almost to posterior end of prosome. Right antennule (Fig. 10B) geniculate, 22-segmented. Armature as follows: segment 1 (ancestral segment I) 2 setae + aesthetasc; segment 2 (corresponding to compound ancestral segments II–IV) 6 + 2 ae; segment 3 (V) 2 + ae; segment 4 (VI) 2; segment 5 (VII) 2 + ae; segment 6 (VIII) 2; segment 7 (IX) 2 + ae; segment 8 (X) 1 seta; segment 9 (XI) 1 + ae; segment 10 (XII) 1; segment 11 (XIII) 1 + ae; segment 12 (XIV) 1 + ae; segment 13 (XV) 1 + ae; segment 14 (XVI) 2; segment 15 (XVII) 2; segment 16 (XVIII) 1 + ae; segment 17 (XIX) 1 modified, fused spine; segment 18 (XX) 1 modified, fused spine + 1 seta; segment 19 (XXI–XXVII) 2 setae + ae; segment 20 (XXIV–XXV) 2 + 2 + ae; segment 21 (XXVI) 1 + 1, segment 22 (XXVII–XXVIII) 5 + ae.

Leg 5 (Fig. 10C) strongly asymmetrical; coxae and intercoxal sclerite fused to form common base. Left leg biramous: basis with slender outer basal seta located on posterior surface; exopod 3-segmented; first segment with long (63 μ m), bilaterally serrate outer spine; second segment modified, bearing strongly reflexed spine on outer margin; third segment highly transformed bearing multiple short processes and one long, naked, modified setal element. Right leg biramous, basis with slender outer basal seta located on posterior surface; exopod unsegmented; segment with 2 outer spines (proximal spine 33–35 μ m, distal spine 62–63 μ m in length) and terminal spine 34–35 μ m long, plus a short spine vestige located subdistally towards the medial surface; endopod forming an elongate lobe, about 61 μ m long, by 17–18 μ m wide (3.6 times longer than wide); armed with 2 slightly sigmoid spines, apical spine 17–19 μ m long, subapical spine 12–13 μ m (Fig. 10C).

Remarks. The family Boholinidae and the genus *Boholina* were established by Fosshagen & Iliffe (1989) on the basis of two new species discovered in a brackish pool in San Vincente Cave on Bohol Island in the Philippines. In the original description, the urosome of the females and males were described as 3- and 4-segmented respectively (Fosshagen & Iliffe 1989). We find that the anal somite has been overlooked in both sexes and this has been verified by examination of the types of *B. crassicephala* Fosshagen & Iliffe, 1989 (Reg. No. BMNH 1989.58–67). It is represented by a short hoop of cuticle, which lies more-or-less completely telescoped within the preceding free abdominal somite. The presence of the extensive hyaline membrane around the entire posterior margin of that

somite adds to the concealment. A similarly tiny and concealed anal somite was noted for genera such as *Edaxiella* Fosshagen, Boxshall & Iliffe, 2001, *Oinella* Fosshagen, Boxshall & Iliffe, 2001 and *Gloinella* Fosshagen, Boxshall & Iliffe, 2001 within the closely related Epacteriscidae (Fosshagen *et al.* 2001).

Both sexes of the type species, *B. crassicephala*, were fully described in 1989 but the description of the second species, *B. purgata* Fosshagen & Iliffe, 1989, focused only on differences and several illustrations were provided to facilitate comparison of key characters between the two species. The species differed in several fine scale characters: the most important of which were, the posterior angles of the tergites on the second and third pedigerous somites are rounded in *B. crassicephala* but pointed in *B. purgata*, the lengths of the antennules relative to the prosome, and the armature of the male leg 5. On the exopod of the right leg 5 of the male there are four strong spines in *B. crassicephala* but only three strong spines plus a vestige in *B. purgata*. In all of these characters the new species closely resembles *B. purgata*.

The new species can be distinguished from *B. purgata* by the length and shape of the endopod of the female leg 5. In the former the distal tip of the endopod extends beyond the level of the origin of the proximal spine on the outer margin of the third exopodal segment, and the distal endopodal segment is 2.6 times longer than wide, whereas in the latter the distal tip only extends as far as the articulation between second and third exopodal segment is only 1.1 times longer than wide. Also in the female leg 5, the terminal spine on the exopod is longer than the segment in the new species but shorter than it in *B. purgata*.

The differences between the males are similarly fine scale: the unsegmented endopod of the right leg 5 of the male is 2.6 times longer than wide in *B. purgata* and the outer margin is evenly convex so the maximum width is about at mid-length, whereas in the new species the endopod is 3.6 times longer than wide and the maximum width is in the distal third. This endopod bears two slender setae in the former species but two sigmoid spines in *B. para-purgata* **n. sp.**

Such fine scale differences are sometimes difficult to interpret. Little is known about variability in *Boholina* and, like *Paracyclopina*, the genus appears to prefer low salinity, brackish waters. We consider that the *B. parapurgata* population from Muna (Indonesia) and the *B. purgata* population from Bohol (Philippines) are distinct species, but we strongly recommend sampling at suitable intermediate locations to search for additional *Boholina* populations.

Boholina munaensis n. sp.

(Figs. 11-12)

Type material. Holotype female, 2 paratype females, 2 paratype males, collected from Lawou cave spring in Walengkabola village, Muna Island, Indonesia located at 5° 10.950'S 122° 34.720'E; salinity 1.8 ppt, on 13 September 2007 by G.A. Boxshall and D. Jaume. Registration numbers: holotype female [MZB.Cru Cop.107], 2 paratype females and 1 paratype male [MZB.Cru Cop. 108] in Museum Zoologicum Bogoriense; 1 paratype male in Natural History Museum, London [BMNH 2011.1178].

Additional material. 13 copepodid stages with same collection data: tentatively identified as *B. munaensis* **n. sp.**

Etymology. The name of the new species is based on its type locality, Muna Island in Indonesia.

Description of adult female. Body (Fig. 11A) length ranging from 0.70 to 0.77 mm, with mean of 0.74 mm (based on 3 specimens). Prosome 5-segmented; postero-lateral corners of second and third pedigerous somites forming acute posteriorly-directed points; postero-lateral corners of posterior double-somite rounded, symmetrical. Ratio of prosome to urosome length about 2.2:1. Urosome 4-segmented as in *B. parapurgata*. Genital double-somite (Fig. 11B) symmetrical, with strongly convex lateral margins widest about at mid-length; posterior border ornamented with finely serrate hyaline membrane dorsally and strongly but slightly irregularly dentate hyaline membrane ventrally (Fig. 11B); genital openings paired, more widely separated on mid-ventral surface than in *B. parapurgata*; 1 pair of sensillae present on ventral surface near posterior margin of double-somite. First and second free abdominal somites subequal in length; first with finely serrate hyaline membrane all around posterior margin, second with posterior margin hyaline membrane expanded mid-ventrally and mid-dorsally, to form double-pointed flap functioning as pseudoperculum concealing anal opening. Hoop of integument representing anal somite, irregular in thickness (arrowed in Fig. 11B). Caudal rami as for *B. parapurgata*.

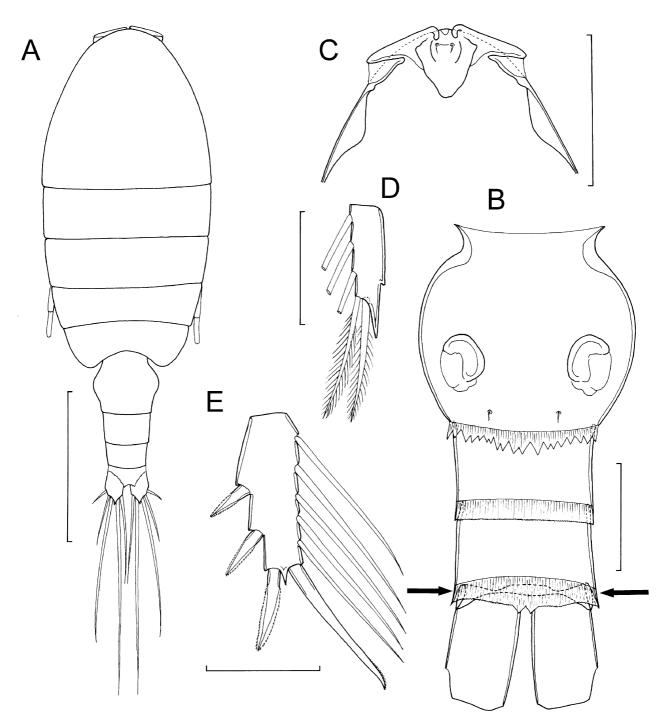


FIGURE 11. *Boholina munaensis* **n. sp.**, adult female. A, habitus, dorsal view of holotype showing tips of antennules reaching mid-length of final prosome somite; B, urosome, ventral, showing paired gonopores and reduced, hoop-like anal somite (arrowed) entirely telescoped within preceding somite and concealed by extensive intersomitic membrane (only outline of caudal rami shown); C, rostrum and transverse frontal crest, ventral; D, third endopodal segment of leg 1, showing notch on outer margin; E, third exopodal segment of leg 4 showing relative lengths of distal spines. Scale bars: A = $250 \mu m$, B–E = $50 \mu m$.

Frontal margin of dorsal cephalic shield forming elaborate transverse crest of thickened integument; continuous with weakly developed, tapering rostrum (Fig. 11C).

Antennules, antennae, maxillules, maxillae and maxillipeds all as in *B. parapurgata*. Legs 1–4 as for *B. parapurgata* except leg 1 with distinct notch on outer margin of third endopodal segment (Fig. 11D), and leg 4 with terminal spine on third exopodal segment (Fig. 11E) 68 μ m long, about 1.9 times longer than adjacent distolateral spine (36 μ m) and about same length as segment (= 68 μ m).

Leg 5 (Fig. 12A) biramous, with 3-segmented exopod and 2-segmented endopod, intercoxal sclerite smooth

and unornamented. Setal formula as in *B. parapurgata*. Basis of leg 5 with acute process located on posterior surface near base of exopod. Exopod longer than endopod: tip of endopod reaching almost to level of proximal outer spine on third exopodal segment; distal endopodal segment 2.6 times longer (39 μ m) than wide (16 μ m). Outer spines on exopod each with serrate membrane bilaterally. Terminal spine on exopod with serrate membrane externally and finely serrate membrane internally, inner distal spine (46 μ m) about 1.4 times longer than terminal spine (34 μ m), both shorter than segment (56 μ m).

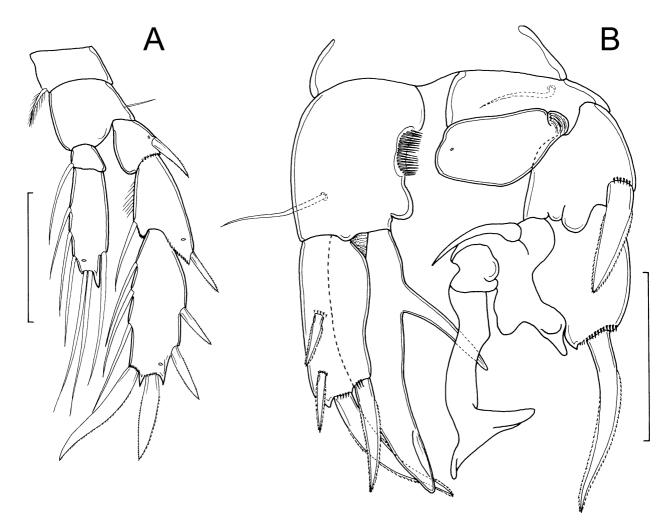


FIGURE 12. Boholina munaensis n. sp., A, female leg 5, anterior; B, male leg 5, anterior: All scale bars = 50 µm.

Description of adult male. Body 0.68 mm in length (based on 1 complete specimen). Prosome 5-segmented, as in female: postero-lateral corners of second and third pedigerous somites forming acute posteriorly-directed points, as in female. Ratio of prosome to urosome length about 2.3:1. Urosome 5-segmented. Genital somite slightly asymmetrical, with single gonopore opening posterolaterally on left side; genital and first to third free abdominal somites similar in length, although often variably telescoped within preceding somite; each with hyaline membrane around posterior border, except membrane lacking ventrally on genital somite. Anus opening terminal, located between caudal rami, concealed beneath pseudoperculum formed by hyaline membrane on third free abdominal somite. Caudal rami as in female.

Antennules as in male *B. parapurgata*; antenna to maxillipeds and legs 1–4, as described above for female *B. parapurgata*.

Leg 5 (Fig. 12B) strongly asymmetrical; coxae and intercoxal sclerite fused to form common base. Left leg biramous: basis with slender outer basal seta located on posterior surface; exopod 3-segmented; first segment with bilaterally serrate outer spine 35 μ m in length; second segment modified, bearing slightly curved spine, about 56 μ m long on outer margin; third segment highly transformed bearing several rounded processes, one modified setal element, and a long process terminating in a T-shaped distal expansion (Fig. 12B); endopod an unarmed lobe, about

1.9 times longer (40 μ m) than wide (21 μ m). Right leg biramous, basis armed with slender outer basal seta located on posterior surface and ornamented with medial comb of spinules in mid-margin; inner distal corner of basis forming rounded lobe; exopod unsegmented with 2 spines on outer margin; proximal spine (18 μ m) shorter than distal spine (23 μ m long); curved apical spine 47 μ m in length, subapical inner spine 37 μ m long; endopod forming an elongate lobe, about 81 μ m long, tapering distally, bearing strong, spiniform process originating medially on inner surface (Fig. 12B).

Remarks. The new species shares with *B. crassicephala* the presence of four well developed spines on the exopod of the male right leg 5, but can be distinguished from it by the relative lengths of these spines and by the presence of a large spinous process on the medial surface of the endopod of the right leg 5. In the female leg 5, the two distal spines on the exopod are more dissimilar in size than in *B. crassicephala*. The inner distal spine is 1.4 times longer than the outer terminal spine in the new species but only 1.1. times longer in *B. crassicephala*. In addition *B. munaensis* has pointed corners on the tergites of the second and third pedigerous somites in both sexes, as in *B. parapurgata*, whereas these corners are rounded in *B. crassicephala* according to Fosshagen & Iliffe (1989).

It is interesting to note that *Boholina* tends to occur in cave pools. In Bohol the two species co-occurred in the pools in San Vincente Cave (Fosshagen & Iliffe 1989). On Muna, a dense population of *B. munaensis* was found in Lawou, a cave spring with a water depth of up to 100 cm and a salinity of only 1.8 ppt. Villagers were washing clothes there every time we visited and the air smelled of soap and detergent yet the copepods were abundant. Interestingly, the pool in San Vicente cave on Bohol where *B. purgata* and *B. crassicephala* were originally found, was also used for clothes washing and the water was described as "milky and opaque from an overload of detergents" (Fosshagen & Iliffe 1989). Other crustaceans occurred in these caves including an atyid shrimp of the genus *Caridina* H Milne Edwards, 1837 (Wowor, pers. comm.), a cymothoid isopod parasitic on the *Caridina*, and a melitid amphipod.

Key to species of Boholina

All four species of *Boholina* are astonishingly similar in their mouthparts and legs 1 to 4. Copepodids of the *Boholina* species from Muna co-occurred in at least one site and, on the basis of morphology alone, it was not possible to distinguish between the copepodids of the two species. This key therefore functions for adults only:

1.	Female genital apertures well separated on genital double somite, located ventrolaterally; male leg 5 with four well-developed
	spines on right exopod. 2
-	Female genital apertures located close together on genital double somite, either side of ventral midline; male leg 5 with one
	vestigial and three well-developed spines on right exopod
2.	Postero-lateral corners of tergites of second and third pedigerous somites pointed in both sexes; endopod of male right leg 5
	with large inner spinous process
-	Postero-lateral corners of tergites of second and third pedigerous somites rounded in both sexes; endopod of male right leg 5
	lacking inner spinous process
3.	Endopod of right leg 5 of male 2.6 times longer than wide, bearing two slender setae; distal segment of endopod of female leg
	5 only 1.1 times longer than wide; two distal spines on exopod of female leg 5 shorter than segment
-	Endopod of right leg 5 of male 3.6 times longer than wide, bearing two sigmoid spines; distal segment of endopod of leg 5 of
	female 2.6 times longer than wide; terminal spine on exopod of female leg 5 longer than segment, inner distal spine just shorter
	than segment

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