

Two new species of *Pupulina* van Beneden, 1892 (Copepoda: Siphonostomatoida: Caligidae) from mobulid rays off South Africa

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Abstract The caligid genus *Pupulina* van Beneden, 1892 currently has three accepted species. Two new species, *Pupulina cliffi* n. sp. and *P. merira* n. sp., are described from *Mobula kuhlii* (Müller & Henle) and *M. eregoodootenkee* (Bleeker) (Mobulidae) caught along the east coast of South Africa. *Pupulina cliffi* can be distinguished from all the other species by the absence of posterolateral processes on the genital complex, whereas *P. merira* has very short, rounded posterolateral processes on the genital complex compared to the three previously known species. Additionally, *P. merira* is the only species with the abdomen only about two-thirds the length of the genital complex and the caudal rami about the same length as the abdomen. A dichotomous key to distinguish the five species of *Pupulina* is provided.

Introduction

Pupulina van Beneden, 1892 currently consists of three accepted species (Walter & Boxshall, 2008): *P. flores* van Beneden, 1892; *P. minor* M. S. Wilson, 1952; and *P. brevicauda* M. S. Wilson, 1952. *Pupulina flores* was reported from *Manta* spp. (Wilson, 1935, 1952) and *Mobula* sp. (Wilson, 1952), whereas

P. minor and *P. brevicauda* were both reported from *Mobula* spp. (Wilson, 1952; Dojiri & Ho, 2013; Pillai, 1985). *Pupulina* females can be distinguished from the other caligid copepods by the presence of posterolateral processes on the genital complex, the presence of processes posteromedial to the dentiform projection of the maxillule and immediately posterior to the maxilliped, the absence of a sternal furca, the well-developed endopod of leg 1, the presence of a lateral expansion (velum) on the first and second endopodal segments of legs 2 and 3, and the distinctly 3-segmented rami of leg 3 (Wilson, 1952; Dojiri & Ho, 2013; Pillai, 1985). The present report describes two new species of *Pupulina* collected from mobulid rays captured off the east coast of South Africa.

Materials and methods

Copepods were collected from the gill rakers and filaments of mobulid rays caught in the shark nets routinely set off the coast of South Africa by the KwaZulu-Natal Sharks Board (KZNSB), and host identification was undertaken by KZNSB staff. Collected copepods were fixed and preserved in 70% ethanol and studied using the wooden slide technique (Humes & Gooding, 1964) after being cleared in lactic acid with a small amount of dissolved lignin pink. Measurements were made using an ocular micrometer and drawings made with the aid of a drawing tube. One adult female was prepared for scanning electron

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microscopy by dehydrating through a series of ethanol concentrations (70, 80, 90, 100 and 100% for one hour each) followed by immersion in hexamethyldisilazane (1 day). Before sputter-coating (gold-palladium), critical point drying was achieved by placing the specimen under a slight vacuum to remove the remainder of the hexamethyldisilazane. The anatomical terminology used conforms to Kabata (1979), Boxshall (1990) and Huys & Boxshall (1991).

Pupulina cliffi n. sp.

Type-host: *Mobula kuhlii* (Müller & Henle) (Mobulidae).

Additional host: *Mobula eregoodootenkee* (Bleeker).

Type-locality: Indian Ocean, off Umdloti (29°40'S, 31°08'E), KwaZulu-Natal, South Africa.

Material examined: From *M. kuhlii* caught during April 2004 (1♀), December 2010 (1♀) and January 2011 (2♀♀ and 7♀♀, respectively); off Durban (29°07'S, 31°38'E) during April 2004 (11♀♀), December 2009 (2♀♀), November 2010 (1♀) and December 2010 (3♀♀); off Winklespruit (30°06'S, 30°51'E) during December 2010 (3♀♀); off Karridene (30°07'S, 30°51'E) during January 2011 (4♀♀ and 6♀♀, respectively); off Park Rynie (30°19'S, 30°44'E) during January 2000 (1♀) and 2011 (4♀♀); off Hibberdene (30°34'S, 30°34'E) during April 2009 (6♀♀); off Umzumbe (30°32'S, 30°37'E) during January 2011 (3♀♀). From *M. eregoodootenkee* caught off Richards Bay (28°48'S, 32°06'E) during September 2004 (8♀♀ and 1♀, respectively).

Type-material: Holotype female (SAMC A45920) and 3 paratypes (SAMC A45921–A45923) deposited in the Iziko South African Museum, Cape Town, South Africa.

Etymology: The species epithet is for Mr Jeremy Cliff of the KwaZulu-Natal Sharks Board (KZNSB), who always accommodated us during many collecting trips to the facilities of the KZNSB.

Description (Figs. 1–3)

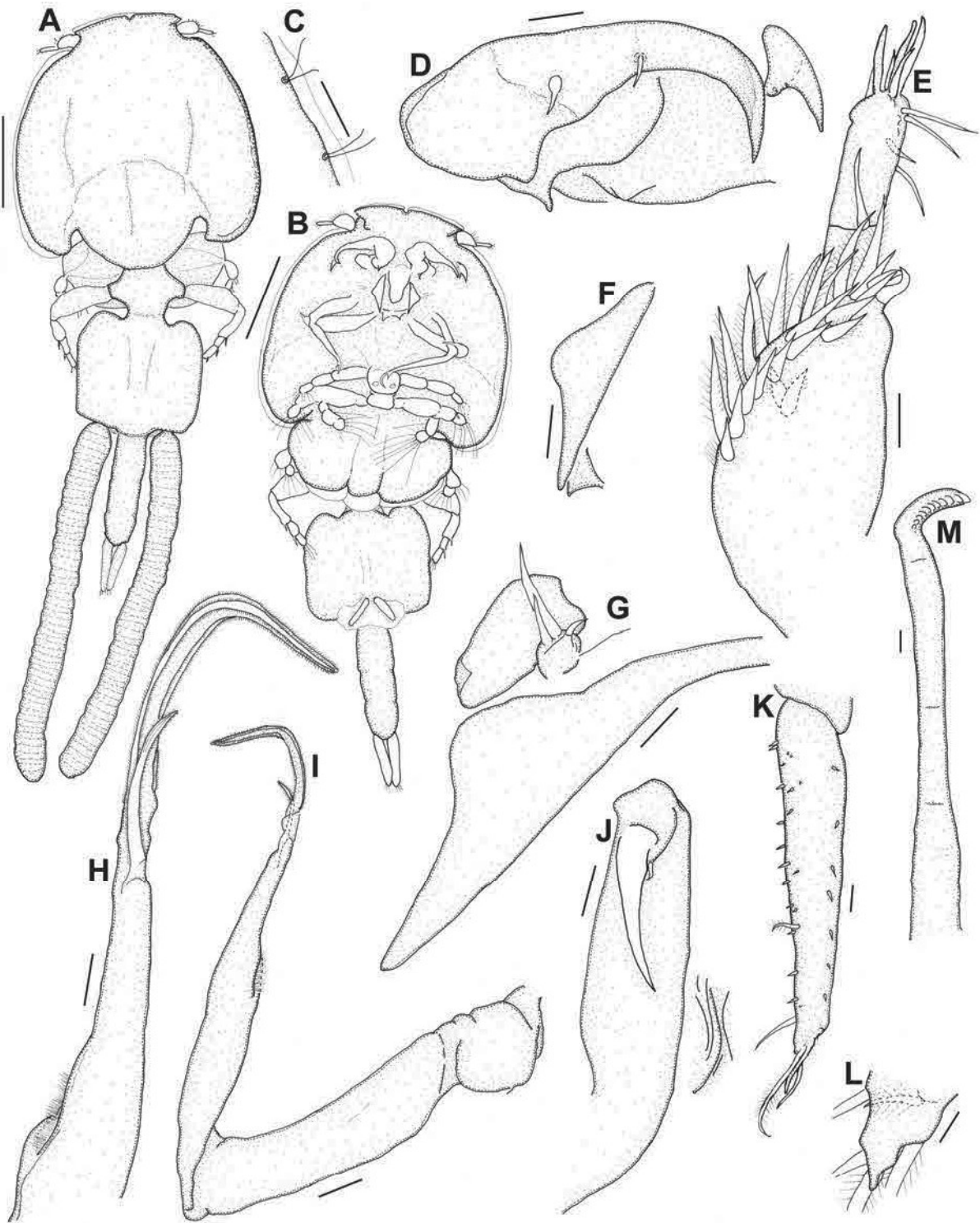
Adult female

Overall length (from anterior margin of frontal plate to tip of caudal rami), without egg sacs, c.6.5 mm. Cephalothorax composed of cephalosome and first 3 thoracic somites (Fig. 1A,B). Carapace

Fig. 1 *Pupulina cliffi* n. sp., adult female. A. habitus, dorsal view; B. same, ventral view; C. lateral border of cephalothorax, dorsal view; D. antenna and postantennal process; E. antennule; F. dentiform process of maxillule and postoral process; G. maxillule with lateral sclerotised plate; H. distal half of maxilla; I. maxilla; J. maxilliped and post-maxillipedal process; K. caudal ramus; L. tip of caudal ramus; M. mandible. *Scale-bars*: A,B, 1 mm; C,E,G,H,K,L, 50 µm; D,F,I,J, 100 µm; M, 10 µm

almost circular, slightly longer than wide, with obvious paired frontal plates (without lunules) and shallow posterior sinuses; posterior margin convex (Fig. 1A). Anterior and lateral margins, as well as posterior sinuses of carapace, rimmed with transparent, striated membrane (Fig. 1A). Pairs of setules present at regular intervals along lateral margins (Fig. 1C). Antennules visible posterolaterally to frontal plate. Fourth free thoracic somite about twice as wide as long. Genital complex (Fig. 1A) about half length and width of cephalothorax, subquadrate with anterolateral corners slightly protruded and without posterolateral processes; ventral surface with irregular pattern of small spinules and vestigial leg 5 posterolaterally. Spermatophores elongate, attached posteromedially on genital complex (Fig. 1B). Abdomen (Fig. 1A,B) indistinctly 3-segmented, about same length and 1/3 width of genital complex. Caudal ramus (Fig. 1K,L) slender, about 1/2 length of abdomen, covered with spinules, slightly protruded at apex; armed with 1 pinnate seta laterally about 2/3 from base, 1 naked seta distolaterally, 1 naked and 2 pinnate setae terminally and 1 small pinnate seta distomedially. Egg sacs (Fig. 1A) uniseriate, each approximately 4 mm long.

Antennule (Fig. 1E) 3-segmented. First segment armed with at least 25, mostly pinnate, setae around anterodistal margin; second segment with 1 naked seta distally; apical segment with 2 naked setae along length of the segment and at least 6 naked setae and 2 aesthetascs around apex. Antenna (Figs. 1D,2A) 4-segmented, piercing. First segment unarmed; second segment with short, posteriorly directed spine-like process; third segment unarmed; subchela claw-like, ornamented with membranous flap near terminal hook, and armed with seta in basal region and 2 slender, naked setae laterally at mid-length (Figs. 1D,2A). Postantennal process (Fig. 1D) with posteriorly directed tine and basal sclerites with branched setules. Mouth tube with intrabuccal stylet and strigil; mandible (Fig. 1M) bears 11 apical teeth. Maxillule (Figs. 1G,2B) consists of palp



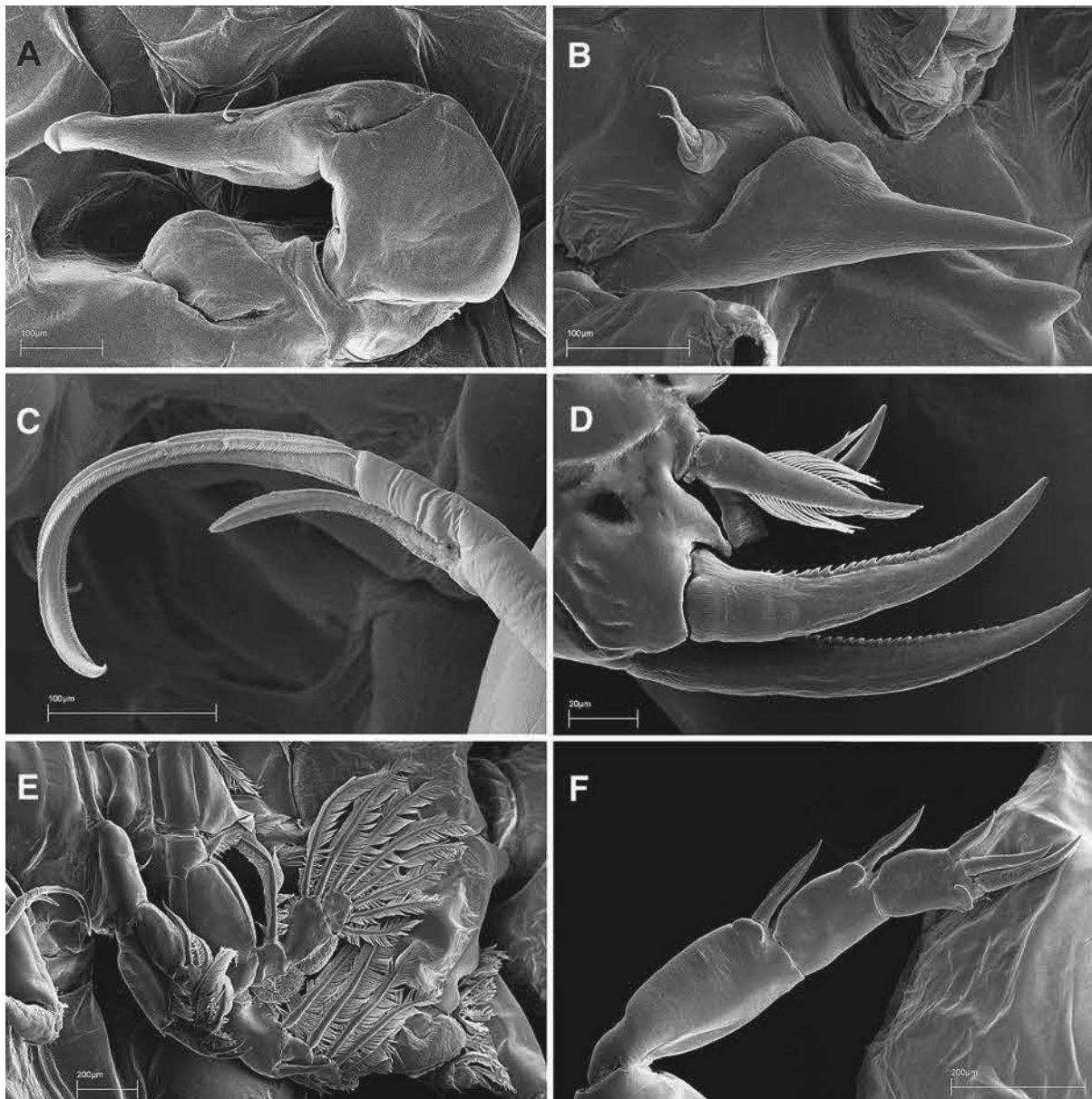


Fig. 2 *Pupulina cliffi* n. sp., adult female. A. antenna; B. maxillule and postoral process; C. tip of maxilla; D. apical armature on second exopod segment of leg 1; E. leg 2. F. leg 4 exopod

with 1 long and 2 shorter naked setae and large, subtriangular dentiform process (praecoxal endite). Sclerotised plate lateral to base of palp with rounded distal margin almost reaching middle of bulging area of dentiform process. Cuticular spine-like process (postoral process) (Figs. 1F,2B) present medial to tip of dentiform process. Maxilla (Figs. 1H,I) brachiform; syncoxa (lacertus) broader than basis (brachium); basis with flabellum at about mid-length and distally with

calamus and canna (both rimmed with serrate membranes) (Fig. 2C). Maxilliped (Fig. 1J) with fairly slender, unarmed corpus; subchela (claw), slender and relatively straight, with small naked seta near base. Post-maxillipedal process (Fig. 1J) present, consisting of raised, crescentic sclerite.

Leg 1 (Fig. 3A) biramous; sympod with small outer and inner pinnate setae. Exopod 2-segmented; first segment with fringe of setules on inner margin and

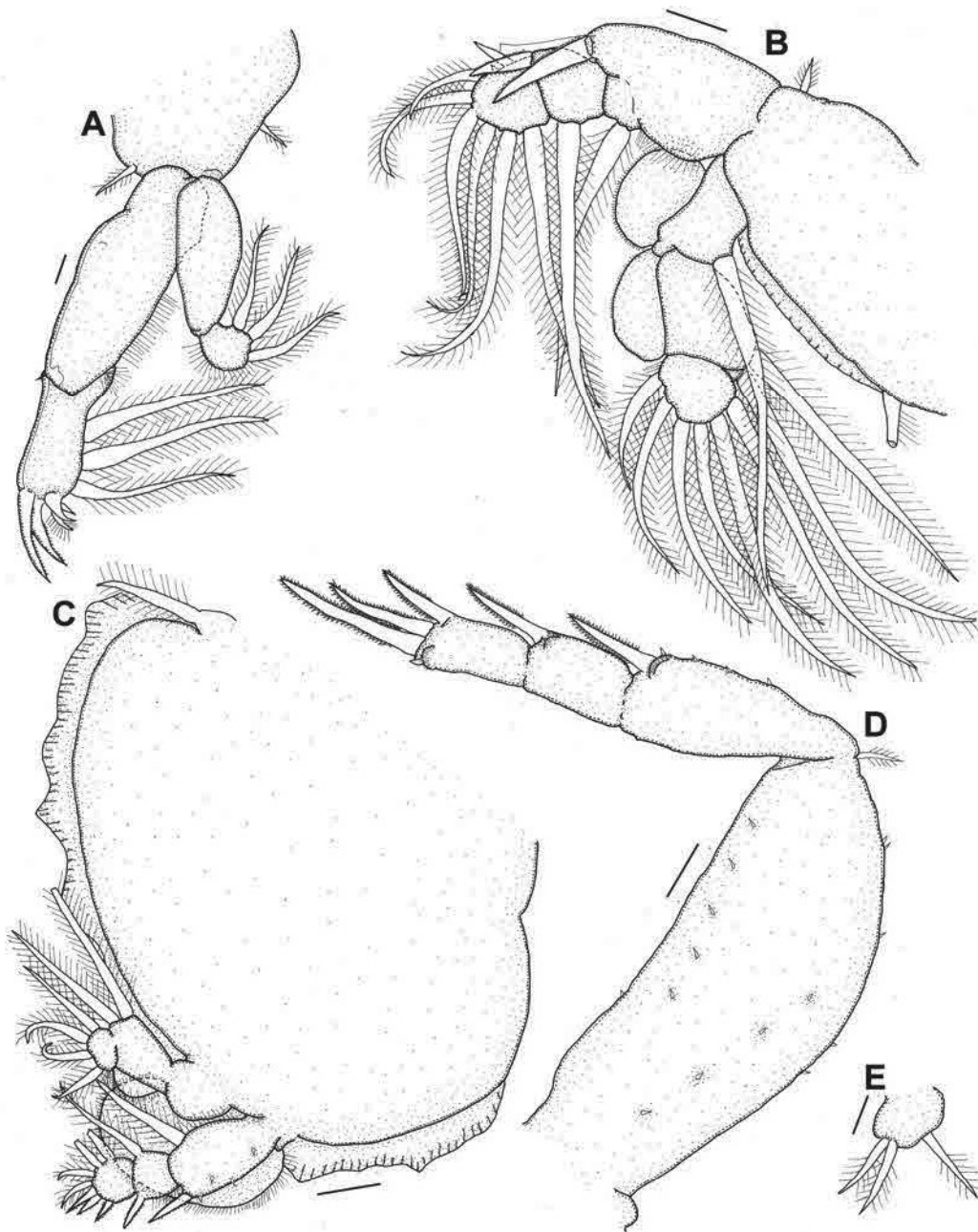


Fig. 3 *Pupulina cliffi* n. sp., adult female. A. leg 1; B. leg 2; C. leg 3; D. leg 4; E. leg 5. Scale-bars: A,E, 50 μ m; B,C,D, 100 μ m

short, naked seta distolaterally; second segment with patch of setules proximally on medial surface, 3 pinnate setae along medial margin and 2 large spines with serrate margins, 1 smaller spine with accessory setiform process and short pinnate seta terminally (Fig. 2D). Endopod 2-segmented, about same length as first exopod segment; first segment unarmed;

second segment with 3 inner pinnate setae and setules around lateral and distolateral margins. Leg 2 (Figs. 2E,3B) biramous; sympod with short outer pinnate seta and long, inner pinnate seta and fringed with membrane along inner margin. Exopod 3-segmented; first segment with fringe of setules on medial margin, long, pinnate seta distomedially and

long, stout, serrate spine distolaterally; second segment bears shorter, serrate spine distolaterally and long, medial pinnate seta; third segment with 6 pinnate setae decreasing in length toward outer margin and 1 minute spine followed by 1 longer, naked spine on lateral margin. Endopod 3-segmented; first segment with long pinnate seta distomedially and velum fringed with short setules; second segment with 2 long pinnate setae distomedially and velum fringed with short setules; third segment bearing 6 long pinnate setae decreasing in length toward outer margin and few setules proximolaterally. Leg 3 (Fig. 3C) biramous; sympod with large, pinnate seta medially and fringed with membrane along margins. Exopod 3-segmented; first segment with distolateral serrate spine and distomedial pinnate seta, setules along medial margin and few spinules scattered on surface; second segment with distolateral serrate spine, distomedial pinnate seta and setules along lateral margin; third segment with 3 distolateral serrate spines and 4 (shorter than leg 2) pinnate setae decreasing in length toward outer margin. Endopod 3-segmented; first segment with very large velum, covering first 2 exopod segments and most of velum on second endopod segment, and fringed with short setules; second segment with 2 long pinnate setae distomedially and small velum fringed with short setules; third segment bearing 4 short pinnate setae decreasing in length toward outer margin and few setules proximolaterally. Leg 4 (Fig. 3D) uniramous; sympod with spinules scattered on surface and pinnate seta distolaterally. Exopod (Figs. 2F,3D) 3-segmented; first segment with distolateral spinulate spine, pectinate membrane at base and spinules scattered along lateral margin; second segment with distolateral spinulated spine and pectinate membrane at base; third segment with 1 distolateral and 2 terminal spinulated spines and pectinate membrane at base of small, naked distomedial seta. Leg 5 (Fig. 3E) located posterolaterally on ventral surface of genital complex, vestigial, represented by 3 small pinnate setae on small papilliform process.

Pupulina merira n. sp.

Type-host: *Mobula kuhlii* (Valenciennes, in Müller & Henle) (Mobulidae).

Additional host: *Mobula eregoodootenkee* (Bleeker).

Type-locality: Indian Ocean, off Karridene (30.07°S, 30.51°E), KwaZulu-Natal, South Africa.

Fig. 4 *Pupulina merira* n. sp., adult female. A. habitus, dorsal view; B. antenna and postantennal process; C. maxillule with lateral sclerotised plate and postoral process; D. posterolateral border of cephalothorax, dorsal view; E. posterior margin of genital complex with spermatophores attached, ventral view; F. maxilliped and post-maxillipedal process; G. leg 4; H. leg 3 exopod; I. tip of caudal ramus; J. caudal ramus. Scale-bars: A,E, 0.5 mm; B,C,F,G,H,I,J, 50 µm; D, 100 µm

Material examined: From *M. kuhlii* caught during January 2011 (2♀♀). From *M. eregoodootenkee* caught off Richards Bay during September 2004 (1♀).

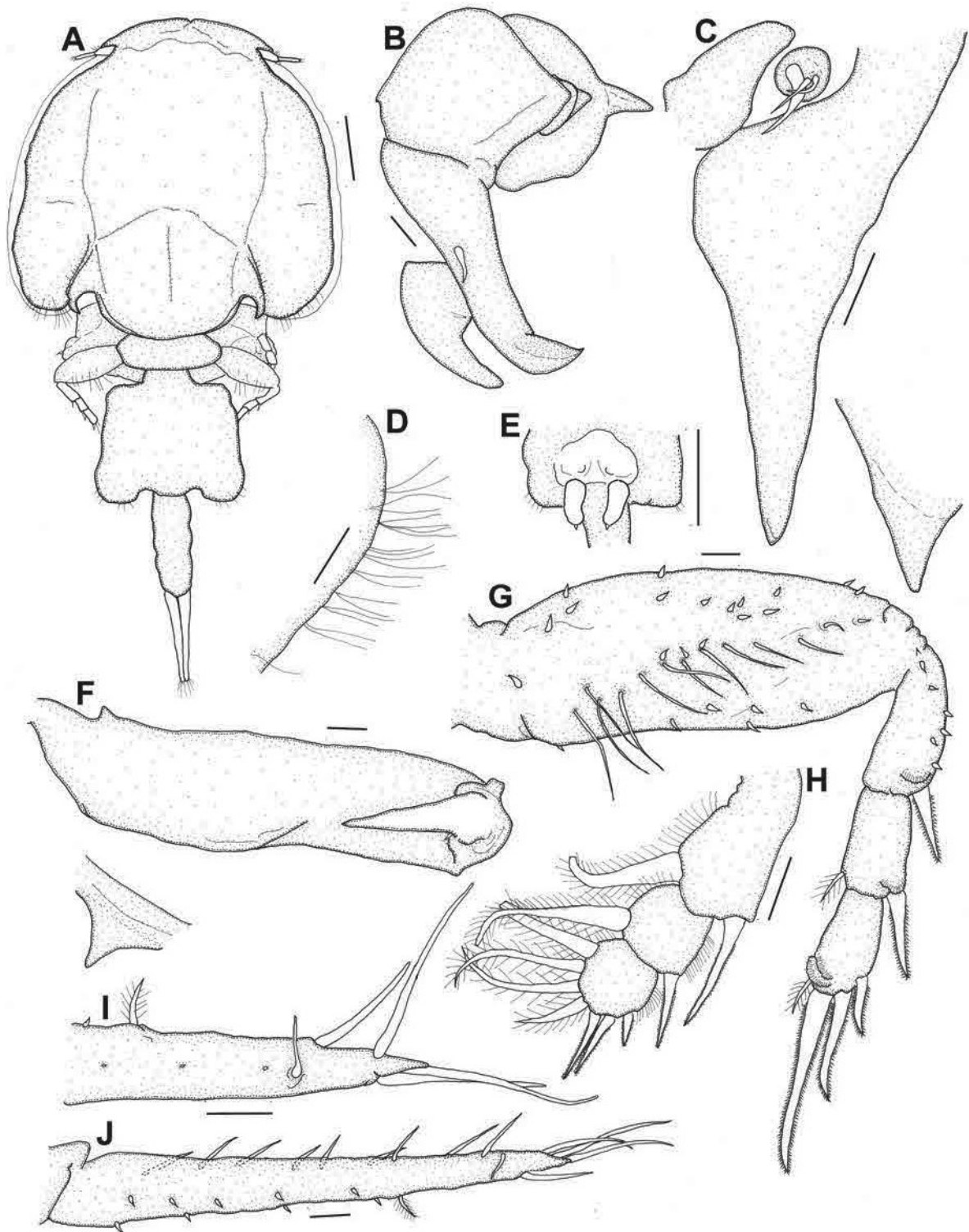
Type-material: Holotype female (SAMC A45924) and 1 paratype (SAMC A45925) deposited in the Iziko South African Museum, Cape Town, South Africa.

Etymology: The species epithet is a latinised version of *meriri* (= a term used in Sepedi for hair), referring to the setules occurring on the posterolateral corners of the cephalothorax and genital complex as well as on the sympod of leg 4 and medial margins of the caudal rami. It is treated as a noun.

Description (Fig. 4)

Adult female

Overall length *c.*5.3 mm. Carapace almost circular, with convex posterior margin, but less protruded than in previous species. Anterior and lateral margins, as well as posterior sinuses of carapace, rimmed with transparent, striated membrane (Fig. 4A). Clusters of long setules present at regular intervals along lateral borders. Very long setules present dorsally on posterolateral corners of cephalothorax (Fig. 4D) and posterolateral corners of genital complex (Fig. 4A). Antennules visible posterolateral to frontal plate. Fourth free pedigerous somite short, *c.*3 times as wide as long. Genital complex (Fig. 4A) less than half length and width of cephalothorax, squarish, with posterolateral corners only slightly protruded, not forming obvious processes; ventral surface with small spinules and vestigial fifth legs posterolaterally. Spermatophores bean-shaped, attached posteromedially on genital complex (Fig. 4E). Abdomen (Fig. 4A) indistinctly 3-segmented, shorter than genital complex and less than 1/3 its width. Caudal rami (Fig. 4A,J) long, slender, about same length as abdomen, ornamented with short spinules and with long setules along medial margin; distal margin (Fig. 4I) slightly extended into pointed process, armed with 1 pinnate seta laterally *c.*2/3 distance from base, 1 naked seta



distolaterally, 3 naked setae terminally and 1 small naked seta distomedially.

Appendages similar to those of *P. cliffi* n. sp., except for the following. Antenna (Fig. 4B) with more acuminate posteriorly-directed, spine-like process on coxa. Postantennal process (Fig. 4B) with wider tine. Sclerotised plate (Fig. 4C) lateral to maxillary palp smaller than that of *P. cliffi*. Post-maxillipedal process (Fig. 4F) consisting of triangular cuticular spine (cf. Dojiri & Ho, 2013). Leg 3 (Fig. 4H) without spinules on first exopodal segment and outer spine longer than that of *P. cliffi*; second exopodal segment with additional setules along medial margin and outer spine slightly longer than that of *P. cliffi*; third exopodal segment with setules along lateral and medial margins. Dorsal surface of leg 4 (Fig. 4G) sympod with spinules and longitudinal row of long setules decreasing in length from proximal to distal part of segment and distolateral seta not observed; second exopodal segment with additional small pinnate seta distomedially; distomedial seta on third segment pinnate with double pectinate membrane at base, longer than that of *P. cliffi*.

Discussion

According to Wilson (1952) and Dojiri & Ho (2013), the adult females of the known *Pupulina* species, i.e. *P. flores*, *P. minor* and *P. brevicauda*, can be distinguished from the other caligids by, amongst other things, the presence of posterolateral processes on the genital complex. However, *P. cliffi* n. sp. differs from all the other species by the absence of these processes on the genital complex (Fig. 1A,B), and thus the presence of posterolateral processes on the genital complex is not a distinguishing feature of *Pupulina* spp. Additionally, *P. cliffi* differs from all the other species by having reduced post-maxillipedal processes (see Fig. 1J) consisting only of raised, crescentic sclerites.

Pupulina merira n. sp. can be distinguished from all the other species by: having very short, rounded posterolateral processes on the genital complex (Fig. 4A); possessing an abdomen only about two-thirds the length of the genital complex and caudal rami about the same length as the abdomen; and leg 4 (Fig. 4G) having the transverse row of long setules dorsally on the sympod (that of *P. flores* has only five long setules (Dojiri & Ho, 2013)), a double pectinate

membrane at the base of the small distomedial seta on the third segment (there is only a single one in the other species), and pinnate distomedial setae on the second and third segments (there is a naked seta/spine when present in other species).

Pupulina brevicauda is the only species with: very long posterolateral processes extending beyond the caudal rami (Wilson, 1952); an abdomen that is longer than the genital complex, but with caudal rami that are only about a quarter of the length of the abdomen (see Wilson, 1952, plate 14, fig. 1); and the two distolateral spines of the last exopodal segment of leg 2 of about the same size (first slightly shorter than second) and the second spine is “setiform and sparsely plumose” (see Wilson, 1952, plate 15, fig. 12), whereas the first is longer than the second and are naked in both *P. flores* and *P. minor* (see Wilson, 1952, plate 12, fig. 18 and plate 13, fig. 13; Dojiri & Ho, 2013, fig. 38a). Additionally, the last exopodal segment of leg 3 has an additional seta (see Wilson, 1952, plate 15, fig. 16) and leg 4 has additional small spines distomedially on both first and second segments and “narrow marginal laminae” laterally on the second and third segments (Wilson, 1952).

Pupulina flores can be distinguished from the other species of the genus by its unique, long, sharply pointed, sclerotised plates, accompanying the maxillule, which reach beyond the bulging area of the praecoxal endite (Wilson, 1952, plate 12, fig. 8; Dojiri & Ho, 2013, fig. 35e), and the post-maxillipedal processes that are broad, stout, chitinous spines, triangular in shape, with a sharp posteriorly pointed tip (Wilson, 1952, plate 12, fig. 11; Dojiri & Ho, 2013, fig. 36c).

Pupulina minor has an abdomen that is almost the same length as the genital complex, but the caudal rami are longer than the abdomen (Wilson, 1952), and leg 4 has an additional small spine distomedially on the second segment (Wilson, 1952, plate 13, fig. 18).

Identification key to adult females of *Pupulina* spp.

1. Distolateral processes on genital complex absent.....*P. cliffi* n. sp.
- Distolateral processes on genital complex present.....2
2. Distolateral processes on genital complex long, extending beyond caudal rami.....*P. brevicauda*

- Distolateral processes on genital complex not extending beyond caudal rami.....3
- 3. Distolateral processes on genital complex very short, rounded.....*P. merira* n. sp.
- Distolateral processes on genital complex longer, reaching around mid-length of genital complex.....4
- 4. Distolateral processes with rounded tips; genital complex with a squarish shape and posterior border almost straight until abrupt change into distolateral processes; abdomen almost same length as genital complex; caudal rami longer than abdomen; sclerotised plate lateral to maxillulary palp, small, not extending to bulging area of praecoxal endite with posteriorly rounded protrusion.....*P. minor*
- Distolateral processes with pointed tips; genital complex with more rounded shape and posterior border gradually extending into distolateral processes; abdomen longer than genital complex; sclerotised plate lateral to maxillulary palp, long, sharply pointed, reaching beyond bulging area of praecoxal endite.....*P. flores*

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