

Two new species of the genus *Bryocyclops* Kiefer, 1927 (Copepoda: Cyclopoida: Cyclopidae) from southern Thailand

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Abstract. *Bryocyclops muscicoloides*, new species, and *B. trangensis*, new species, are described from caves in southern Thailand. They have a morphologic resemblance to *B. muscicola* (Menzel, 1926), a member of Group II sensu Lindberg (1954) in their armature formulae of Exp-2 P1–P4 (3.3.3.3 and 5.5.5.4 for spines and setae, respectively), intercoxal plates of P1–P4 acute, Enp P4 of female and male with one- and two-segmented, respectively, and urosomites and anal operculum serrated. However, *B. muscicoloides*, new species, is clearly distinguishable by the absence of inner coxal seta on P1, the absence of blunt setae on Exp-2 P2–P3, the shape of female Enp P4 and the unique transformed spine on the male Enp-2 P3 (the outstretched part tapers distally, and is located above the middle of the spine). *Bryocyclops trangensis*, new species, is characterised by the armature on Enp-2 P2 and Exp-2 P2–P3, the presence of spiniform processes on the male Enp-1 P4 and the transformed spine on the male Enp-2 P3 (the outstretched part is less-produced, and is located near the tip of the spine).

Key words. taxonomy, cave, epikarst, stygobiont, Chumphon, Nakhon Sri Thammarat

INTRODUCTION

The genus *Bryocyclops* Kiefer, 1927 currently contains 22 valid species including the two new species, *B. muscicoloides*, new species, and *B. trangensis*, new species. This number excludes *B. feei* Shen, 1956, which was originally placed in *Bryocamptus* Chappuis, 1929, but now repositioned in *Cletocamptus* Schmankevitch, 1875; *B. arenicolous* (Fryer, 1956), the fifth leg of which was accurately re-described, and assigned back to the original genus *Metacyclops* Kiefer, 1927; *B. correctus* Kiefer, 1960, which is now regarded as a junior synonym of *Haplocyclops pauliani* Kiefer, 1955. *Bryocyclops soqotraensis* Mirabdullayev, Van Damme & Dumont, 2002 was recently re-examined and reallocated to the genus *Thalamocyclops* Fiers & Van Damme, 2017. Fiers (2002) considered that *Haplocyclops parvulus* Kiefer, 1928 should be allocated to the genus *Bryocyclops* (typical in its setation on the fifth leg, the ratio of length: width on the genital double-somite and the longitudinal keel on the caudal ramus), but since the original description is inadequate, it is remained as a species inquirendae (Rocha et al., 1998; Shen, 1956; Fiers, 2002, 2012; Gómez, 2005; Dussart & Defaye, 2006; Watiroyram et al., 2012, 2015a; Fiers & Van Damme, 2017). This genus is distributed in peculiar habitats in inland waters such as leaf-litter, wet mosses, phytotelmata, interstitial habitats and groundwater (wells, springs, and caves) in Eurasia and Africa, with the

exception of *B. campaneri* Rocha & Bjornberg, 1987 (from Brazil), *B. caroli* Bjornberg, 1985 (from Brazil and Puerto Rico), and *B. muscicola* (Menzel, 1926) (from Florida, USA) (Bjornberg, 1985; Reid, 1999; Watiroyram et al., 2012; Jocque et al., 2013). However, *B. campaneri* and *B. caroli* restrictively dwelled in America, while *B. muscicola* was presumably introduced from Asia via the ornamental plant trade (Reid & Hribar, 2006).

Members of the genus *Bryocyclops* are found prominently in tropical zones, and 65% of records for valid species are documented in Southeast Asia (SEA) (eight species) and Africa (seven species) approximately. To date, species reported from SEA are in Indonesia (*B. anninae* (Menzel, 1926), *B. bogoriensis* (Menzel, 1926), *B. chappuisi* Kiefer, 1928, *B. muscicola*), Malaysia (*B. cf. anninae*), Vietnam (one unidentified species), and Thailand (*B. maewaensis* Watiroyram, Brancelj & Sanoamuang, 2012, *B. maholarnensis* Watiroyram, Brancelj & Sanoamuang, 2015, *B. muscicoloides*, new species, and *B. trangensis*, new species), and only species in Vietnam and Thailand were found in subterranean dwellings (i.e. cavernous species) (Watiroyram et al., 2012, 2015a; Alekseev et al., 2013, 2016; Tran et al., 2015).

The knowledge on this genus in Thailand was gleaned from studying cave-dwelling Copepoda, which is greatly recognised along with the genus *Elaphoidella* Chappuis, 1929, compared to other genera (Brancelj et al., 2010; Watiroyram et al., 2015b, 2017; Watiroyram & Brancelj, 2016). The number of described copepods in Thailand increased in 2010 and 2017 when new samples were investigated, especially in non-studied areas in South Thailand. *Bryocyclops muscicoloides*, new species, and *B.*

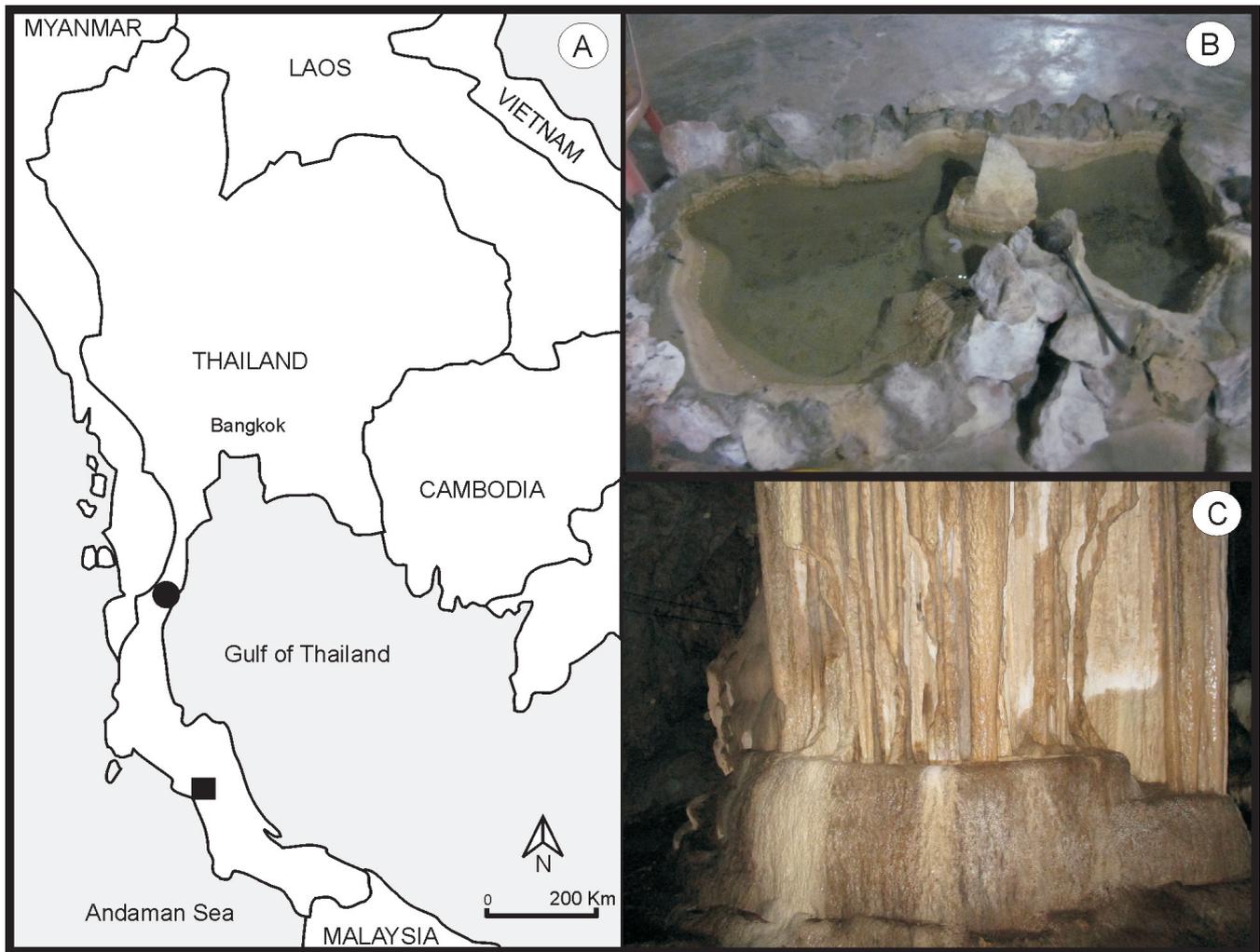


Fig. 1. Sampling localities and habitats for new species in southern Thailand (A–C). A, sampling localities, black circle (●) = Khao Plu cave from Chumphon Province for *B. muscicoloides*, new species, and black square (■) = Khao Pina cave from Trang Province for *B. trangensis*, new species; B and C, habitat for *B. muscicoloides*, new species and *B. trangensis*, new species, respectively.

trangensis, new species, are two newly cavernous species of the genus *Bryocyclops* reported from Thailand.

MATERIALS AND METHODS

Samples were collected from the cave’s pools fed directly by dripping water from the ceiling (Fig. 1). Water from individual pools was filtered through plankton net having a mesh size of 60 µm. Samples were fixed and preserved by 70% ethanol in plastic bottles. Animals were sorted under a stereomicroscope, transferred into plastic vials and stored in 70% alcohol with a drop of glycerol. Animals were placed in a mixture of glycerol and 70% ethanol (ratio ~ 1:10 v/v), which was later replaced with pure glycerol. Animals were dissected at 100× magnification under an Olympus SZ51 stereomicroscope. Undissected and dissected animals were taxonomically examined at a magnification of 1000× under

an Olympus CX31 compound microscope. Morphology and ornamentation of adult males and females were observed by a scanning electron microscope (SEM, FEI Helios NanoLab G3 CX). Drawings were made at the same magnification with the aid of a drawing tube (Olympus U-DA) mounted on an Olympus compound microscope. The final version of the drawings was made using CorelDRAW 12. For permanent slides, all dissected specimens were mounted in glycerol and sealed with clear nail polish. Material was deposited at the Faculty of Science, Nakhon Phanom University, Thailand (NPU) and the Natural History Museum, London (NHMUK).

The following abbreviations are used throughout the text and figures: Enp = endopod; Exp = exopod; Exp/Enp-1 = exopod/endopod segment 1; Exp/Enp-2 = exopod/endopod segment 2; P1–P6 = first to sixth thoracic appendages. The nomenclature and descriptive terminology follows that of Huys & Boxshall (1991).

TAXONOMY

Cyclopoida Sars, 1886*Cyclopidae* Burmeister, 1834*Bryocyclops* Kiefer, 1927*Bryocyclops muscicoloides*, new species

(Figs. 2–7)

Type locality. Khao Plu cave in Wat [=temple] Tham Khao Plu located in Chumko Subdistrict, Pathiu District, Chumphon Province, southern Thailand (Fig. 1A, 1B). The coordinates of the entrance are 10°43'49.06"N, 99°19'12.52"E; altitude 27 m a.s.l. The cave is about 50 m long and situated on a small hill, with a single pool filled exclusively with dripping water in the dark zone. The type locality was made by a mixture of stone and cement in order to collect the dripping water for observance. The water temperature during sampling was 24.0°C, pH 7.8, and conductivity was 310 $\mu\text{S cm}^{-1}$.

Material examined. Holotype: an adult female was dissected and mounted on a slide in glycerol and sealed with nail polish, NHMUK 2017.503. Allotype: an adult male was dissected and mounted on a slide in glycerol and sealed with nail polish, NHMUK 2017.504. Paratypes: three undissected females (one female with egg sacs) and males were preserved by 70% ethanol in a 1.5 mL microtube, NHMUK 2017.505–510; three undissected females and males were preserved by 70% ethanol in a 1.5 mL microtube, NPU 2017.002. Additional materials: four undissected females and males were mounted on SEM Stub (NPU 2017.002S).

All specimens were collected from Khao Plu cave by author on 25 November 2016.

Diagnosis. Adult female: anal operculum is ovate, and serrated. P1–P4 show acute projection on distal margins, without inner coxal seta, P1 has an inner basal spine. P1–P3 have two-segmented Enp and Exp, P4 has one-segmented Enp and two-segmented Exp. Setal and spine formula are present on swimming legs: Exp-2 P1–P4 as 5.5.5.4 and 3.3.3.3, respectively; Enp-2 P1–P3 as 3.3.5 and 1.1.1, respectively. Enp P4 has four setae and one spine, without spiniform process on the segment.

Adult male: P1, P2, Exp P3–P4 are the same as in females. Enp-2 P3 has three pinnate and one smooth seta. The transformed spine has a well-produced, outstretched part tapering distally and located slightly above the middle of the spine. P4 Enp is two-segmented, and Enp-1 has one inner pinnate seta and one spiniform process on the inner distal margin; Enp-2 has three pinnate setae and one spiniform spine, without a spiniform process.

Description. Female (Figs. 2, 3), body length: 550–625 μm (mean: 610 μm ; $n = 10$), preserved specimens are colourless. Naupliar eye not discernible. Cephalosome wider than rest of body, with transverse scar indicating the fusion region of

cephalosome and pediger 1 (Figs. 2A, 3A, 3B). Cephalosome, pedigers 2–5, genital double-somite, urosomites and anal somite (incl. anal operculum) covered with fine refractile points. Posterior dorsal margins of cephalosome and pedigers 2–4 with smooth hyaline membrane, and with a serrated transverse row of dorsal hyaline frills above its posterior margin; pediger 5 with small serrated hyaline frill. Genital double-somite and urosomite 2–3 with irregularly serrate hyaline membrane on entire posterior margins; with a transverse row of small grooves on dorso-lateral surface (Figs. 2A, 3C, 3D). Genital double-somite symmetrical, about 1.5 times wide as long; with a pair of dorsal sclerotised; single copulatory pore situated ventrally near middle of segment. Anal somite (Figs. 2A–C, 3C) with strong spinules along entire posterior margin.

Anal operculum (Figs. 2C, 3C) ovate, coarsely serrated along its free margin, reaching beyond the middle of caudal ramus length, with a pair of small sensillum above the base.

Caudal rami (Figs. 2A, 3C) symmetrical sub-rectangular, about 1.3 times long as wide. Dorsal keel long, orientated nearly the entire caudal ramus length. Seta I absent, seta II–V pinnate. Lateral seta (II) shorter than the caudal ramus, inserted at about mid-length of caudal ramus. Outermost terminal seta (III) longer than dorsal seta, about 1.5 times as long as caudal ramus with strong spinules near its insertion on lateral and ventral views. Seta IV and V with fracture plane: outer terminal seta (IV) and inner terminal seta (V) about 4.0 and 6.0 times as long as caudal ramus, respectively. Innermost terminal seta (VI) thin and bare, shorter than 1/2 of caudal ramus. Dorsal seta (VII) articulated and bare, about 1.2 times as long as caudal ramus, inserted on distal end of keel with tiny spinules at insertion.

Rostrum (Fig. 4A) fused to cephalosome, free margin round, unornamented.

Antennule (Figs. 3A, 4B) short, reaching about 1/2 of cephalosome, 11-segmented. Setal formula on each segment as follows (A = aesthetasc): 6.2.5.2.0.2.3.1+A.2.2.7+A. All setae smooth.

Antenna (Fig. 4C) four-segmented, comprising coxobasis and three-segmented Enp. Coxobasis with bare seta on distal inner corner. Enp-1 with smooth seta on distal inner margin plus row of spinules on outer margin. Enp-2 with five smooth setae: three lateral and two apical setae. Enp-3 with seven smooth setae on distal end: one inner, four apical and two outer setae.

Mandible (Fig. 4D) short and robust, with five strongly chitinised teeth on gnathobase plus a smooth seta on innermost margin. Mandibular palp reduced, with one bare seta.

Maxillule (Fig. 4E) with praecoxa, coxobasis and one-segmented Enp. Praecoxal arthrite with four strong apical claws fused to arthrite base, with one strong spine, four short and smooth setae and one spinulate seta on frontal side.



Fig. 2. *Bryocyclops muscicoloides*, new species, female. A, habitus, dorsal view; B, genital double-somite and urosome, ventral view; C, pediger 5, genital double-somite and urosome, lateral view; D, P5. Scale bar = 100 μ m.

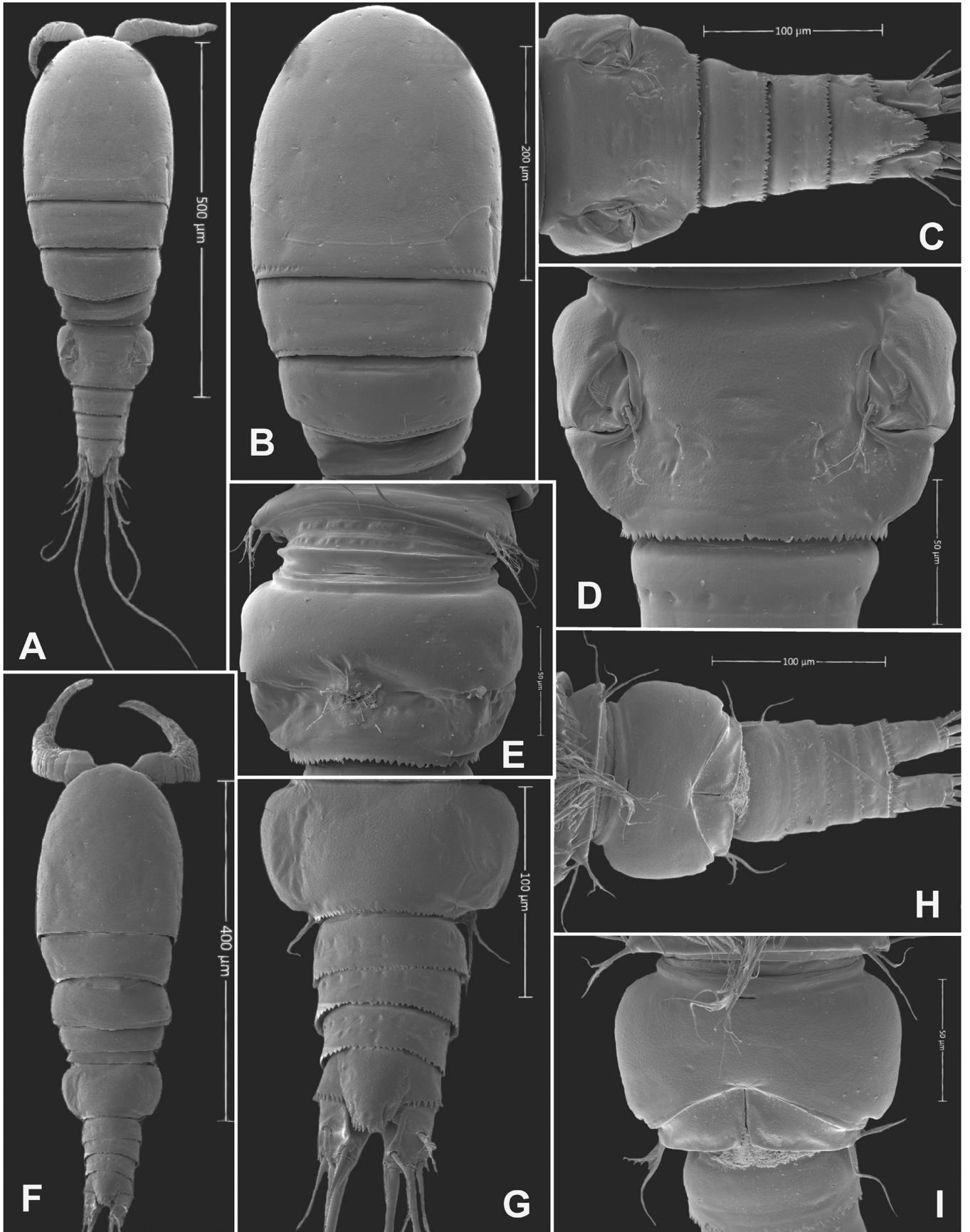


Fig. 3. *Bryocyclops muscicoloides*, new species, female (A–E) and male (F–I). A, habitus, dorsal view; B, prosome, dorsal view; C, urosome, dorsal view; D, genital double-somite and urosome 2, dorsal view; E, pediger 5 and genital double-somite, ventral view; F, habitus, dorsal view; G, genital somite and urosome, dorsal view; H, pediger 5, genital somite and urosome, ventral view; I, genital somite and urosome 2, ventral view.

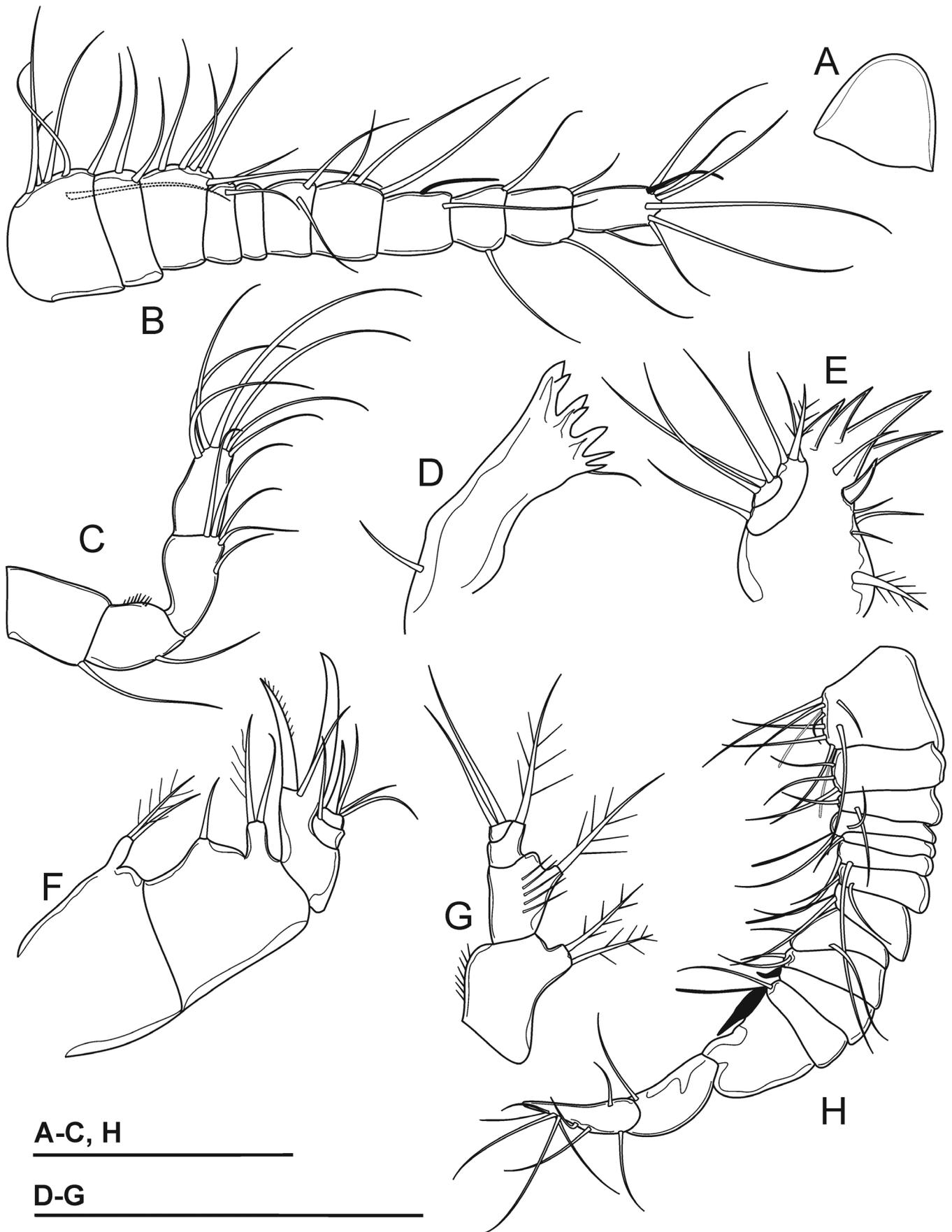


Fig. 4. *Bryocyclops muscicoloides*, new species, female (A–G) and male (H). A, rostrum; B, antennule; C, antenna; D, mandible; E, maxillule; F, maxilla; G, maxilliped; H, antennule. Scale bars = 100 μ m.

Coxobasis with one spiniform and two smooth setae. Exp reduced into one smooth seta. Enp with three smooth setae.

Maxilla (Fig. 4F) with praecoxa, coxa, basis, and two-segmented Enp. Praecoxal endite with two apical setae. Coxa with one seta at near middle of inner margin and two setae on distal endite. Basis with two claw-like expansions plus one seta at insertion. Enp-1 with one seta and Enp-2 with three thin setae and one robust seta.

Maxilliped (Fig. 4G) syncoxa and basis with two and one spiniform setae, respectively, each with a row of strong spinules on inner margin. Two-segmented Enp, Enp-1 with spiniform seta. Enp-2 reduced, with two smooth setae.

Armament of legs 1–4 as follows (legend: outer-inner seta/spine; outer-apical-inner; Arabic numerals indicate setae; Roman numerals indicate spines):

	Coxa	Basis	Exopod	Endopod
P1	0-0	1-I	I-0; III-2-3	0-1; 1-I+1-1
P2	0-0	1-0	I-0; III-2-3	0-1; 1-I+1-1
P3	0-0	1-0	I-0; III-2-3	0-1; 1-I+1-3
P4	0-0	1-0	I-0; III-2-2	1-I+1-2

P1 (Fig. 5A) intercoxal plate with acute projections on distal margin. Coxa without seta on inner corner. Basis with bare, slender outer seta and robust, stout inner spine with a cluster of spinules at spine insertion. Two-segmented Exp and Enp. Exp-1 smaller than Exp-2, with outer spiniform spine. Exp-2 twice as long as wide with three outer spiniform spines, two apical pinnate setae and three inner pinnate setae. Enp-1 with one inner pinnate seta with spiniform process at distal outer corner. Enp-2 with three subequal pinnate setae and one spiniform spine; one outer seta located between two spiniform processes, one apical spine and one apical seta, and one inner seta.

P2 (Fig. 5B) intercoxal plate, coxa, basis, and Exp similar to P1, but basis without spine on inner margin. Two-segmented Enp, and Enp-1 slightly smaller than Enp-2. Enp-1 with one inner pinnate seta and spiniform process at distal outer corner. Enp-2 with four subequal elements: one apical spiniform spine and three pinnate setae (outer seta located between two spiniform processes, apical seta and inner seta).

P3 (Fig. 5C) intercoxal plate, coxa, basis, Exp, and Enp-1 similar to P2. Enp-2 with one apical spine and five pinnate setae: shortest outer seta located between two spiniform processes, three inner setae about as long as spine and longest apical seta.

P4 (Fig. 5D) intercoxal plate with acute projections on distal margin. Coxa without inner seta. Basis with slender outer seta. Two-segmented Exp, Exp-1 with outer spiniform spine and Exp-2 with three outer spiniform spines, two apical pinnate setae and two inner pinnate setae. One-segmented Enp, with one spine and four pinnate setae; outer seta about

as long as two inner setae, apical one longest, about 3.0 times as long as spine.

P5 (Figs. 2C, 2D, 3E) completely fused to somite, with three pinnate setae: outermost seta longest, inserted on small prominence; middle and innermost seta directly inserted on somite, former seta shorter than latter one.

P6 (Figs. 2C, 3D) reduced to simple plate on genital double-somite laterodorsally, with two minute spinules and short pinnate seta.

Adult females each have a pair of egg sacs, each with three large eggs.

Male (Figs. 3F–I, 6) smaller than female. Body length, excluding caudal setae, 460–550 μm (mean: 530 μm ; $n = 10$). Body shape similar to female, except in genital segment and urosomal segmentation (i.e. the males have one more urosomite). Antenna and mouthparts (not figured), caudal rami (Fig. 6), P1 (Fig. 7A), P2 (Fig. 7B), P3–P4 exopods (Figs. 7C, 7D) and P5 (Fig. 6D) similar to those in female.

Anal operculum (Figs. 3G, 6B, 6D) slightly shorter than in female, about mid-length of caudal ramus.

Antennule (Fig. 4H) 15-segmented, geniculate. Setal formula as follows (A = aesthetasc, S = spine): 8+2A.3.2.2+A.1.2.2.1+A.2.2.S.1+A.S.1.8+A. All setae smooth.

P3 (Fig. 7C) two-segmented Enp, proximal segment smaller than distal one. Enp-1 with inner pinnate seta and spiniform process on distal outer margin. Enp-2 with small outer pinnate seta between two spiniform processes, apical transformed spine and apical bare seta and two inner pinnate setae. Transformed spine with outstretched part tapering distally and well-produced, located slightly above the middle of spine; with several strong spinules on its outstretched part.

P4 (Fig. 7D) two-segmented Enp. Enp-1 with inner pinnate seta and spiniform process on inner distal margin. Enp-2 with one outer pinnate seta, one apical spine and pinnate seta, and one inner pinnate seta.

P6 (Figs. 3I, 6C) reduced to simple plate, with two equal pinnate setae and one longer pinnate seta.

Variability. Not observed.

Etymology. The specific name *muscoloides*, formed with the Latin suffix “-oides” for resembling, refers to the similarity of the new species with *B. muscolola*. The species epitheton is a masculine singular adjective.

Remarks. *Bryocyclops muscoloides*, new species, fits to the generic characters as the P5 is completely fused to the fifth thoracic somite, with three setae, P2–4 have no inner coxal seta, the intercoxal plate of P4 has acute protuberances on the distal margin, and the male P3 Enp-2 has a transformed spine. The new species is similar to the members of Group

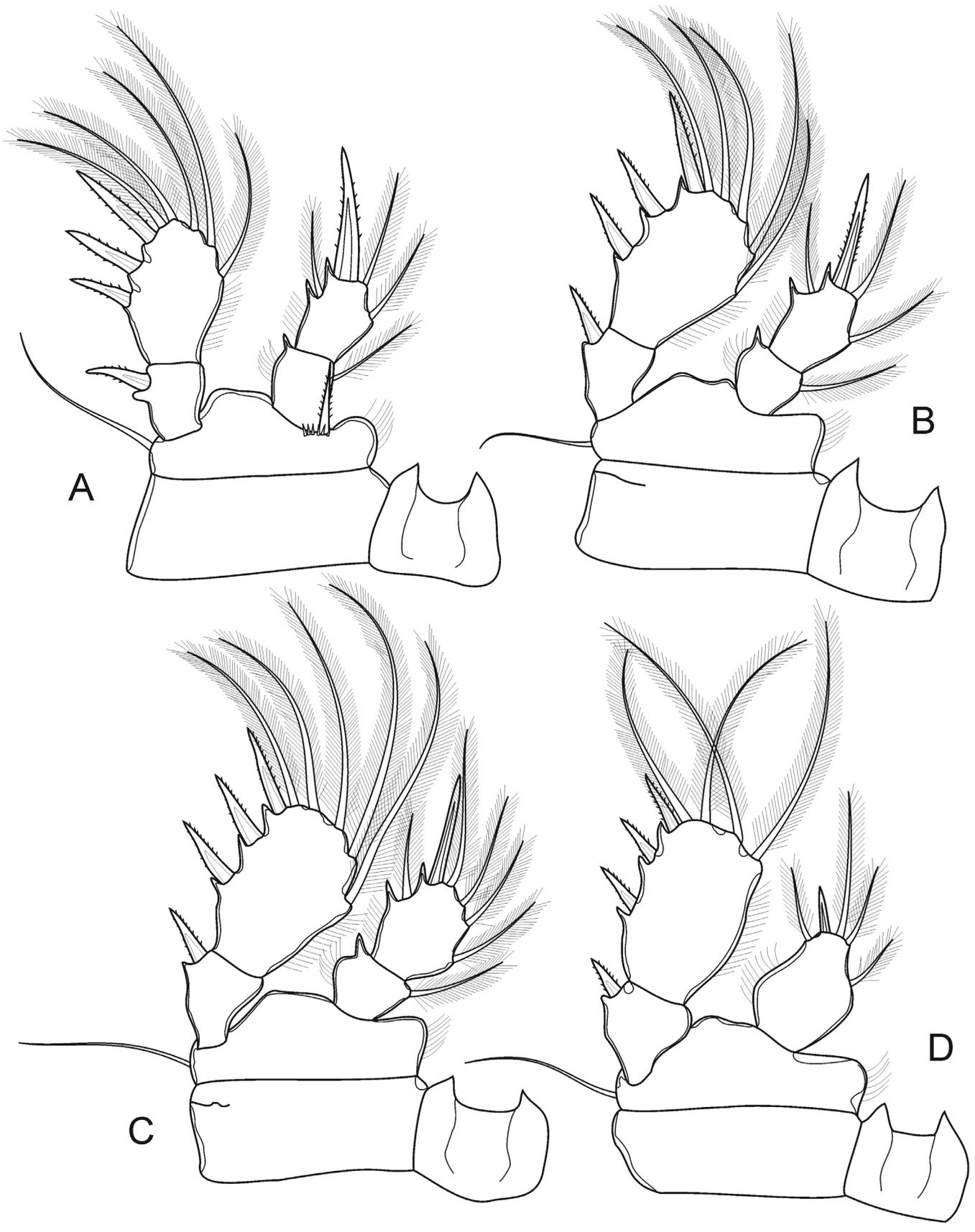


Fig. 5. *Bryocyclops muscicoloides*, new species, female. A, P1; B, P2; C, P3; D, P4. Scale bar = 100 μ m.

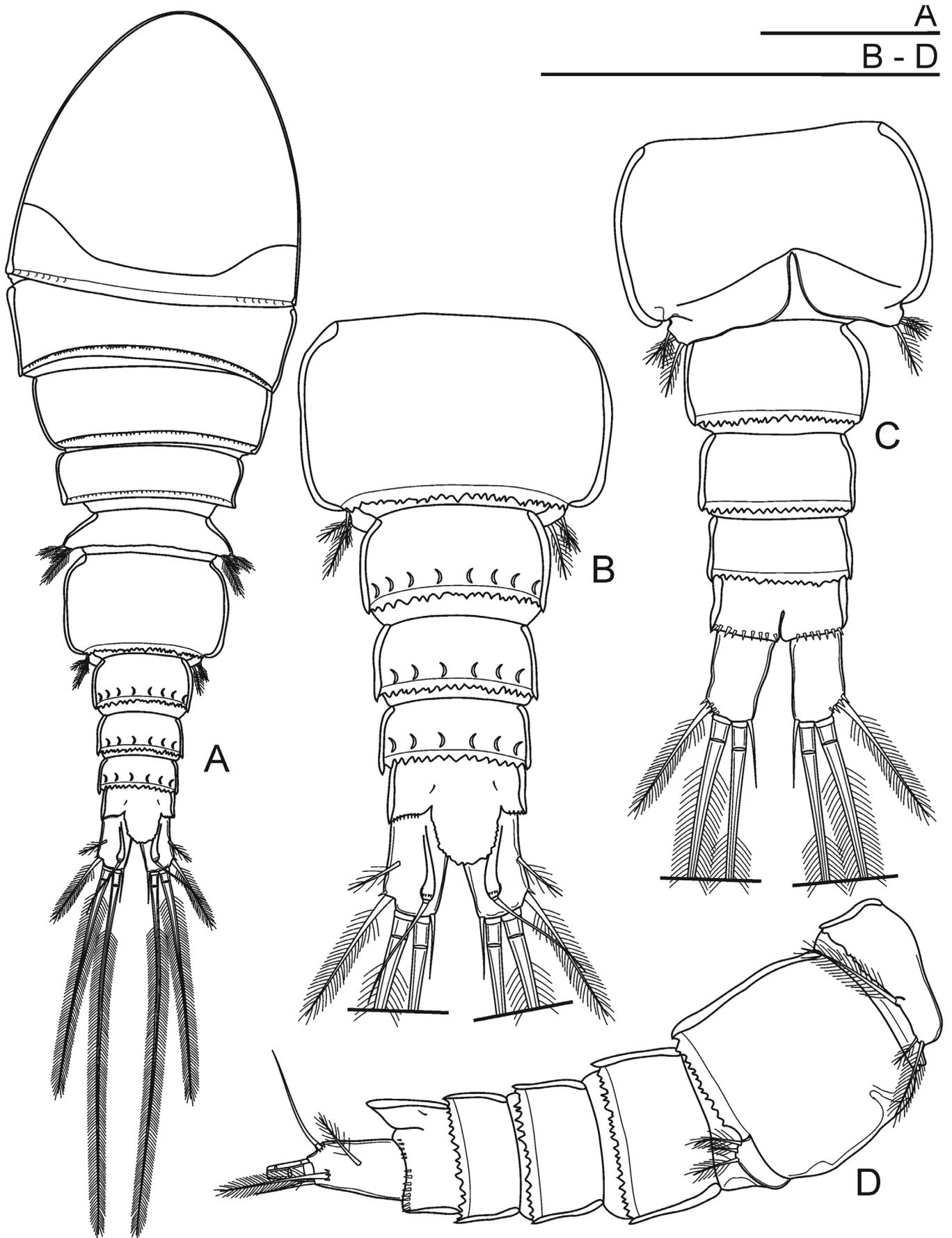


Fig. 6. *Bryocyclops muscicoloides*, new species, male. A, habitus, dorsal view; B, genital somite and urosome, dorsal view; C, genital somite and urosome, ventral view; D, pediger 5, genital somite and urosome, lateral view. Scale bar = 100 μ m.

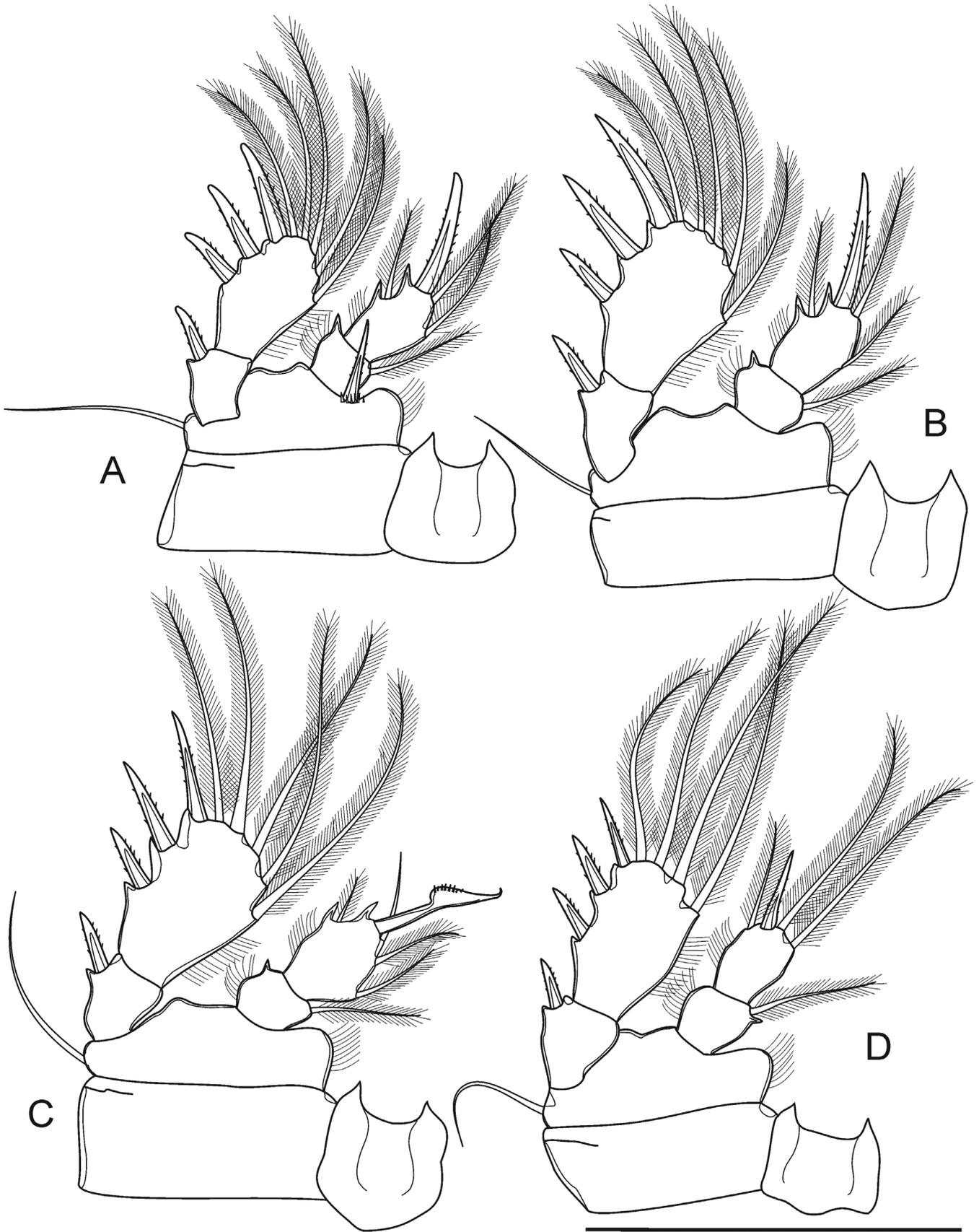


Fig. 7. *Bryocyclops muscicoloides*, new species, male. A, P1; B, P2; C, P3; D, P4. Scale bar = 100 μ m.

II sensu Lindberg (1954) (except that P1 coxa has no inner seta in *B. muscicoloides*, new species) in the following characters: (1) sexual dimorphism occurs on P3 Enp-2 and P4 Enp-2, (2) P1 basis has an inner spine, (3) intercoxal plates of P1–P4 have acute protuberances on the distal margin, (4) the setal and spine formulae of P1–P4 Exp-2 are 5.5.5.4. and 3.3.3.3, respectively and (5) the P4 of female has two-segmented Exp and one-segmented Enp, and there are two-segmented Exp and Enp in the male (Lindberg, 1954; Watiroyam et al., 2015a).

Currently, Group II consists of five species: *B. bogoriensis* (Menzel, 1926), *B. muscicola* (Menzel, 1926), *B. fidjiensis* Lindberg, 1953, *B. caroli* Bjornberg, 1985, and *B. campaneri* Rocha & Bjornberg, 1987 (Reid, 1999). The new species is most similar to *B. muscicola* from Indonesia (Menzel, 1926; Bjornberg, 1985; Rocha & Bjornberg, 1987; Watiroyam et al., 2015a). Apart from the group characteristics as previously mentioned, they share some affinities in the posterior margin of the urosome and anal operculum serrated, the female Enp P4 has four setae and one spine; the setal formulae on Enp-2 P1 and P3 in female have four and six elements, respectively, and Enp-2 P1 of males has four elements. *Bryocyclops muscicoloides*, new species, differs markedly from *B. muscicola* in the following characteristics: in both sexes, P1 has no inner coxal seta (present in *B. muscicola*) and Exp-2 P2–P3 have normal setae (each with two blunt setae in *B. muscicola*); in males, the transformed spine on Enp-2 P3 shows the outstretched part gradually tapering to the tip, which is located above the middle of its length (semi-circular and located at about halfway along the length in *B. muscicola*); in female, Enp P4 has no spiniform process on the outer margin (present in *B. muscicola*). Regarding species descriptions by Reid (1999), the other differences between the species are (a) the prosome and urosome have refractile points including anal operculum (only prosome in female and included urosomite 2 in male with respective characters in *B. muscicola*), (b) caudal ramus has spinules at the insertion of dorsal seta, and (c) caudal ramus of male has dorsal keel (both absent in *B. muscicola*).

Bryocyclops muscicoloides, new species, differs from *B. bogoriensis* in that the female of the new species has three setae on P2 Enp-2 but there are five setae in *B. bogoriensis*; P4 Enp has no spiniform process on the outer margin of *B. muscicoloides*, new species, whereas it is present in *B. bogoriensis*; and apical seta are longest in the new species but are almost as long as the distal inner seta in *B. bogoriensis*. The transformed spine on the male P3 Enp-2 has an outstretched part gradually tapering to the distal end, but it is rounded in *B. bogoriensis*.

The new species has a serrated and rounded anal operculum, but this is triangular and smooth in *B. fidjiensis*. *Bryocyclops muscicoloides*, new species' P4 Exp and Enp are two- and one-segmented in female and two-segmented in male, but are one- and two-segmented in both sexes of *B. fidjiensis*. There are three spines on the distal exopod of P4 in the new species but four spines on the same segment in *B. fidjiensis*.

Bryocyclops muscicoloides, new species, can be distinguished from *B. caroli* by P1 of the new species: it has no coxal inner seta, which is present in the later species. The anal operculum is regularly serrated in the new species compared to those in *B. caroli*. The shape of the female P4 Enp differentiates the new species from *B. caroli* by lack of a spiniform process on the outer margin and the transformed spine of male P3 Enp-2 has well-developed outstretched part and acute tip in the new species, while those spine in *B. caroli* has less-developed outstretched part and blunt tip.

Bryocyclops muscicoloides, new species, is different from *B. campaneri* in that the new species has no inner coxal seta on P1, which is present in *B. campaneri*; the P1 Exp-2 of the new species has five setae compared to four for *B. campaneri*; the female P4 Enp of the new species has no any spiniform process and the apical seta is typically longer than the apical spine while those segment in *B. campaneri* has outer spiniform process and apical seta is slightly longer than the spine; and the transformed spine on the male P3 Enp-2 of the new species has an outstretched part tapering to its tip compared to a semi-circular shape for *B. campaneri*.

The new species is easily distinguished from Group VII, proposed by a single species (*B. maholarnensis* Watiroyam, Brancelj & Sanoamuang, 2015) from Thailand, in that the distal Enp of P4 in *B. muscicoloides*, new species, has four setae and one spine, as in other members of Group II; however, only two setae are present in *B. maholarnensis* and P3 Enp-2 of male in *B. muscicoloides*, new species, have remarkable transformed spine which is normally found in Group II and other *Bryocyclops* species but is absent in *B. maholarnensis*. The new species also differs from *B. maewaensis* Watiroyam, Brancelj & Sanoamuang, 2012, found in Thailand, by the new species lacking inner coxal seta on P1, P2 Enp-2 of the new species having three setae (compared to four setae in *B. maewaensis*), the female P4 Enp being one-segmented and having four setae and one spine in the new species (compared to *B. maewaensis* being two-segmented and having three setae and one spine on the distal Enp), the male P3 Enp-2 of the new species having a longer distance between its tip and the outstretched part in the new species being smaller and differently shaped to *B. maewaensis*.

***Bryocyclops trangensis*, new species**

(Figs. 8–13)

Type locality. Khao Pina cave in Wat [=temple] Tham Khao Pina located in Nawong Subdistrict, Huai Yot District, Trang Province, southern Thailand (Figs. 1A, 1C). The coordinates of the entrance are 07°44'55.30"N, 99°31'36.81"E; altitude 34 m a.s.l. The cave is about 150 m long, with several pools filled exclusively with water dripping from stalactites. A rimstone pool in the dark zone, with about 10–20 L was selected as the location type. The water temperature during sampling was 22.0°C, pH 8.0, and conductivity was 350 $\mu\text{S cm}^{-1}$.

Material examined. Holotype: an adult female was dissected and mounted on a slide in glycerol and sealed with nail

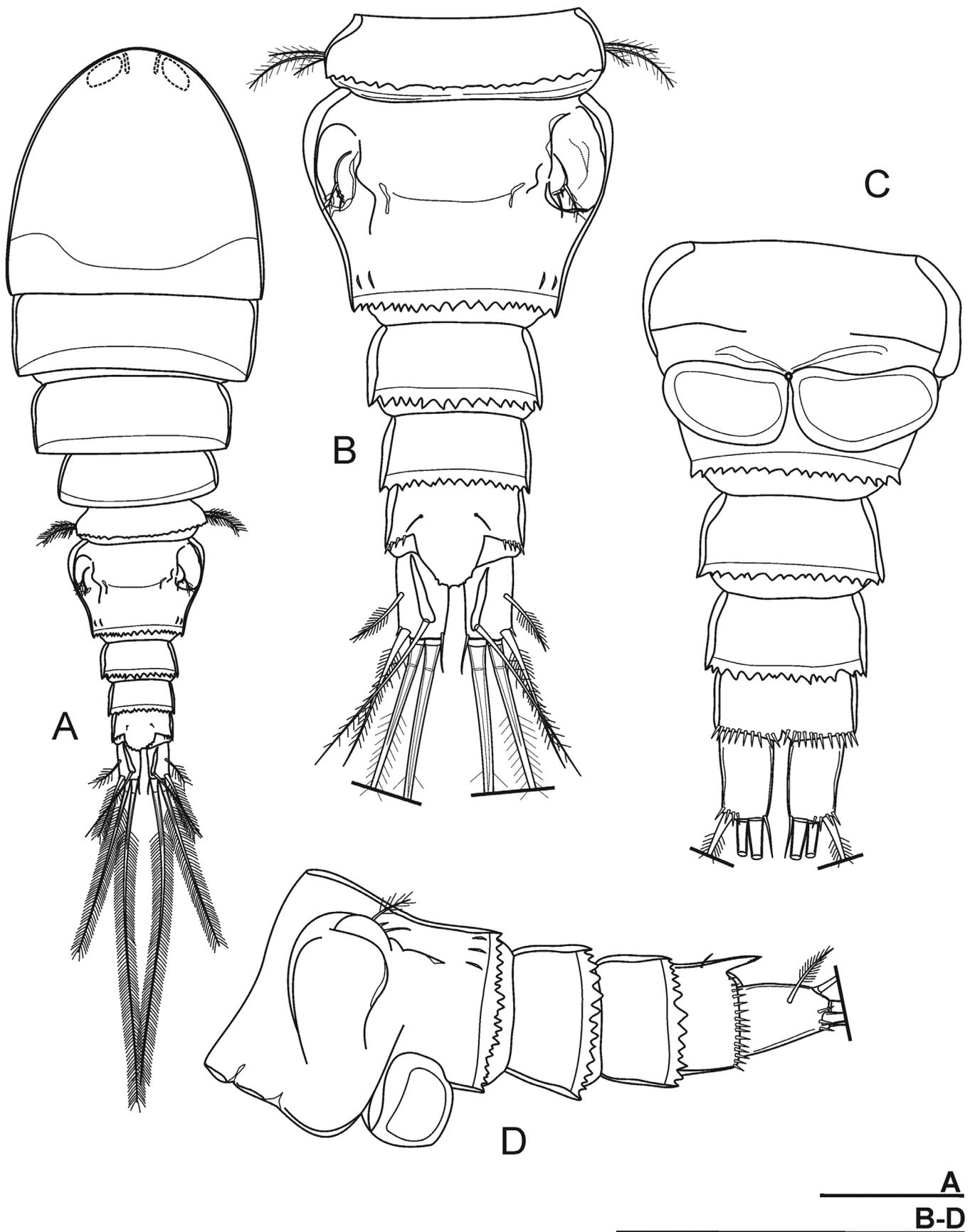


Fig. 8. *Bryocyclops trangensis*, new species, female. A, habitus, dorsal view; B, pediger 5, genital double-somite and urosome, dorsal view; C, genital double-somite with spermatophores and urosome, ventral view; D, genital double-somite with spermatophores and urosome, lateral view. Scale bar = 100 μ m.

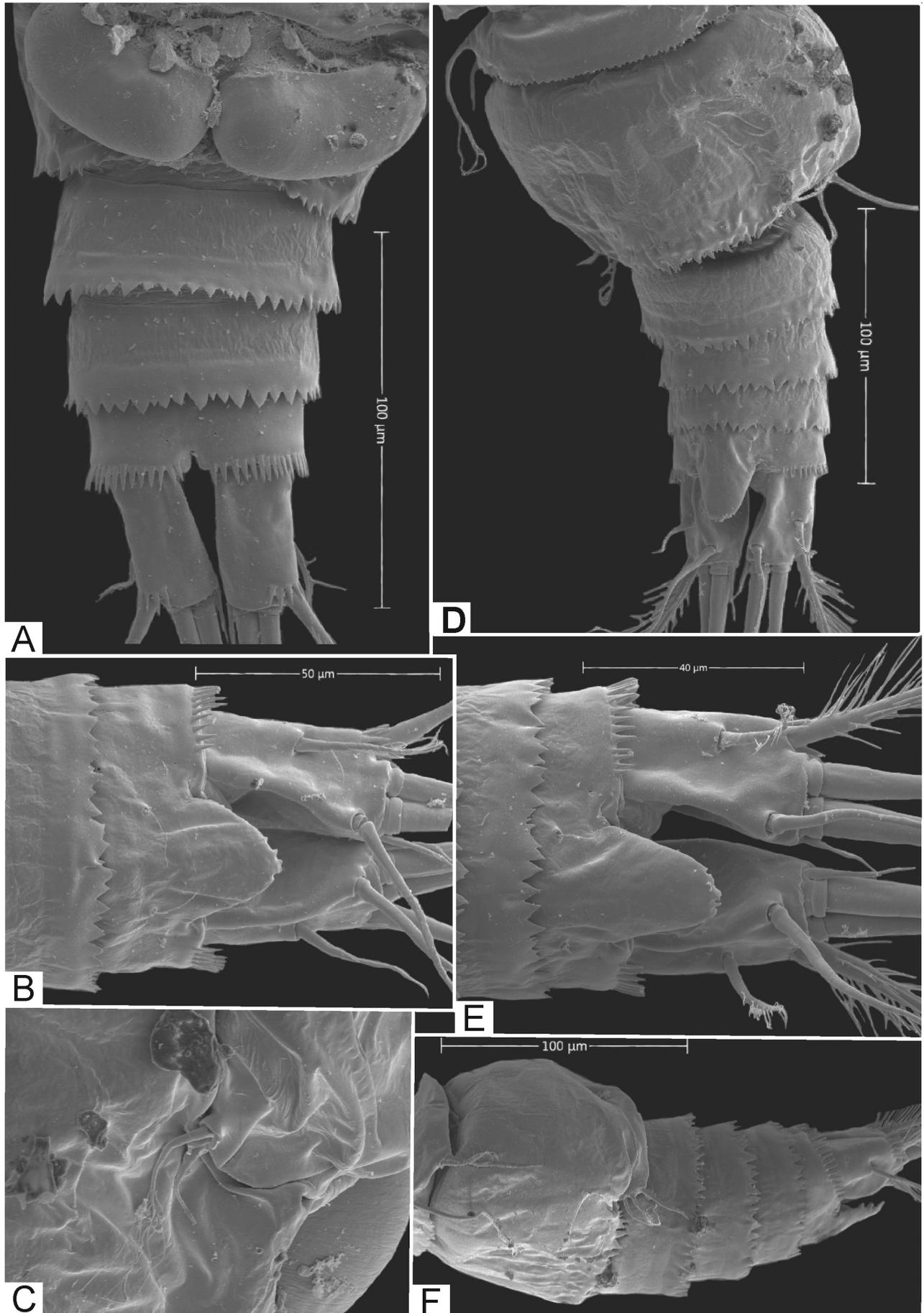


Fig. 9. *Bryocyclops trangensis*, new species, female (A–C) and male (D–F). A, genital double-somite and urosome, ventral view; B, anal somite and caudal ramus, dorsal view; C, P6; D, pediger 5, genital somite and urosome, dorsal view; E, anal somite and caudal ramus, dorsal view; F, pediger 5, genital somite and urosome, lateral view.

polish, NHMUK 2017.511. Allotype: an adult male was dissected and mounted on a slide in glycerol and sealed with nail polish, NHMUK 2017.512. Paratypes: three undissected females and males were preserved by 70% ethanol in a 1.5 mL microtube, NHMUK 2017.513–518; three undissected females with egg sacs and males were preserved by 70% ethanol in a 1.5 mL microtube, NPU 2017.003. Additional materials: four undissected females and males were mounted on SEM Stub (NPU 2017.003S).

All specimens were collected from Khao Pina cave by the author on 27 November 2016.

Diagnosis. Adult female: anal operculum is ovate and serrated. P1–P4 have acute projections on the distal margins, P1 has inner coxal seta, which are lacking on P2–P4. P1 has an inner basal spine. P1–P3 have two-segmented Enp and Exp; P4 has one-segmented Enp and two-segmented Exp. Setal and spine formulae on (1) Exp-2 P1–P4 as 5.5.5.4 and 3.3.3.3, respectively; (2) Enp-2 P1–P3 as 3.4.5 and 1.1.1, respectively; (3) Enp P4 has four setae and one spine, with an outer spiniform process.

Adult male: P1, P2, and Exp P3–P4 are as in female. Enp-2 P3 has four pinnate setae and one strong transformed seta and spine. The transformed spine has a less-produced outstretched part, located near the tip of the spine. P4 Enp is two-segmented; the Enp-1 has one inner pinnate seta and one spiniform process on the outer distal corner plus two strong, short, spine-like processes along the inner margin; Enp-2 has three pinnate setae and one spiniform spine, with a spiniform process on the outer distal corner.

Description. Female (Figs. 8, 9), body length: 490–570 μm (mean: 550 μm ; $n = 10$). Naupliar eye not discernible. Cephalothorax expanded laterally at cephalosome, with transverse scar on posterior end of cephalosome dorsally (Fig. 8A). Cephalosome, pedigers 2–5, and genital double-somite covered with fine refractile points. Posterior dorsal margins of cephalosome and pedigers 2–4 with smooth hyaline membrane; pediger 5 with irregularly serrated hyaline membrane (Figs. 8A, 8B). Genital double-somite and two succeeding urosomites with irregularly serrated hyaline membrane on the entire posterior margin (Figs. 8B–D, 9A). Genital double-somite symmetrical, anterior part expanded laterally, about 1.4 times wide as long, with a pair of dorsal sclerotised; single copulatory pore situated mid-ventrally. Anal somite (Figs. 8B–D, 9A, 9B) with a row of strong spinules on the entire posterior margin except nearly insertion point of anal operculum.

Anal operculum (Figs. 8B, 8D, 9B) ovate, small and irregularly serrated along its distal margin, not reaching the middle of caudal ramus, with a pair of dorsal sensilla on its base.

Caudal rami (Figs. 8B–D, 9A, 9B) symmetrical rectangular, about 2.3 times long as wide, with well-developed longitudinal dorsal keel, extended nearly the entire caudal ramus length. All seta pinnate, except seta VI; seta VII articulated at its base. Anterolateral accessory seta (I) absent. Anterolateral seta (II) shorter than caudal ramus, inserted near the middle of ramus dorsally. Posterolateral seta (III) about 1.5 times as long as caudal ramus, with strong spinules at its insertion laterally and ventrally. Outer terminal seta (IV) and inner terminal seta (V) with fracture plane, about 5.0 and 9.0 times as long as caudal ramus, respectively. Terminal accessory seta (VI) thin, shorter than 1/2 of caudal ramus. Dorsal seta (VII) about 2.0 times as long as caudal ramus, inserted on distal end of keel.

Rostrum (Fig. 10A) fused to cephalosome, free margin round and unornamented.

Antennule (Figs. 10B) short, not reaching posterior margin of cephalosome, 11-segmented. Setal formula as follows (A = aesthetasc, S = spine): 7.2.5.2.0.2.3.1+A.2.2.7+A.

Antenna (Fig. 10C) four-segmented, comprising coxobasis and three-segmented Enp. Coxobasis with smooth seta on inner distal corner. Enp-1 with smooth seta on inner medial margin. Enp-2 with five smooth setae along inner distal margin (one proximal and four distal setae) and row of tiny spinules along outer margin. Enp-3 with seven smooth setae on distal end: one inner, four apical and two outer.

Mandible (Fig. 10D) short and robust, with four strongly chitinised teeth on gnathobase plus a smooth seta on innermost margin. Mandibular palp reduced, with one smooth seta.

Maxillule (Fig. 10E) with three-segmented. Praecoxal arthrite with two strong apical claws, one spine on distal margin and one spine, five setae on inner margin. Maxillular palp two-segmented, with four and three smooth setae on coxobasis and Enp, respectively.

Maxilla (Fig. 10F) with five-segmented. Praecoxal endite with two apical pinnate setae. Coxa with smooth seta on proximal endite and two smooth setae on distal endite. Basis with one apical claw-like expansion, one strong spine and one seta inserted at base of claw. Enp-1 with one smooth inner seta. Enp-2 with one strong and two thin apical setae.

Maxilliped (Fig. 10G) with four-segmented. Syncoxa with one spiniform and one smooth seta on distal inner margin accompanied by row of strong spinules along middle outer surface. Basis with one spiniform seta on middle inner margin and row of strong spinules on distal outer margin. Enp with two-segmented, with one spiniform seta and two smooth setae on proximal and distal segments, respectively.

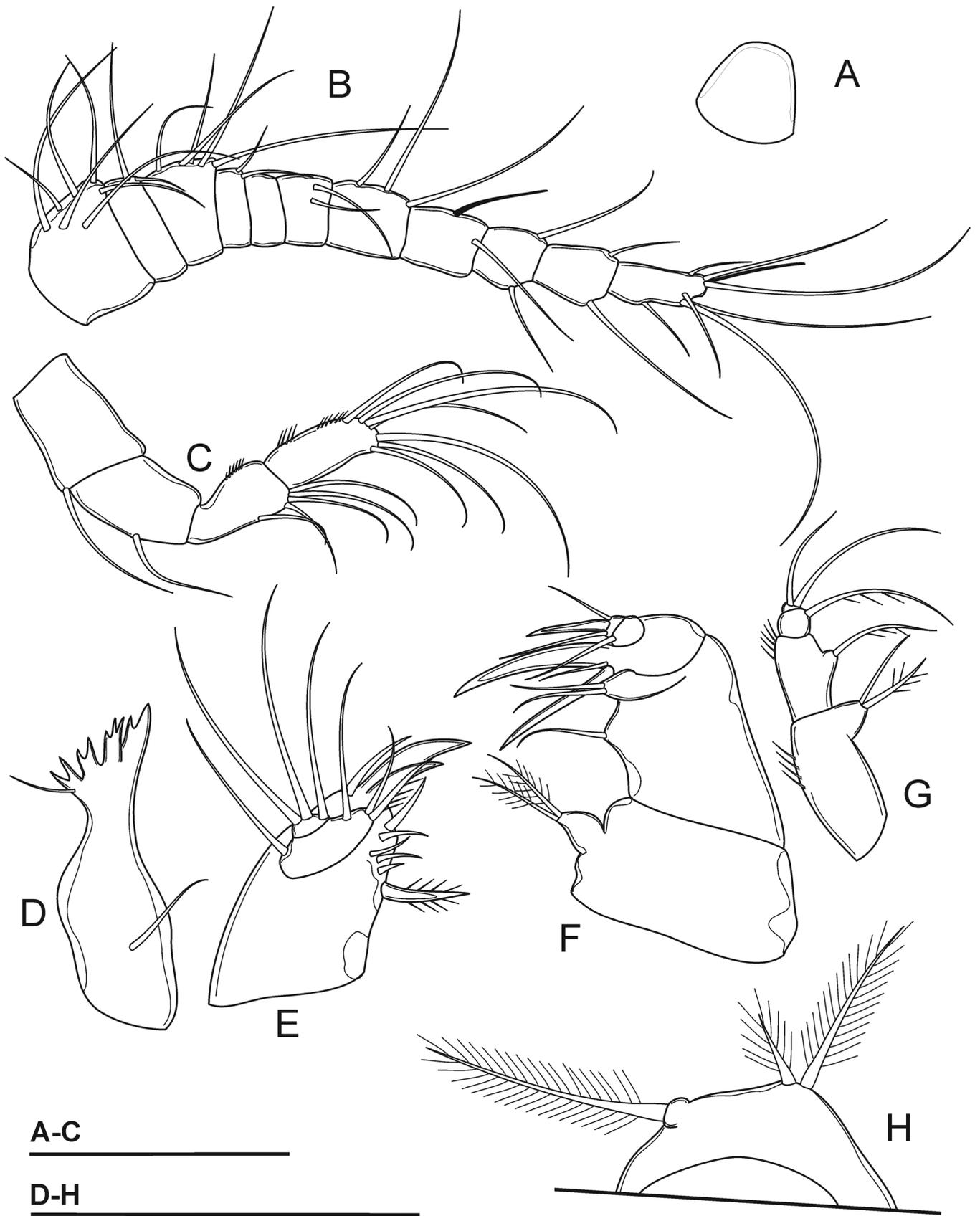


Fig. 10. *Bryocyclops trangensis*, new species, female. A, rostrum; B, antennule; C, antenna; D, mandible; E, maxillule; F, maxilla; G, maxilliped; H, P5. Scale bars = 100 μ m.

Armament of legs 1–4 as follows (legend: outer-inner seta/spine; outer-apical-inner; Arabic numerals indicate setae; Roman numerals indicate spines):

	Coxa	Basis	Exopod	Endopod
P1	0-1	1-I	I-0; III-2-3	0-1; 1-I+1-1
P2	0-0	1-0	I-0; III-2-3	0-1; 1-I+1-2
P3	0-0	1-0	I-0; III-2-3	0-1; 1-I+1-3
P4	0-0	1-0	I-0; III-2-2	1-I+1-2

P1 (Fig. 11A) intercoxal plate with acute projections on distal margin. Coxa with inner seta. Basis with slender seta on outer margin and stout spiniform spine on inner margin. Two-segmented Exp and Enp. Exp-1 much smaller than Exp-2, with one outer spine. Exp-2 twice as long as wide, with three inner pinnate setae, two apical pinnate setae and three outer spines. Enp-1 with one inner pinnate seta. Enp-2 with one apical spiniform spine and three pinnate setae (one inner, one apical and one outer).

P2 (Fig. 11B) intercoxal plate, and Exp similar to P1. Coxa without inner seta. Basis with one slender outer seta. Two-segmented Enp, Enp-1 with inner seta, Enp-2 with one apical spine and four pinnate setae (two inner, one apical and one outer).

P3 (Fig. 11C) intercoxal plate, coxa, basis and Exp similar to P2. Two-segmented Enp, Enp-1 with inner seta, Enp-2 with one apical spine and five setae (three inner, one apical and one outer). All setae pinnate.

P4 (Fig. 11D) intercoxal plate with acute projections on distal margin. Coxa without inner seta. Basis with slender seta on outer margin. Exp with two-segmented, Exp-1 with outer spine. Exp-2 with two inner pinnate setae, two apical pinnate setae and three outer spines. Enp with one-segmented, with spiniform process on outer margin, ornamented with apical spine and four pinnate setae (two inner, one apical and one outer).

P5 (Fig. 10H) fused to fifth thoracic somite, with three pinnate setae: outermost seta longest, inserted on small prominence; middle and innermost setae directly inserted to somite, former seta shorter than latter.

P6 (Figs. 8B, 8D, 9C) reduced to simple plate on genital double-somite laterodorsally, with lateral pinnate seta, middle minute spinule and inner naked seta.

Adult females each have a pair of egg sacs, each with three eggs.

Male (Figs. 12–13) slightly smaller than female. Body length, excluding caudal setae, 500–560 µm (mean = 530 µm; n = 5). Body shape similar to female, except in genital segment and urosomal segmentation (Fig. 12A). Antenna, mouthparts, anal operculum, caudal rami (Figs. 9E, 10C–G), P1–P2, P5 (Figs. 12C, 13A, 13B), P3–P4 exopods (Figs. 13C–D), similar to those in females.

Antennule (Fig. 12D) 15-segmented. Setal formula as follows (A = aesthetasc, S = spine): 8+A.5.2.2+A.0.4.1.0.2.3.S.1+A.S.1.8+A. All setae smooth.

P3 (Fig. 13C) Enp two-segmented, Enp-1 smaller than Enp-2. Enp-1 with inner pinnate seta. Enp-2 with one transformed spine apically, four pinnate setae (two inner, one apical, one outer) and one strong transformed seta on distal inner margin; transformed spine with outstretched part less-produced, located nearly its tip.

P4 (Fig. 13D) Enp two-segmented. Enp-1 bigger than Enp-2, with inner pinnate seta and two short, strong spines on inner surface. Enp-2 smaller, with inner seta, apical seta and spine and outer seta; all setae pinnate.

P6 (Figs. 9F, 12B, 12C) reduced to simple plate, represented by three pinnate setae.

Variability. P1 Exp-2 of male with six setae on one ramus (only one individual of ten examined males).

Etymology. The specific name *trangensis* refers to the place “Trang” where the new species was found, the Latin suffix “-ensis” is an adjective for the place.

Remarks. *Bryocyclops trangensis*, new species, also fits with the generic and Group II characteristics as described for *B. muscicoloides*, new species. *Bryocyclops trangensis*, new species, is most similar to *B. muscicola* by the posterior margin of urosome and anal operculum serrated, the present of inner coxal seta on P1, Enp-2 P1 having four elements, the female Enp-2 P3 having six elements, the shape and setation of the female Enp P4 (five elements), and the male Enp-2 P4 of males having four elements. *Bryocyclops trangensis*, new species, can be easily distinguished from *B. muscicola* in the morphological differences in both sexes: Enp-2 P2 has five elements in *B. trangensis*, new species, but four elements in *B. muscicola*, and Exp-2 P2–P3 of *B. trangensis*, new species, have normal setae but those in *B. muscicola* have two blunt setae. The female P4 Enp of *B. trangensis*, new species, has subequal three setae but *B. muscicola* has apical seta remarkably longer than the others. *Bryocyclops trangensis*, new species, has certain typical characteristics in the male P3–P4: Enp-1 P4 has two strong, spine-like outgrowths on its inner surface, which are absent in *B. muscicola*; Enp-2 P3 of *B. trangensis*, new species, has four pinnate setae and one transformed seta while *B. muscicola* has one smooth and three pinnate setae; the outstretched part of the transformed spine on Enp-2 P3 is not well-produced in *B. trangensis*, new species, but is well-produced in *B. muscicola*.

Bryocyclops trangensis, new species, differs from *B. bogoriensis* by a P1–P4 intercoxal plate with an acute projections on distal margin that is more rounded in *B. bogoriensis* and the P1 Enp having inner coxal seta only in the new species. The male P3 Enp-2 of the new species has larger setae than those in *B. bogoriensis*; there are one transformed and four normal setae in the new species but only four normal setae in *B. bogoriensis*. The outstretched

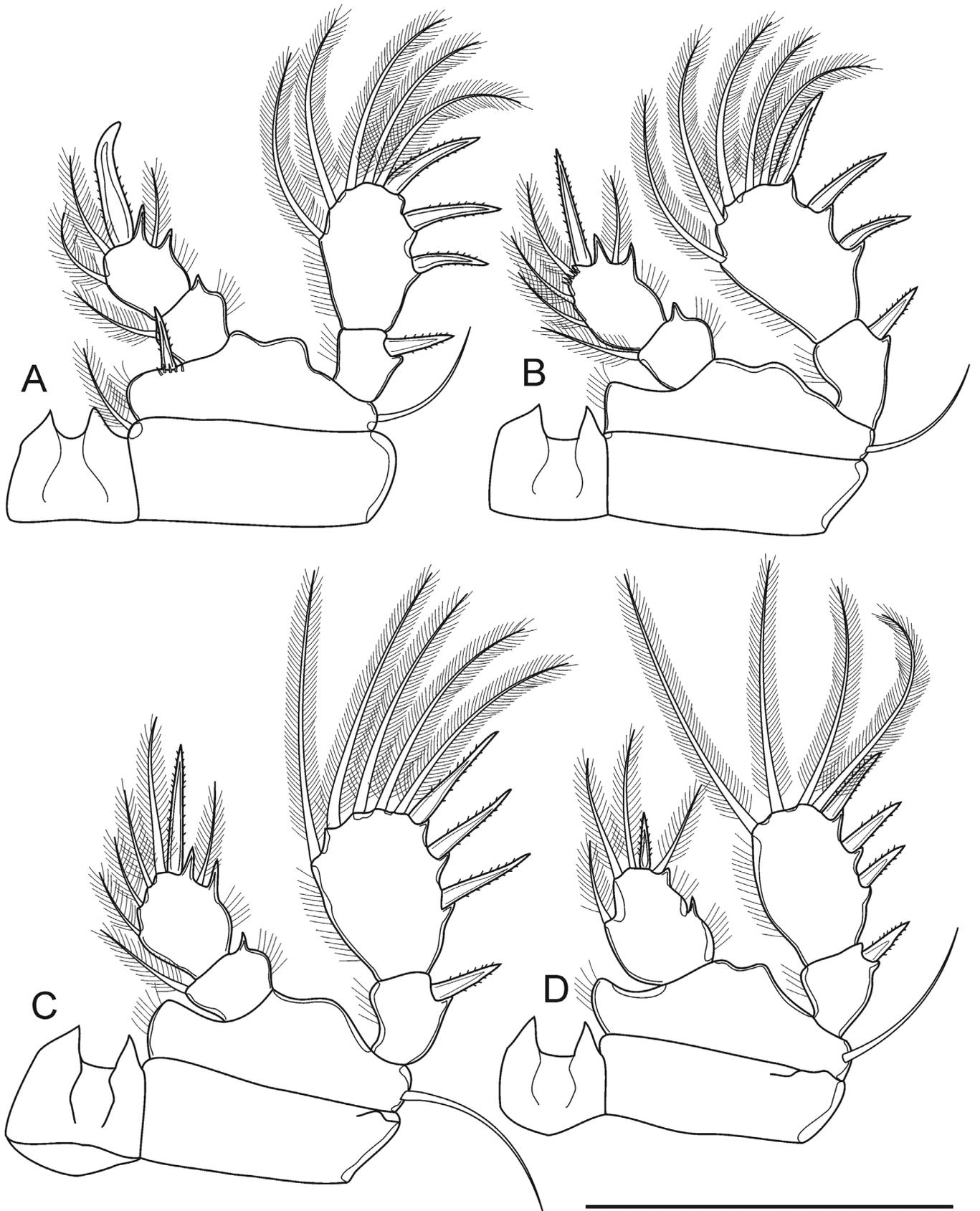


Fig. 11. *Bryocyclops trangensis*, new species, female. A, P1; B, P2; C, P3; D, P4. Scale bar = 100 μ m.

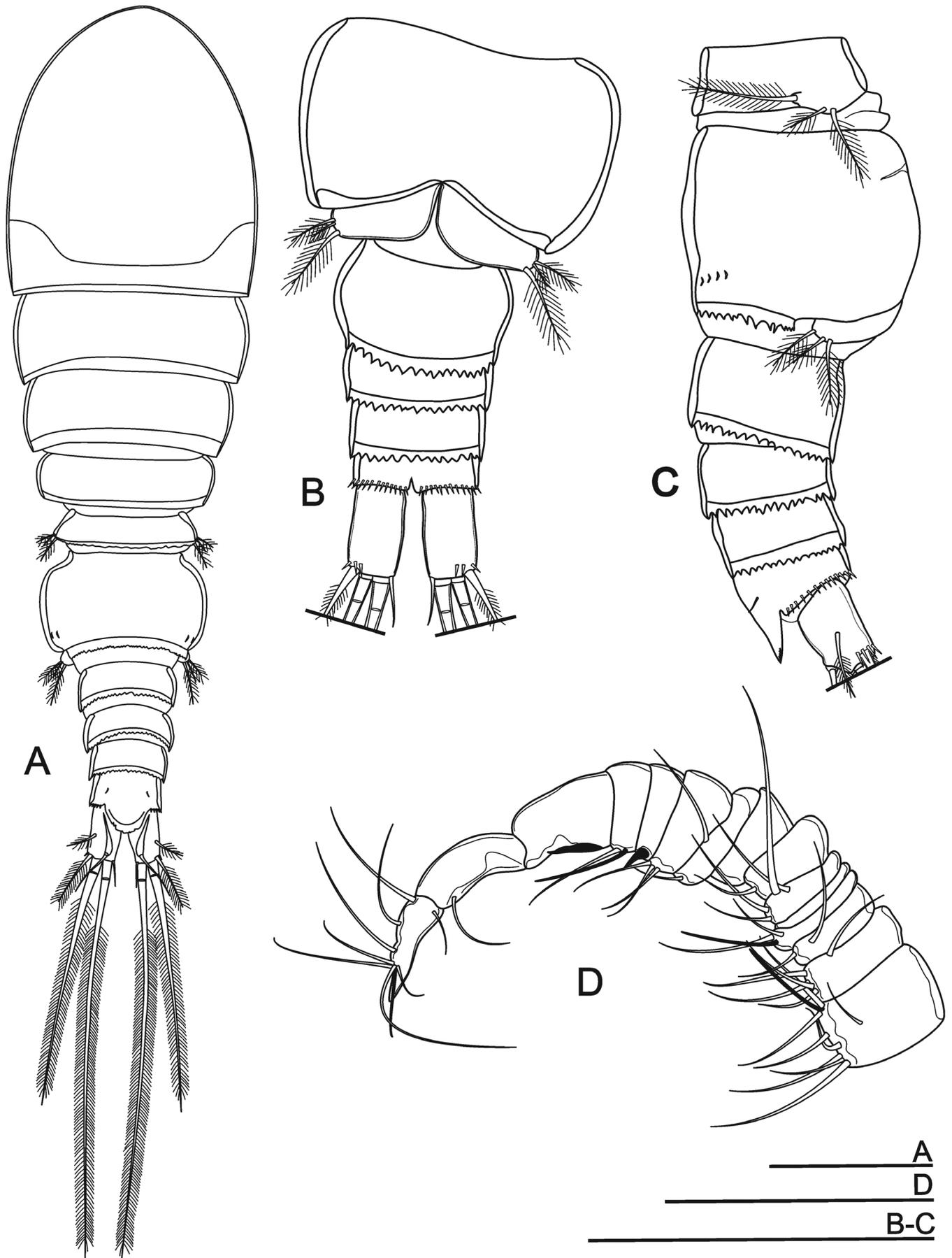


Fig. 12. *Bryocyclops trangensis*, new species, male. A, habitus, dorsal view; B, genital somite and urosome, ventral view; C, pediger 5, genital somite and urosome, lateral view; D, antennule. Scale bar = 100 μ m.

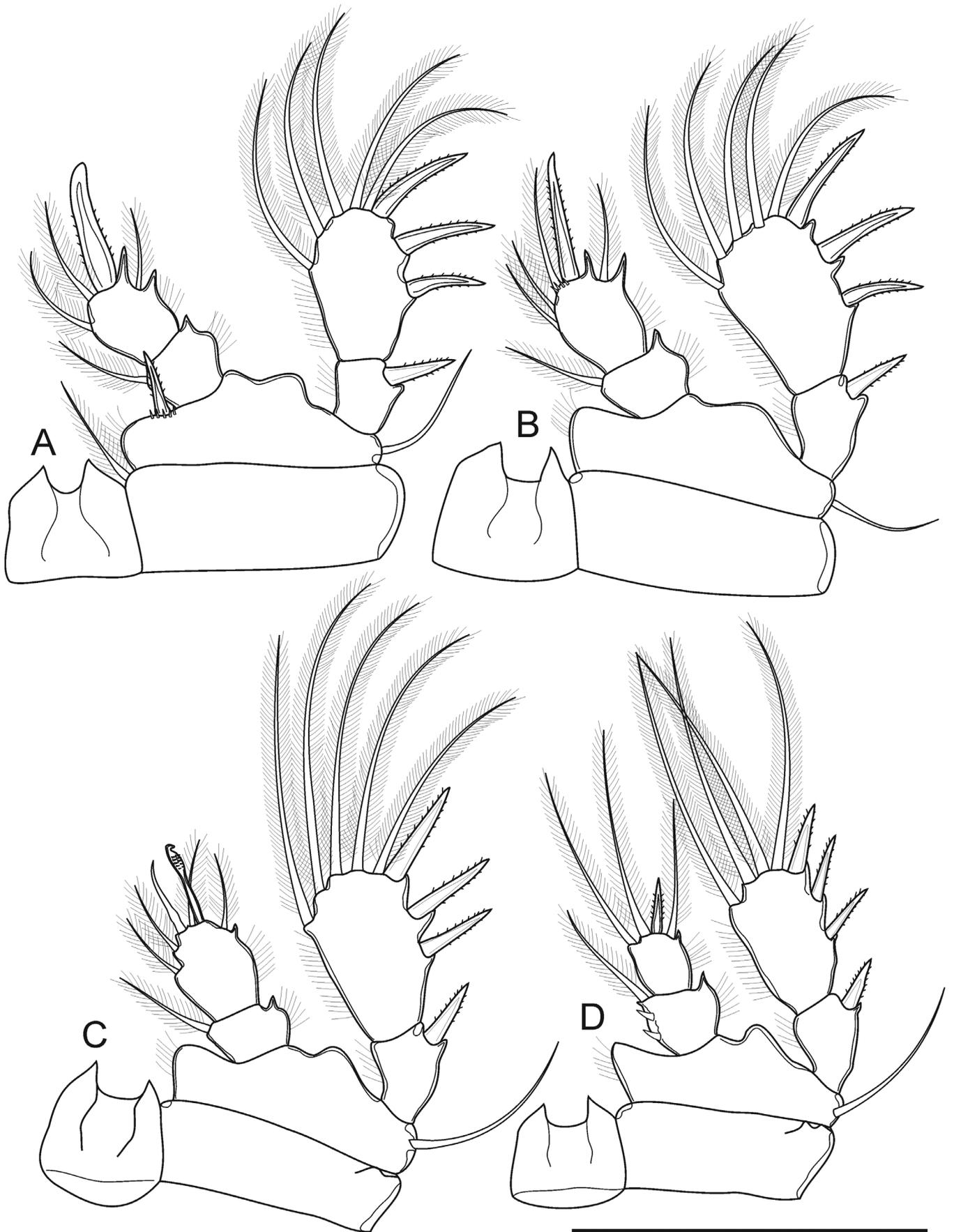


Fig. 13. *Bryocyclops trangensis*, new species, male. A, P1; B, P2; C, P3; D, P4. Scale bar = 100 μ m.

portion of the transformed species on the male P3 Enp-2 is less produced compared to those in *B. bogoriensis*, which are semi-circular. The male P4 Enp-1 of the new species has two spiniform outgrowths on the inner margin but is smooth in *B. bogoriensis*.

Bryocyclops trangensis, new species, is different from *B. fidjiensis* by its anal operculum ovate, which is serrated but triangular and smooth in the later species, and P4's two-segmented Exp and one-segmented Enp in the female in the new species but one-segmented Exp and two-segmented in both sexes in *B. fidjiensis*. The female P4 Enp of the new species has one spine and four setae but one spine and three setae in *B. fidjiensis*; and the distal exopod of P4 has three spines in the new species but four spines in *B. fidjiensis*. The male P4 Enp-1 of the new species has two outgrowths on the inner margin that are absent in *B. fidjiensis*.

Bryocyclops trangensis, new species, differs from *B. caroli* in both sexes by the following: anal operculum is finely serrated in the new species but coarsely serrated in *B. caroli*; Enp-2 P2 of the new species has four setae but three in *B. caroli*; and Exp-2 P2–P3 of the new species have no blunt setae compared to two in *B. caroli*. The female P4 Enp of the new species has four subequal setae but one typically long seta and three equal setae in *B. caroli*. The new species has two spiniform outgrowths on the inner margin in the male P4 Enp-1, but this is absent in *B. caroli*. The male P3 Enp-2 of *B. trangensis*, new species, has four setae, one transformed spine and seta compared to four setae and one transformed spine in *B. caroli*. The transformed spine of the new species has an acute tip and a short distance between the outstretched part and its tip, but there is a blunt tip and a long distance in *B. caroli*.

Bryocyclops trangensis, new species, and *B. campaneri* are different from each other as follows: the posterior margins of pediger 2–4 dorsally are smooth in *B. trangensis*, new species, but *B. campaneri* has transverse crenulated frills; P2 Enp-2 has four setae in the new species but three setae in *B. campaneri*; and P2–3 Exp-2 has no blunt setae compared to two in *B. campaneri*. The female P4 Enp of the new species has no outgrowth near the innermost seta but there is a large rounded outgrowth in *B. campaneri*. The transformed spine on the male P3 Enp-2 of the new species has an outstretched part that is less developed and located close to its tip, but it is well-produced into a semi-circular shaped and located near the mid-length of the spine for *B. campaneri*.

DISCUSSION

Distinguishing characteristics in the *Bryocyclops* species were mainly determined using segmentation, number of spines and presence and length of setae on the swimming legs in both sexes. In addition, the unique transformed spine on the Enp-2 P3 of males is traditionally used to separate species, and it seems to be a generic characteristic (except in *B. maholarnensis* Watiroyram, Brancelj & Sanoamuang, 2015). The following can be observed about the transformed

setae: (a) on Exp-2 P1–P4, it is common in both sexes for each species with this apparatus (absent in both new species). For example, *B. muscicola* (Menzel, 1926), *B. campaneri* Rocha & Bjornberg, 1987, *B. caroli* Bjornberg, 1985, and *B. maewaensis* Watiroyram, Brancelj & Sanoamuang, 2012), each have two blunt-tip setae on Exp-2 P2–P3; *B. maholarnensis* and *B. anninae* (Menzel, 1926) have one and two additional setae on Exp-2 P1, respectively. This characteristic was reviewed by Reid (1999) on *B. muscicola*, *B. campaneri*, *B. caroli*, and *B. anninae*, who noted that the species even live in different zoological regions and show similar traits in their setae (i.e. the presence of blunt seta on Exp-2) (Menzel, 1926; Bjornberg, 1985; Rocha & Bjornberg, 1987; Reid, 1999; Watiroyram et al., 2012, 2015a; author observation in Thai specimens for *B. muscicola*). These modified setae are also present in other cyclopoid copepods. For example, the presence of blunt setae on Enp-3 and Exp-3 of swimming legs was used in an identification key for species in *Eucyclops* Claus, 1893 (Suárez-Morales, 2004; Gutiérrez-Aguirre et al., 2013). On the male Enp-2 P3, the presence of modified seta was known as medial naked seta near the transformed spine, such as *B. muscicola*, *B. campaneri*, *B. caroli*, *B. maewaensis*, and *B. muscicoloides*, new species. However, *B. trangensis*, new species, has special strong transformed seta against its congeners, inserted next to the medial seta (i.e. innermost seta of stead of medial seta).

Other differential characteristics of these two new species were observed in (a) the ornamentation on the body surface, namely the crenulated linear ornamentation above its posterior margin on prosomite 2–3 present in *B. muscicoloides*, new species (see Fig. 3B for SEM photograph), and (b) the female Enp P4 lacking a spiniform processes on outer margin, which differs from *B. muscicola*, *B. trangensis*, new species, and others. The appearance of crenulated lines also occurs in *B. campaneri* and has been used as a differential characteristic from all its congeners (Rocha & Bjornberg, 1987). However, this characteristic in all previously described species except *B. muscicola* may be insufficiently described or overlooked rather than absent. The structure of the Enp P4 in terms of the presence of the spiniform process on the outer margin can also be used as a tool in separating species (Reid, 1999). These two new species can be separated from members of Group II sensu Lindberg (1954) by the P4 Enp ornamentation. Although this group is characterised by one spine and four setae on the female Enp P4 or one spine and three setae on the male Enp-2 P4, its members can be distinguished from others by the presence of spiniform process on its margin. For example, *B. muscicoloides*, new species, and *B. bogoriensis* have no spiniform process, whereas *B. trangensis*, new species, and others have this characteristic. In addition, the male Enp-1 P4 of *B. trangensis*, new species, has two strong spiniform processes on its inner margin, which is absent in all previous known species. This is in line with observation from Reid (1999) that the presence of spiniform processes on Enp P4 are a distinctive characteristic for species levels in Group II, especially for extremely similar species (especially *B. caroli*, *B. muscicola* and *B. muscicoloides*, new species).

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