

A new genus and new species of stygobitic copepod (Crustacea: Copepoda: Cyclopoida) from Thien Duong Cave in Central Vietnam, with a redescription of *Bryocyclops anninae* (Menzel, 1926)

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Abstract. During a faunistic expedition of Thien Duong Cave in Central Vietnam, specimens of two species of cave-dwelling cyclopoid copepods, belonging to two genera, were encountered in a small pool filled by drip water that was well separated from other water bodies within the cave. One group of specimens was tentatively assigned to *Bryocyclops anninae* (Menzel, 1926), representing the first record of the genus in Vietnam. The other species was recognised as a new species, most similar to *Graeteriella longifurcata* Tran & Chang, 2013, which was originally described from a syphon lake in the same cave. Detailed analyses of the morphological characters of both species revealed that they both actually belong to a new genus, *Pseudograeteriella*, established herein. The new genus can be distinguished from the closely related European genus, *Graeteriella* Brehm, 1926, by characters on the P1–P5, in the structure of genital segment in females, and in the armament and proportions of furcal rami. The two species of the *Pseudograeteriella*, new genus, differ considerably from each other in their mouthparts, characters on the P1–P5, and furcal rami. A generic diagnosis for *Pseudograeteriella*, new genus, and a detailed description of *P. longiaesthetascus*, new species and redescription of *B. anninae* are provided.

Key words. cave-dwelling copepod, karstic cave, Southeast Asia, stygobiont, subterranean

INTRODUCTION

Cave-dwelling faunas from unsaturated [= vadose] zones of karstic caves in Southeast Asia have remained relatively unexplored in the past 50 years (Brancelj et al., 2013), with only *Elaphoidella vietnamica* (Copepoda) having been discovered within such habitats (Borutzky, 1967). Recent intensive studies on copepods from the epikarst and adjacent vadose zones of karstic caves in Thailand and Vietnam since 2010 have revealed a rich diversity of both Cyclopoida and Harpacticoida (Watiroyam et al., 2012, 2013, 2017; Watiroyam & Brancelj, 2016). Among Cyclopoida, several new species belonging to known genera (i.e., *Bryocyclops* Kiefer, 1927; *Fierscyclops* Karanovic, 2004; *Graeteriella* Brehm, 1926; *Mesocyclops* Sars, 1914; *Metacyclops* Kiefer, 1927; and *Thermocyclops* Kiefer, 1927) were recorded (Brancelj et al., 2010; Watiroyam et

al., 2012, 2013; Tran & Chang, 2013; Boonyanusith et al., 2013; Tran & Holyńska, 2015; Karanovic et al., 2017). In addition, the first endemic genus, *Siamcyclops* Boonyanusith, Sanoamuang & Brancelj, 2018, was recently recorded from a cave in Thailand (Boonyanusith et al., 2018).

During a sampling campaign in 2012, in the longest cave in Asia, Thien Duong Cave (Central Vietnam), representatives of two cave-dwelling cyclopoid species were collected from a pool filled exclusively with drip water. These two species were preliminarily designated as members of the genera *Graeteriella* and *Bryocyclops*, respectively. However, detailed analyses revealed that the ‘*Graeteriella*’ species should be assigned to a new genus instead, along with another Vietnamese species from the same cave, *G. longifurcata* Tran & Chang, 2013; whereas the second species represents a rare species of *Bryocyclops* Kiefer, 1927, presently known to have a patchy distribution in areas with contiguous connection to the Pacific Ocean.

Representatives of the genus *Graeteriella*, including the two subgenera *Graeteriella* Brehm, 1926 (with five species) and *Paragraeteriella* Rylov, 1948 (with four species), were known only from Europe (France, Germany, Italy, and Slovenia) for a long time, with the most recently discovered European species described 40 years ago (Rylov, 1948; Lescher-Moutoué, 1968a, 1968b; Einsle, 1993; Walter & Boxshall, 2018a; Pesce, 2019). This genus is among the lesser known genera in the subfamily Cyclopinae (Cyclopoida). It is characterised by 1-segmented fifth thoracic leg that is well separated from the pediger bearing it, with two apical setae,

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and no lateral seta on pediger five (vestige of the basis of fifth thoracic leg) (Dussart & Defaye, 2001; Boonyanusith et al., 2018).

In 2013, *Graeteriella* (*G.*) *longifurcata* Tran & Chang, 2013, was described from a cave in Vietnam, and it was the first record of the genus in the Oriental region (Tran & Chang, 2013). The second species from the same cave resembles its congeners, but it has several distinct characters that suggest that it could be established as a new species. In addition, the unique morphological characters of the two *Graeteriella*-like species from Vietnam and the typical species from Europe are sufficiently different to classify the two species from Vietnam as a separate, new genus.

To date, 25 species of the genus *Bryocyclops* Kiefer, 1927 have been recorded worldwide (Walter & Boxshall, 2018b; Watiroyam, 2018a, 2018b; Pesce, 2019). Typically, they are found in semi-terrestrial and subterranean habitats (Watiroyam et al., 2012; Pesce, 2019). The genus has a pantropical distribution (Kiefer, 1937), but each species shows more local/endemic distributions in Africa, Asia, South America, and Central America (Reid, 1999; Pesce, 2019).

At present, nine species of *Bryocyclops* have been described from Southeast Asia, including: *B. bogoriensis* (Menzel, 1926) and *B. chappuisi* Kiefer, 1928, from Java; *B. muscicola* (Menzel, 1926) from Java, Sumatra, and Thailand; *B. maewaensis* Watiroyam, Brancelj & Sanoamuang, 2012, *B. maholarnensis* Watiroyam, Brancelj & Sanoamuang, 2015, *B. muscicoloides* Watiroyam, 2018, *B. trangensis* Watiroyam, 2018, and *B. aetus* Watiroyam, 2018 from Thailand; and, *B. anninae* (Menzel, 1926) from Vietnam (the present study). Four of the species mentioned above (described during 1926–1928) were collected in wet semiterrestrial epigeal habitats, including phytotelmata, but five from Thailand (described after 2012) were found in pools filled with drip water inside caves (Brancelj et al., 2013; Watiroyam et al., 2012, 2015; Watiroyam, 2018a, 2018b). This report of the finding of *B. anninae* in a karstic cave is the first record of this genus in Vietnam.

MATERIAL AND METHODS

Samples were collected from Thien Duong [= Paradise] Cave in Phong Nha-Ke Bang National Park, Quang Bin Province, Central Vietnam – a UNESCO World Natural Heritage site. Coordinates at the entrance are: 17°31'09.46"N, 106°13'17.80"E, altitude: 200 m a.s.l. The cave is 31.4 km long, with running water inside. Samples were collected using a hand net with a 60 µm mesh size from a pool, about 400 m from the tourist entrance. The pool is located well above the river channel and is filled exclusively by drip water.

Samples were fixed on-site in 4% formalin solution. In the laboratory, specimens were sorted under a stereomicroscope and stored in a 70% ethanol. Before dissection, specimens were placed in a mixture of glycerol and 70% ethanol (ratio ~1: 10 v/v), which was replaced with pure glycerol within

30 minutes. Selected specimens were dissected using an Olympus SZH 2 stereomicroscope. For permanent slides, all body parts were put in a drop of glycerol on a microscope slide, covered by a thin glass coverslip, and sealed with nail polish. Appendages and body ornamentation were examined under a compound microscope, Olympus BHS 40 with a magnification of 1000×. All drawings, except for the female and male habitus, were made at the same magnification (1000×) with a drawing tube. Final illustrations were prepared using the graphic program, CorelDRAW®12.

Collection reference numbers of the specimens here examined and information on the collection site are provided in the type material section of the new species below; holotype and allotype have been deposited in the Natural History Museum, London, United Kingdom (NHMUK), the other specimens (paratypes) are deposited in the Khon Kaen University Science Museum, Thailand (KKU).

The nomenclature and descriptive terminology here used follow that of Huys & Boxshall (1991). The following abbreviations are used throughout text and figures: ae = aesthetasc; Endp = endopod; Exp = exopod; Exp1/Endp1 = the first segment of exopod/endopod; Exp2/Endp2 = the second segment of exopod/endopod; P1–P6 = the first to the sixth thoracic legs, Pd1–Pd5 = the first to the fifth pedigerous somites.

TAXONOMY

Order Cyclopoida Burmeister, 1834

Family Cyclopidae Dana, 1846

Subfamily Cyclopininae Burmeister, 1834

Pseudograeteriella, new genus

Diagnosis. Small Cyclopininae with slightly elongated habitus and greatest width at posterior margin of cephalothorax. Antennule 11-segmented in female, and 15-segmented in male. Female A1 short, hardly reaching posterior margin of cephalosome. Genital double-somite as long as wide, incision between anterior and posterior half of segment moderate. Seminal receptacle well developed; anterior expansion wide, short; posterior expansion well developed, longer than anterior expansion. P5 with protopodal segment incorporated into Pd5, lacking outer basal seta; exopodal segment fused to Pd5, with one spine apically and one plumose seta dorsally, similar in length. P6 in female small semicircular plate, armed with two short spines. Anal operculum short, broadly rounded, free margin smooth. Basis of antenna without seta representing Exp. Mandibular palp with two very long plumose setae and one short, bare. Coxae of P1–P3 with plumose seta on inner corner, but absent on P4. P1–P4 with 2-segmented Exp and Endp, similar in length. Spine and setae formula on terminal segments of Exp P1–P4 observed to be 3.3.3.3 and 5.5.5.4, respectively. Male genital somite as long as wide.

Table 1. Differences between genera *Graeteriella* Brehm, 1926 (including subgenera *Graeteriella* Brehm, 1926 and *Paragraeteriella* Rylov, 1948) and *Pseudograeteriella* new genus.

Character	<i>Graeteriella</i> Brehm, 1926	<i>Pseudograeteriella</i> new genus
Length of antennule	Middle of cephalosome	Close to end of cephalosome
Receptaculum seminis	Anterior part wide, well developed; posterior part reduced/absent	Anterior part wide, short; posterior part long, well developed
Female: No. of segments P1–P4 Exp/Endp	<i>Graeteriella</i> s.str.: 2.2/2.2/2.2/2.2 <i>Paragraeteriella</i> : 2.2/3.2/3.3/3.3	2.2/2.2/2.2/2.2
Inner seta on basis of P4	Present	Absent
P5 Endp	Separated from pediger	Fused with pediger
P5 spine: seta length	1: 3–4	1: 1–1.2
Fu width : length	1: 1.5–3	1: 5–6

Type species. *Pseudograeteriella longifurcata* (Tran & Chang, 2013); Syn: *Graeteriella* (*Graeteriella*) *longifurcata* Tran & Chang, 2013; by present designation.

Etymology. The genus name is composed of the Latin preposition “pseudo” (meaning “false”) and the existing generic name *Graeteriella*. The gender is feminine.

Remarks. To date, only four genera within the subfamily Cyclopinae Burmeister, 1834 have been identified with the absence of outer basal seta on the protopodal segment of P5 (Dussart & Defaye, 2001). Two of those have only one element representing P5. In *Bacillocyclops* Lindberg, 1956, P5 is reduced to a short, thick, weakly barbed appendix; whereas, P5 is reduced to a tiny, slender seta in *Austriocyclops* Kiefer, 1964 (Dussart & Defaye, 2001).

Two elements on P5 End are present in *Graeteriella* Brehm, 1926 [with subgenera *Graeteriella* s. str. Brehm, 1926 and *Paragraeteriella* Rylov, 1948 (1963) (Dussart, 1969)] and *Pseudograeteriella* new genus. Both genera differ in the characters presented in Table 1.

Boonyanusith et al. (2018) considered the species from Vietnam and Europe as the members of a single genus, *Graeteriella*, separated into two subgenera. However, our new findings and the data that we report herein suggest that two clearly distinct genera exist: *Graeteriella* from Europe and *Pseudograeteriella*, new genus, from Asia (Vietnam). Accordingly, the following amendment should be made to the key in Boonyanusith et al. (2018: 23), where paragraph “7” should be modified and a new paragraph “7a” to be added, as follows:

- 7. Pd5 without lateral seta (vestige of the basis of P5)..... 7a
- Pd5 with lateral seta..... 9
- 7a. P5 with distal segment fused to Pd5; with two setae/spines apically..... *Pseudograeteriella* new genus
- P5 with one free distal segment on Pd5; with two setae/spines apically..... *Graeteriella* (8)

Similarities: P6 in male and female are similar in shape and size in both *Graeteriella* and *Pseudograeteriella*, new genus.

Conclusions: *Pseudograeteriella*, new genus, is the older lineage of the two genera based on reductions of inner seta on basis of P4, and fusion of P5 Endp with pediger.

***Pseudograeteriella longiaesthetascus*, new species**
(Figs. 1A–G, 2A–F, 3A–D [female]; 4A–D [male])

Type locality. A pool at Thien Duong Cave in Phong Nha-Ke Bang National Park, Quang Bin Province, Vietnam, located approximately 400 m from the tourist entrance. Coordinates of the entrance are 17°31'09.46"N, 106°13'17.80"E, altitude: 200 m a.s.l.

Material examined. Holotype: adult female, total body length 973 µm, completely dissected, mounted in glycerol on a slide and sealed with nail polish, NHMUK 2018.1013, collected on 21 March 2012 by L. Sanoamuang. Allotype: adult male, total body length 796 µm, completely dissected, mounted in glycerol on a slide and sealed with nail polish, NHMUK 2018.1014, collected on 21 March 2012 by L. Sanoamuang. Paratypes: two females without egg sacs and two males, sampled with the holotype, whole specimens are stored in 70% ethanol and deposited at the Khon Kaen University Science Museum; KKU–COP–2018.001–004. All specimens were collected at the same place and on the same date as the holotype.

Description. Female (Figs. 1–3): total body length, measured from tip of rostrum to posterior margin of caudal rami, 960–973 µm (mean: 970 µm; n = 3), prosome/urossome ratio about 1.6 (Fig. 1A). Preserved specimens colourless, naupliar eye not discernible. Total body length/width ratio about 3.2, prosome with numerous sensilla, in bilaterally symmetrical pattern. Rostrum triangular, with rounded tip, bearing two sensilla (Fig. 4B). Integument smooth, not strongly chitinised, without any visible cuticular windows. Cephalothorax slightly shorter than wide, representing 43% of body length, with the greatest width near posterior margin. Posterior margins of Pd1–Pd5 smooth. Genital double-somite as long as wide, slightly tapering posteriorly (Fig. 1A, C, D). Copulatory pore small and oval-shaped, positioned at 1/3 length of somite; copulatory duct short, narrow, and sclerotised. Seminal

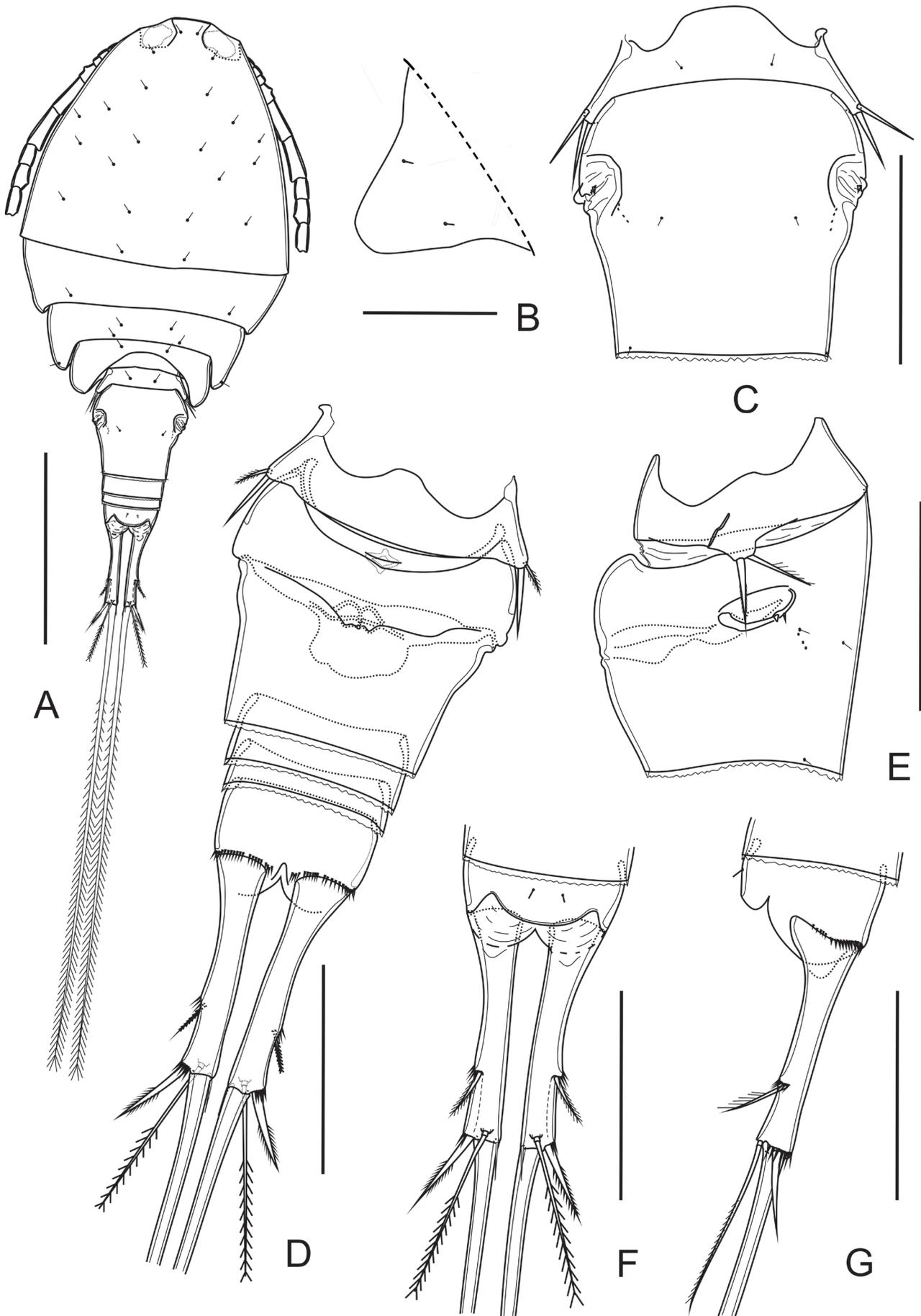


Fig. 1. *Pseudograeteriella longiaesthetascus* new genus, new species, female. A, habitus, dorsal view; B, rostrum, frontal view; C, genital double-somite, dorsal view; D, urosome, ventral view; E, genital double-somite, lateral view; F, caudal rami, dorsal view; G, caudal ramus, lateral view. Scale bars: A, 250 μ m; B, 50 μ m; C–G, 100 μ m.

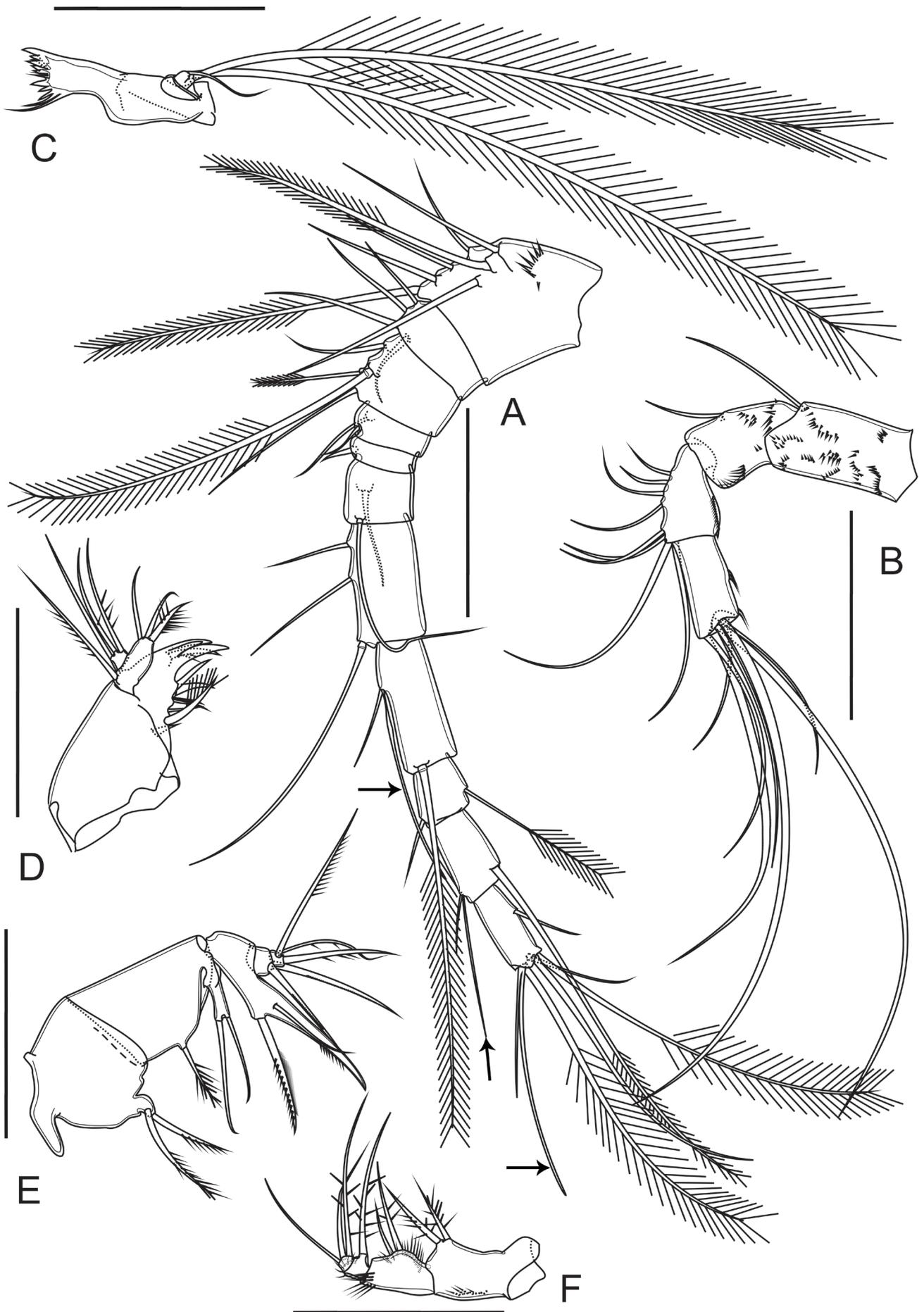


Fig. 2. *Pseudograeteriella longiaesthetascus* new genus, new species, female. A, antennule (arrow indicates the aesthetasc); B, antenna; C, mandible; D, maxillule; E, maxilla; F, maxilliped. Scale bars: 100 μ m.

receptacle about 1/4 of double-somite length, with short and wide anterior portion and well-developed, semi-circular posterior part (Fig. 1D). Ovipores situated dorsolaterally at 1/3 length of somite, covered with reduced P6 (Fig. 1E). Hyaline fringes of genital double-somite and two subsequent somites irregularly serrate both ventrally and dorsally. Anal somite with rows of spinules on posterior margin ventrally and laterally. Anal operculum reaching anterior margin of caudal ramus, free margin of operculum broadly rounded and smooth (Fig. 1F), two large sensilla present anteriorly to operculum.

Caudal rami (Fig. 1D, F, G): elongate and parallel, about 4.2 times as long as wide, with spinules at insertion of anterolateral seta (II) and posterolateral seta (III) (Fig. 1F, G). Anterolateral accessory seta (I) reduced. Anterolateral seta (II) pinnate, inserted at 3/4 length of ramus. Posterolateral seta (III) spiniform. Outer terminal seta (IV) completely reduced. Inner terminal seta (V) without breaking plane. Terminal accessory seta (VI) short and weak. Dorsal seta (VII) articulated, inserted near inner terminal seta. Length ratio of caudal setae II–VII to ramus length (excluding reduced seta IV): 0.2, 0.4, 5.2, 0.1 and 0.8, respectively. Relative length of caudal setae II–VII (excluding reduced seta IV): 1.0 : 2.3 : 29.9 : 0.8 : 4.8.

Antennule (Fig. 2A): 11-segmented, not reaching posterior margin of cephalothorax (Fig. 1A). Armature formula: 7.4.4.2.1.2.3.2+ae.2.2+ae.6+ae. Fourth and fifth segments short, fifth segment with spiniform seta. Aesthetascs on the eighth and penultimate segments inserted near anterodistal seta. Aesthetascs on segments 8 and 10, three and two times as long as corresponding anterodistal seta, respectively. The terminal segment with acrothek subapically, ae is about twice as long as adjacent seta.

Antenna (Fig. 2B): four-segmented, comprising coxobasis and three-segmented Endp. Coxobasis with one smooth seta on inner distal corner, without seta representing Exp, caudal surface adorned with several groups of spinules. Endp-1 with one smooth seta at 1/2 length of inner margin, adorned with several groups of spinules. Endp-2 about 1.5 times as long as wide, with eight smooth setae (six laterally, one subapically, one apically). Endp-3 about twice as long as wide, with seven smooth apical setae of different lengths, two of them robust, about seven times as long as segment bearing them.

Mandible (Fig. 2C): with coxa and short basis partly fused with coxa. Gnathobase with strongly chitinised teeth, ventral-most teeth very robust and slightly obtuse, with smooth seta dorsally. Mandibular palp small, one short, smooth seta, two long, plumose setae representing Exp and Endp, respectively, long setae about ten times as long as short seta.

Maxillule (Fig. 2D): with robust praecoxa and two-segmented palp, proximal segment of palp coxobasis, lateral one Endp. Arthrite of praecoxa bearing seven lateral spines, five of them smooth, proximal one minute, subproximal one robust and pinnate, three strong claw-like spines decreasing in length

apically and one strong curved seta subapically. Coxobasis with three elements: one smooth and one robust bipinnate seta apically, smooth seta subapically. Endp with three smooth setae apically. Exp represented by one seta.

Maxilla (Fig. 2E): five-segmented. Endite of praecoxa with two unipinnate setae. Proximal endite of coxa with one pinnate seta, distal endite elongated, with two setae unequal in length apically. Basis with slim claw-like basal endite, with a row of minute spinules along concave margin. Two setae at base of claw, longer one as long as claw and robust, other one short and slim. Endp two-segmented, proximal segment with two robust setae, distal segment with one robust seta apically, accompanied by two shorter setae subapically.

Maxilliped (Fig. 2F): four-segmented, composed of syncoxa, basis and two-segmented Endp. Syncoxa with a group of spinules on lateral margin, distal endite with two pinnate setae, unequal in length. Basis with row of long spinules on proximal medial margin, group of spinules at distal outer corner, two setae along medial margin unequal in length. Endp-1 with one long, strong pinnate seta. Endp-2 with three setae, one pinnate, two smooth.

Intercoxal sclerites in P1–P4 (Fig. 3A–D): unornamented, distal margin broadly concave, minute rounded projections only slightly overreaching distal margin of sclerite. Coxa rectangular, cuticular pore present anteriorly, close to medial margin. Long plumose seta present at inner distal angle of P1–P3 coxa, corresponding seta absent in P4. Basis with plumose seta laterally. Exp and Endp two-segmented. Basis triangular, with rounded tips. Exp-2 and Endp-2 of P2–P4 with cuticular pore anteriorly, near insertion of subapical and apical spine, respectively. Exp-2 spine and seta formula: 3.3.3.3 and 5.5.5.4, respectively. Endp-2 spine and seta formula: 1.1.1.1 and 4.5.5.4. Complete armament of P1–P4 is shown in Table 2.

P1 (Fig. 3A): basis with strong medial spine reaching middle of Endp-2. Exp-1 and Exp-2 as long as wide. Spine on Exp-1 blunt. Endp-2 about 1.5 times as long as wide, apical spine as long as segment bearing it.

P2 (Fig. 3B): Exp-1 as long as wide, Exp-2 about 1.3 times as long as wide. Endp-1 slightly shorter than its width, Endp-2 1.8 times as long as wide; apical spine as long as segment bearing it.

P3 (Fig. 3C): Exp and Endp identical to P2 in shape, armament and proportions. Apical spine on Endp-2 shorter than segment, blunt and slightly curved.

P4 (Fig. 3D): Exp and Endp similar to P2 and P3 in shape and proportions; apical spine on Endp-2 slightly longer than segment, straight.

P5 (Fig. 1D): reduced to cuticular lobe completely fused to Pd5; two setae present apically, equal in length; dorsal seta uni-pinnate, ventral seta smooth and spiniform.

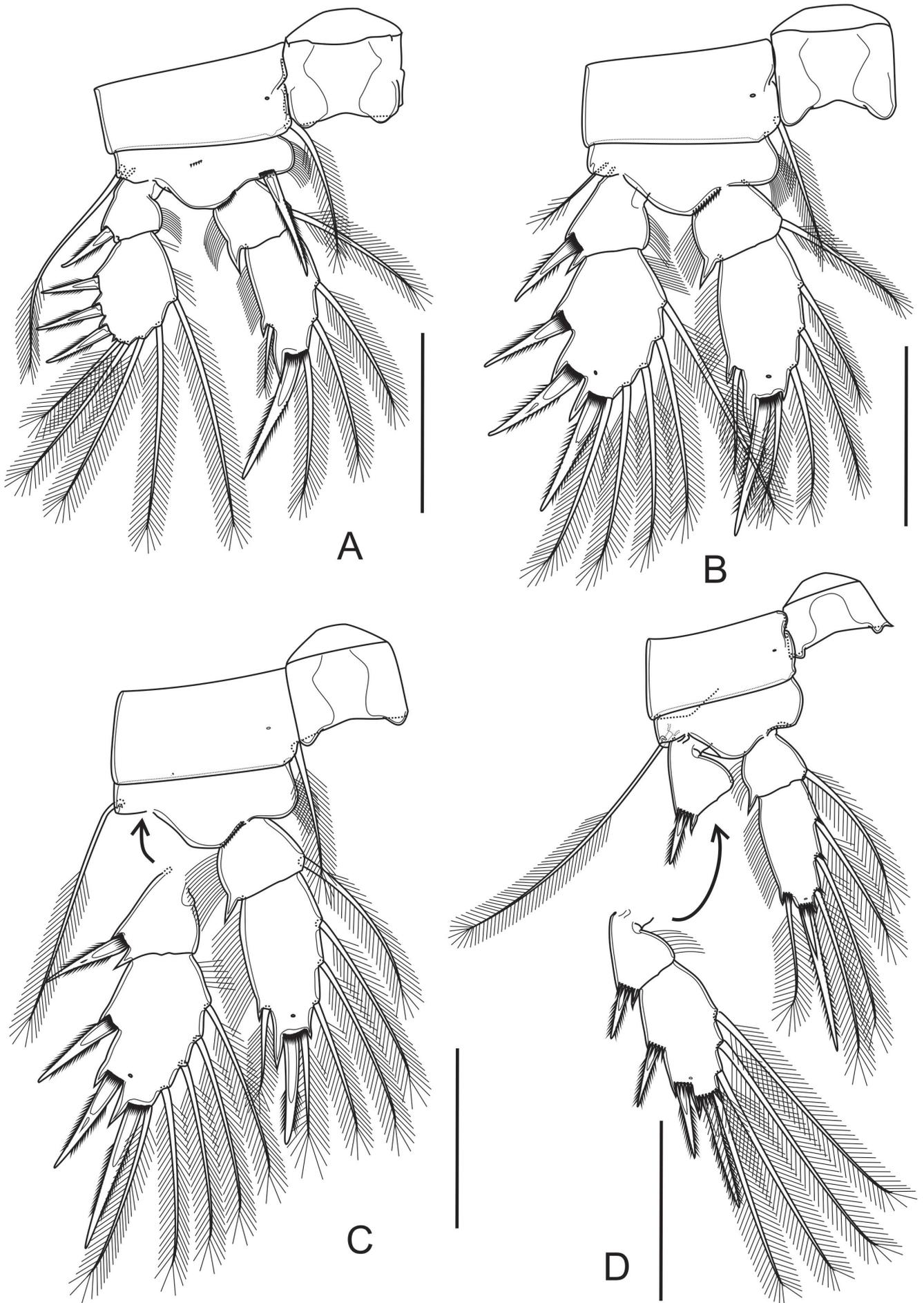


Fig. 3. *Pseudograeteriella longiaesthetascus* new genus, new species, female. A, P1; B, P2; C, P3; D, P4. Scale bars: 100 μ m.

Table 2. Armament of thoracic legs P1–P4 in *Pseudograeteriella longiaesthetascus* new genus, new species (Roman numbers = spines; Arabic numbers = setae).

Thoracic leg	Coxa	Basis	Exopod		Endopod	
			1	2	1	2
P1	0–1	1–I	I–0	III–2–3	0–1	1–I,1–2
P2	0–1	1–0	I–0	II–I,1–4	0–1	1–I,1–3
P3	0–1	1–0	I–0	II–I,1–4	0–1	1–I,1–3
P4	0–0	1–0	I–0	II–I,1–3	0–1	1–I–3

P6 (Fig. 1D): small, simple cuticular plate positioned laterodorsally on genital double-somite, with two minute spines.

Male (Fig. 4): total body length, measured from tip of rostrum to posterior margin of caudal rami, 796–880 μm (mean: 859 μm ; $n = 3$). Habitus slender than in female (Fig. 4A). Body length/width ratio about 3.1, prosome/urosome ratio about 1.5 (including caudal rami), naupliar eye not discernible. Prosome with numerous sensilla, in bilaterally symmetrical pattern along body axis. Rostrum as in female.

Cephalothorax anteriorly oval, as long as wide, representing 33% of body length, with greatest width at posterior end. Posterior margins of all pedigerous somites smooth. Genital somite barrel-shaped (Fig. 4A, B), about 21% of length of urosome (including caudal rami). Genital somite and three subsequent somites with hyaline fringes with irregular serration both ventrally and dorsally. Anal somite and anal operculum as in female.

Caudal rami slightly shorter than in female, about 4.1 times as long as wide. Armament and ornamentation similar to those in female. Length ratio of caudal setae II–VII to ramus length (excluding reduced seta IV): 0.2, 0.4, 6.0, 0.1, and 0.8, respectively. Length ratio of caudal setae II–VII (excluding reduced seta IV): 1.0 : 2.0 : 28 : 0.5 : 3.7.

Antennule (Fig. 4C): 15-segmented, geniculate. Armature formula as follows (however, number of elements on some segments couldn't be verified): 7+3ae.4.2.2+ae.2.2.0.2.0.2+ae.1+I.2+ae.1.2.11+ae. All aesthetascs except aesthetasc on segment 15 very long. Terminal segment with acrothek.

Antenna, mouthparts, P1–P5 similar to those in female, but P4 Endp-2 bearing two inner setae only. P4 Endp-2 twice as long as wide, apical spine slightly longer than segment.

P6 (Fig. 4B): trapezoidal plate, with two sub-equal spiniform setae inserted laterally.

Variability. In one male specimen, the proximal most medial seta on the terminal endopodal segment of P1 was missing on the left side, but it was observed to be normally developed on the right side. Other differences were not observed, except for some minor variations in size, shape, and number of spinules ornamenting the limbs and somites.

Etymology. The specific epithet alludes to very long aesthetascs located on the male antennules. It is used as a noun in apposition.

Remarks. *Pseudograeteriella longiaesthetascus*, new species, shares some characters with *P. longifurcata* (Tran & Chang, 2013), which was described from the same cave in Central Vietnam. The characters that distinguish the new species from *P. longifurcata* are, as follows: i) in *P. longiaesthetascus*, new species, the outer terminal caudal seta (seta IV) is completely reduced (Fig. 1D, F); however, in *P. longifurcata*, seta IV is present and appears as a stout spine slightly shorter than the posterolateral seta (III) (Tran & Chang, 2013: fig. 1E, F); ii) in *P. longiaesthetascus*, new species, the anterolateral seta (II) is inserted laterally at 3/4 length of the ramus (Fig. 1G); however, in *P. longifurcata*, it is “inserted dorsally at about the middle of the caudal ramus” (Tran & Chang, 2013: fig. 1E); iii) in *P. longiaesthetascus*, new species, the ventral spiniform seta of P5 is robust and slightly longer than dorsal seta (Fig. 1D, E); however, it is slim and equal to the dorsal seta in *P. longifurcata* (Tran & Chang, 2013: fig. 1C, D); iv) there are eight setae on Endp-2 of antenna in *P. longiaesthetascus*, new species (Fig. 2B), but five in *P. longifurcata* (Tran & Chang, 2013: fig. 2B); v) aesthetascs on the first and fourth segments of the male antennule overreach the geniculate section in *P. longiaesthetascus*, new species (Fig. 4C), whereas they are much shorter in *P. longifurcata* (Tran & Chang, 2013: fig. 4C); vi) the apical spines on the terminal endopodal segments of P1, P2, and P4 are as long as corresponding segments in *P. longiaesthetascus*, new species (Fig. 3A, B, D), but they are distinctly shorter in *P. longifurcata* (Tran & Chang, 2013: fig. 3A, B, D); vii) prominences on the intercoxal sclerites are minute in *P. longiaesthetascus*, new species (Fig. 3A–D), but conspicuous in *P. longifurcata* (Tran & Chang, 2013: fig. 3A–D).

Both species were collected in different habitats. *P. longifurcata* was collected in a syphon lake located about 1,200 m from the tourist entrance, and *P. longiaesthetascus*, new species was collected in a pool filled with drip water located approximately 400 m from the tourist entrance. Although physical and chemical parameters in these two habitats were not measured at the time of specimen collection, records from locations in Europe indicate significant differences between the two habitats (Brancelj, 2002). It is possible that populations, both in the syphon lake and in the

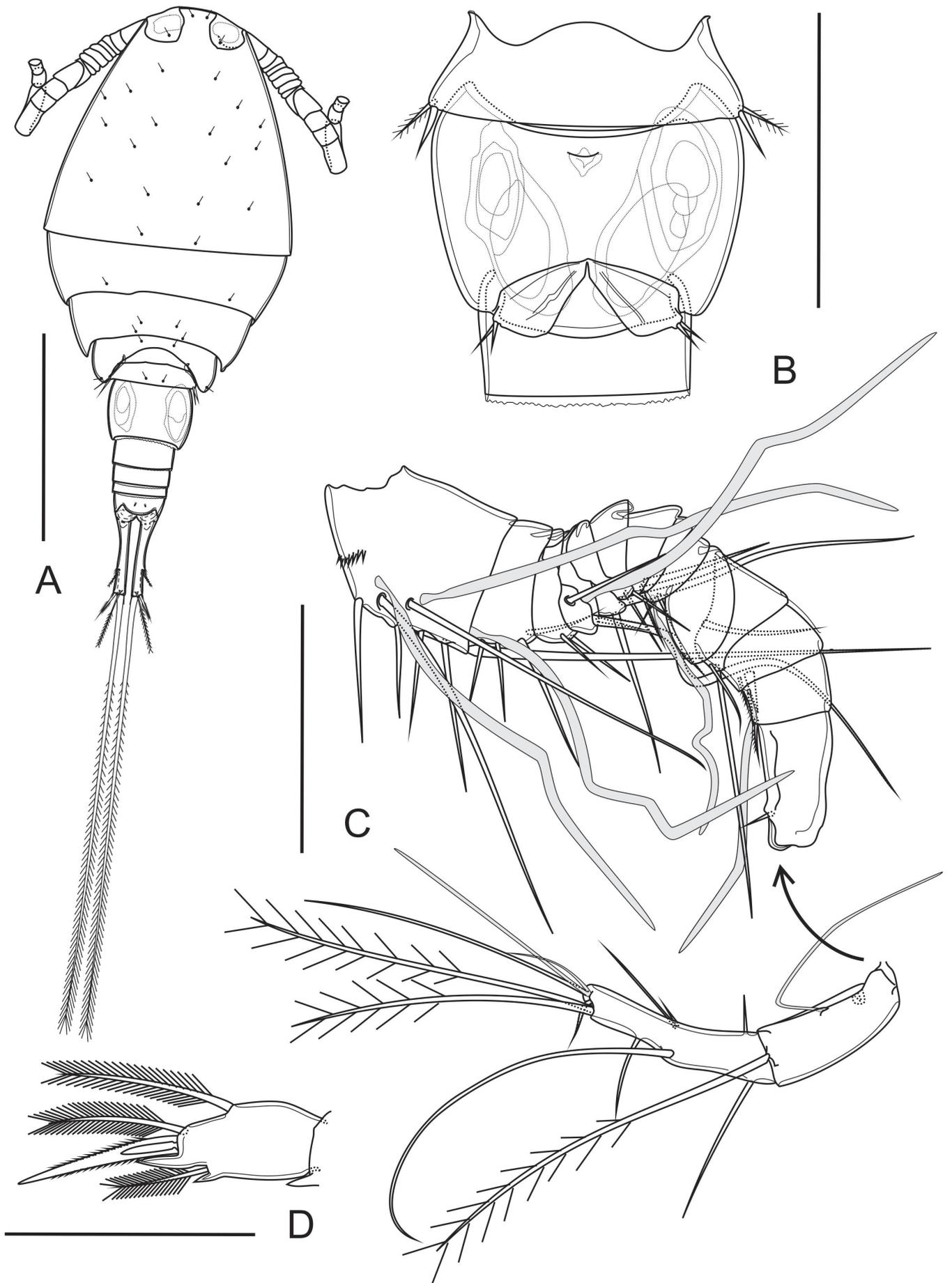


Fig. 4. *Pseudograeteriella longiaesthetascus* new genus, new species, male. A, habitus, dorsal view; B, genital somite, ventral view; C, antennule; D, Endp-2 of P4. Scale bars: A, 250 μm ; B–D, 100 μm .

pools, represent “a sink population” that is washed by dripping water from the epikarst zone, with each drip point acting as an “island” due to very limited lateral communication between and among adjacent drip points (Brancelj, 2006). Coexistence of two closely related species within a small geographical area indicates that sympatric speciation might occur within ancestral populations. The reduction in outer apical caudal seta (seta IV) and the remarkably elongated aesthetascs on the male antennule in *P. longiaesthetascus*, new species, suggests a more specialised and older lineage in both of these subterranean dwellers.

Bryocyclops Kiefer, 1927

***Bryocyclops anninae* (Menzel, 1926)**

(Figs. 5A–G, 6A–F, 7A–D, F [female]; 7E, 8A–D [male])

Material examined. Three females and three males were collected from a water pool in Thien Duong Cave on 21 March 2012 by L. Sanoamuang; (Quang Bin Province, Phong Nha-Ke Bang National Park, Quang Bin Province, central Vietnam. Coordinates of the cave entrance were: 17°31'09.46"N, 106°13'17.80"E, altitude: 200 m a.s.l.). One female (total body length 850 µm) and one male (total body length 755 µm) completely dissected, each of them mounted on one slide, NHMUK 2018.1015 (female), NHMUK 2018.1016 (male). Two adult females, two adult males, stored in 70% ethanol, deposited in the Khon Kaen University Science Museum, KKU–COP–2018.005–008.

Description. Female (Figs. 5–7): total body length measured from tip of rostrum to posterior margin of caudal rami, 811–850 µm (mean: 830 µm; n = 3), prosome/urosome ratio about 1.9 (Fig. 5A). Preserved specimens colourless, naupliar eye not discernible. Body length/width ratio about 3.2, slightly compressed dorsoventrally. Integument not strongly chitinised, with conspicuous refractile points throughout body surface, without cuticular windows. Cephalothorax about 0.8 times as long as wide, representing 32% of body length, greatest width at posterior end. Posterior margins of Pd1–Pd5 smooth. Genital double-somite well developed, about 0.8 times as long as wide (Fig. 5A, B). Copulatory pore small, oval-shaped, situated at 1/2 length of somite, copulatory duct short, narrow and well sclerotised. Seminal receptacle with well-developed anterior part, representing about 1/4 of double-somite length, posterior part very small (Fig. 5B). Ovipores situated laterally, at 1/2 length of somite, covered by a reduced P6 (Fig. 5B, C). Genital double-somite and two subsequent somites with serrate hyaline fringes with irregular serration both ventrally and dorsally. Anal somite with rows of spinules on posterior margin ventrally. Anal operculum well-developed, triangular, with smooth free margin, reaching 1/2 length of caudal rami, at the base of operculum two large sensilla (Fig. 5D).

Caudal rami (Fig. 5A, B, D, E): parallel, each ramus about twice as long as wide, with low ridge on dorsal side along longitudinal axis. Anterolateral accessory seta (I) reduced. Anterolateral seta (II) bare, inserted at 1/2 length of ramus. Posterolateral seta (III) bipinnate. Outer terminal seta (IV)

plumose, with breaking plane; inner terminal seta (V) longest, plumose, with breaking plane, about 0.5 times as long as body length. Terminal accessory seta (VI) stout and bare, very short. Dorsal seta (VII) articulate, bare, inserted near insertion of inner terminal seta (V). Length ratio of caudal setae II–VII to ramus length: 0.8, 1.4, 4.1, 7.4, 0.2 and 1.6, respectively. Length ratio of caudal setae II–VII: 1.0 : 1.8 : 3.7 : 9.5 : 0.3 : 2.0.

Antennule (Fig. 6A): 11-segmented, nearly reaching posterior margin of cephalothorax, second and third segments partly fused. Setal formula: 6.2.4.2.0+1.2.3.2+ae.2.3.7+ae. Fifth segment with short spine on anterodistal margin. Terminal segment with acrothek subapically.

Antenna (Fig. 6B): 4-segmented, comprising coxobasis and 3-segmented Endp. Coxobasis with one smooth seta on distal inner corner, without seta representing Exp. Endp-1 with group of spinules on outer margin, one smooth seta at 1/2 length of inner margin. Endp-2 about twice as long as wide, with five smooth setae on inner margin (three laterally, two apically; apical setae robust and recurved). Endp-3 twice as long as wide, with seven smooth setae apically.

Labrum (Fig. 5F): trapezoidal, cutting edge strongly chitinised, with two large indentations laterally, concave row of a dozen teeth medially, two rows of spinules ventrally.

Mandible (Fig. 6C): with strongly chitinised gnathobase, cutting edge with pinnate seta dorsally. Mandibular palp completely reduced, represented by one short, bare seta.

Maxillule (Fig. 6D): with robust praecoxa and 2-segmented palp, proximal segment of palp coxobasis, distal one Endp. Arthrite of praecoxa bearing: six armature elements along inner margin, five of them smooth, proximal one robust and pinnate, distal one robust and curved; three claw-like extensions apically; and one curved seta laterally. Coxobasis with proximal seta representing Exp, two setae apically, one slender and one robust, bipinnate, and one slender seta subapically. Endp with two setae apically, equal in length, one shorter seta subapically.

Maxilla (Fig. 6E): 5-segmented. Endite of praecoxa conspicuous, with two plumose setae. Proximal endite of coxa with one smooth seta apically, distal endite elongate, with one unipinnate seta and one smooth seta. Basis with claw-like expansion, with row of spinules along concave margin, two setae at base of claw, longer seta as long as claw, robust, other one short and slim, 0.3 times as long as longer seta. Endp 2-segmented, proximal segment with two robust setae, distal segment with one robust seta apically, accompanied by two slender setae, unequal in length, longer one as long as robust seta, shorter half of it.

Maxilliped (Fig. 6F): 4-segmented, comprised syncoxa, basis and 2-segmented Endp. Syncoxa with two setae, shorter one pinnate, longer one smooth, twice as long as shorter seta, row of few spinules along outer margin. Basis with one bipinnate seta, with row of spinules along inner margin and

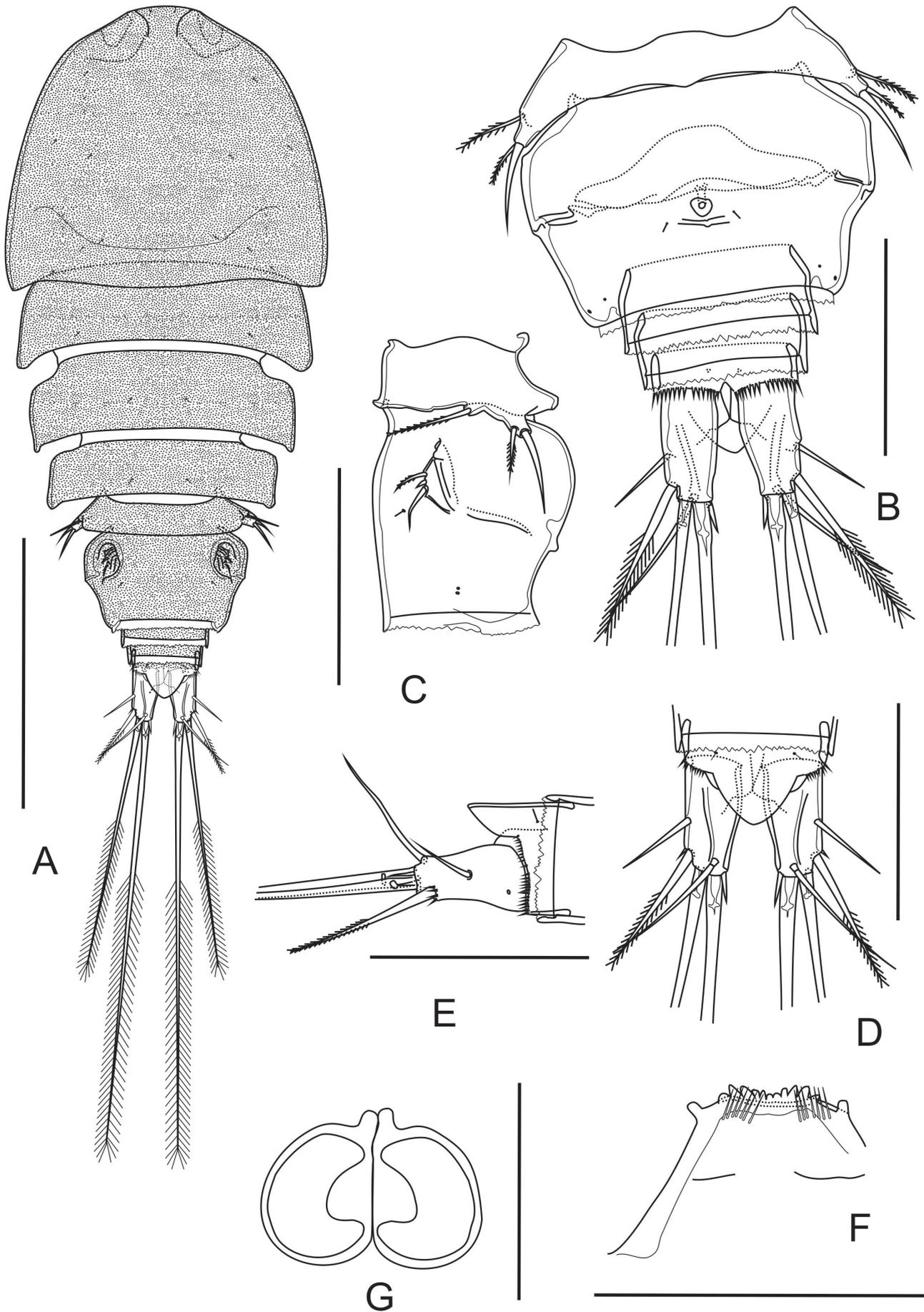


Fig. 5. *Bryocyclops anninae*, female. A, habitus, dorsal view; B, urosome, ventral view; C, genital double-somite, lateral view; D, caudal rami, dorsal view; E, caudal ramus, lateral view; F, labrum, ventral view; G, spermatophore, ventral view. Scale bars: A, 250 μ m; B–G, 100 μ m.

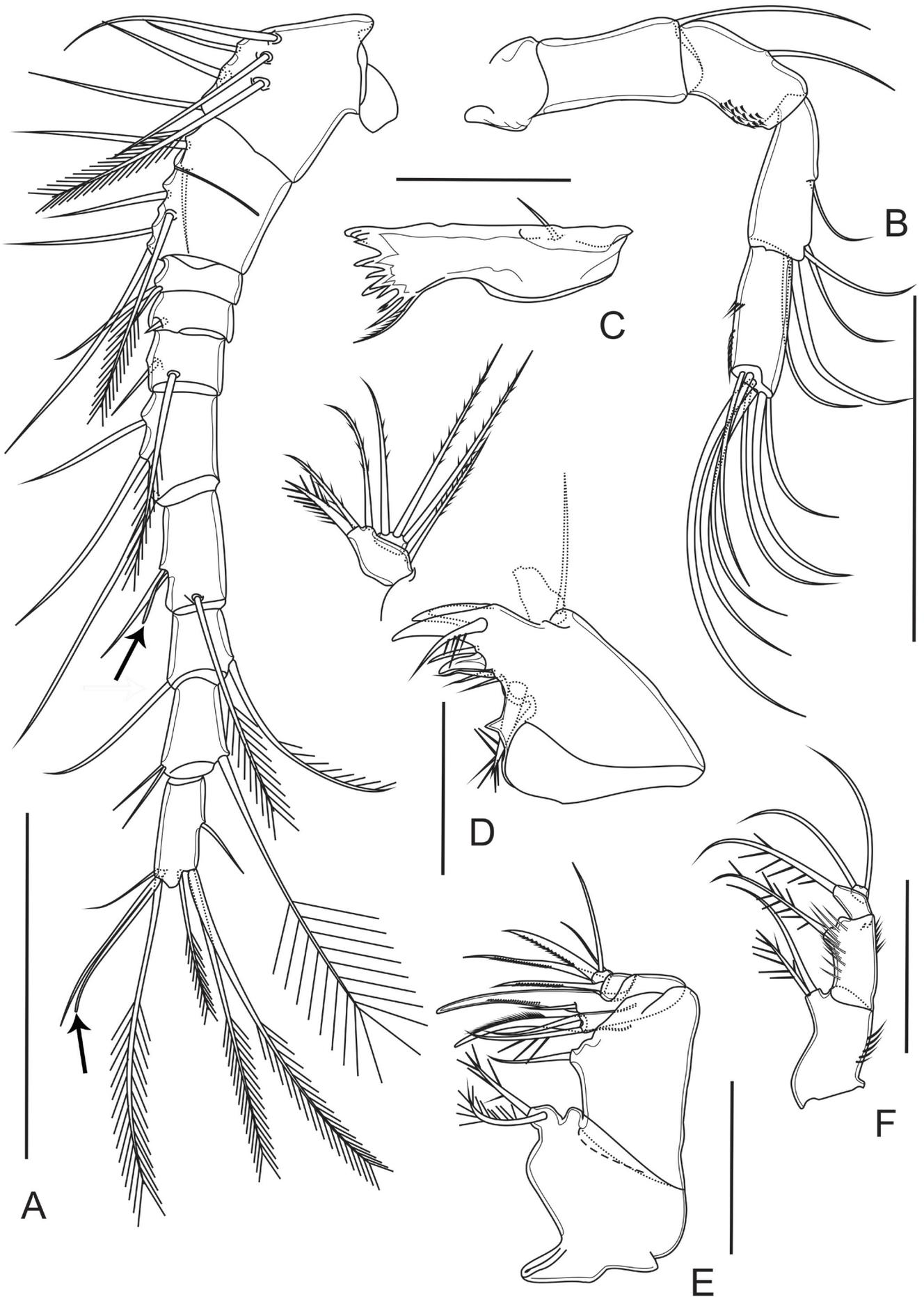


Fig. 6. *Bryocyclops anninae*, female. A, antennule (arrow indicates the aesthetasc); B, antenna; C, mandible; D, maxillule; E, maxilla; F, maxilliped. Scale bars: A–B, 100 μ m; C–F, 50 μ m.

Table 3. Armament of thoracic legs P1–P4 in *Bryocyclops anninae* (Menzel, 1926).

Thoracic leg	Coxa	Basis	Exopod		Endopod	
			1	2	1	2
P1	0–1	1–I	I–0	II–I, 1–4	0–1	1–I–2
P2	0–0	1–0	I–0	II–I, 1–4	0–1	1–I, 1–2
P3	0–0	1–0	I–0	II–I, 1–4	0–1	1–I, 1–3
P4	0–0	1–0	I–0	II–I, 1–3	0–1	1–I, 1–1

row of spinules on distal outer margin. Proximal segment of Endp with one strong, pinnate seta. Distal segment armed with 2 smooth setae.

Intercostal sclerites of P1–P4 (Fig. 7A–D, F): unornamented, free margin of sclerite concave, distal projections rounded in P1 and acute in P2–P4. Coxa rectangular, one cuticular pore present anteriorly, close to medial margin. Long plumose seta present on inner distal corner of P1, coxal seta absent in P2–P4. Basis with medial expansions rounded, long plumose outer seta present on P1, P3 and P4, corresponding seta short and smooth on P2. Exp and Endp 2-segmented. Exp-2 spine and setae formula: 3.3.3.3 and 5.5.5.4. Endp-2 spine and setae formula: 1.1.1.1 and 3.4.5.3. Complete armament of P1–P4 is shown in Table 3.

P1 (Fig. 7A): Exp-1 small, as long as wide, with one blunt and slightly curved spine. Exp-2 about 1.8 times as long as wide. Endp-2 about 1.3 times as long as wide, apical spine as long as segment, slightly curved and blunt.

P2 (Fig. 7C): Exp-1 small, as long as wide. Exp-2 1.8 times as long as wide, with blunt spines, medial setae with blunt tips. Endp similar to that of P1, but terminal segment with two setae on inner margin, apical spine as long as terminal segment, straight, with sharp tip.

P3 (Fig. 7D): identical to P2 but with three setae on inner margin of Endp-2, apical spine straight, as long as segment bearing it. Setae on Exp without blunt tips.

P4 (Fig. 7F): Exp-2 about 1.8 times as long as wide, similar in shape to P2 and P3 but with relatively weak spines. Endp-1 robust, slightly wider than long, with cuticular expansion on proximal inner corner. Endp-2 rectangular, slightly longer than wide, apical spine slim, as long as segment bearing it.

P5 (Fig. 5B, C): reduced to two cuticular lobes, completely fused to Pd5, both lobes well separated. Dorsal lobe minute, with one slender seta oriented dorsally. Ventral lobe more conspicuous, dorsal seta short and plumose, ventral seta longer, robust and smooth; ventral seta about twice as long as dorsal one.

P6 (Fig. 5C): small, forming simple cuticular plate, inserted laterodorsally on genital double-somite, armed with three

elements: anterior seta plumose, middle one shortest, spiniform posterior one longest and slightly longer than anterior one.

Spermatophore (Fig. 5G): paired, kidney-shaped; with thick walls.

Male (Fig. 8): total body length, measured from tip of rostrum to posterior margin of caudal rami, 713–755 μm (mean: 730 μm ; $n = 3$). Habitus slightly slender than in female (Fig. 8A), body length/width ratio about 3.2, prosome/urosome ratio about 2.1, naupliar eye not discernible. Cephalothorax and Pd2–Pd4 similar to female. Cephalothorax slightly wider than long, representing 33% of body length. Genital somite large, globular (Fig. 8A, B), about 0.3 times as long as whole urosome, including caudal rami. Hyaline structures on dorsal and lateral margin of genital somite well developed (Fig. 8A, B). Subsequent three urosomites distinctly narrower than genital somite, with irregularly serrated hyaline fringes ventrally and laterally. Anal somite and anal operculum as in female (Fig. 8A). Caudal rami (Fig. 8A) similar to that of female.

Antennule (Fig. 8C): 16-segmented, geniculate. Armament formula (however, number of elements on some segments couldn't be verified): 7+ae.4.2.2+ae.1.3.2.2.0.2.1.1+I.2.1.1.10+ae. Terminal segment with acrothek.

Antenna, mouthparts, P1, P2, P4 and P5 similar to those in female, but some details variable on P4 (see below).

P3: Exp similar to female. Endp-2 bearing modified apical spine with distal half curved and slightly widened, with finely serrated section, tip acute and hooked. Inner terminal seta of Endp-2 short, spiniform and smooth, half length of modified spine; subterminal inner seta short and also spiniform (Fig. 7E).

P6 (Fig. 8B): modified to large elongated subtrapezoidal plate with three setae laterally, subequal in length, middle one slim and longest, ventral one strong and spiniform, dorsal one slim and shortest.

Variation. In one male, P4 bears additional terminal seta (Fig. 8E). No other variations were observed, except for some minor variations in size, shape, and number of spinules ornamenting any limbs.

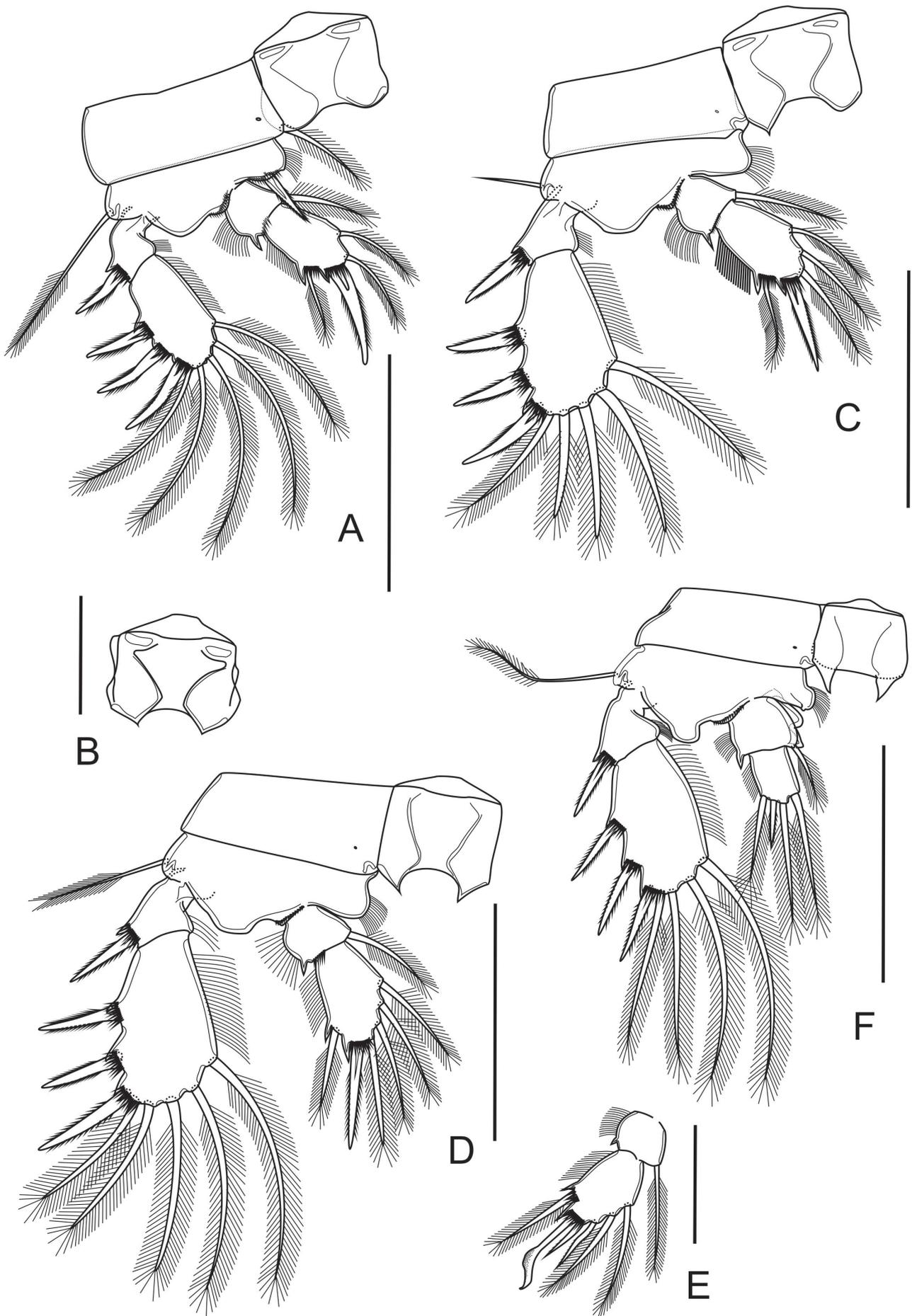


Fig. 7. *Bryocyclops anninae*. A, C, D, F, female. B, E, male. A, P1; B, intercoxal sclerite; C, P2; D, P3; E, End of P3; F, P4. Scale bars: A, C–D, F, 100 μ m; B, E, 50 μ m.

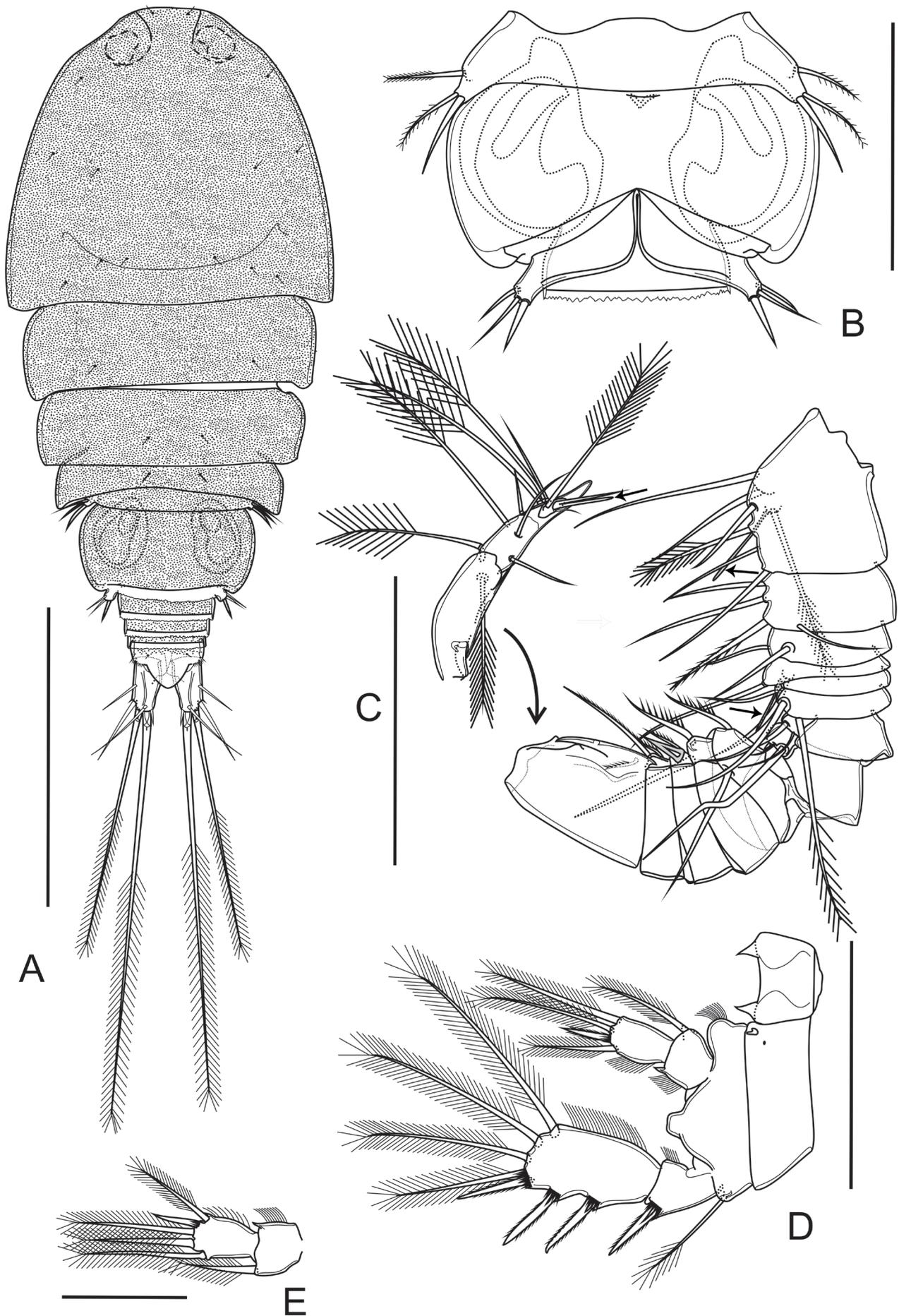


Fig. 8. *Bryocyclops anninae*, male. A, habitus, dorsal view; B, genital somite, ventral view; C, antennule (small arrow indicates the aesthetasc); D, P4; E, Endp of right P4. Scale bars: A, 250 μ m; B–D, 100 μ m; E, 50 μ m.

Remarks. The following characters were used for generic identification: i) 11-segmented antennule (Fig. 6A); ii) thoracic legs with 2-segmented rami (Fig. 7A, C, D, F); iii) P5 completely fused with Pd5 (Fig. 5B, C) (baseopodal seta inserted on a small laterodorsal lobe, a remnant of the proximal segment of P5, i.e., coxa, basis, and Endp; two setae inserted on ventral lobe, a remnant of the Exp P5); iv) acute prominences of the intercoxal sclerites in P2–P4 (Fig. 7C, D, F); v) absence of coxal seta in P2–P4 (Fig. 7C, D, F); vi) sexual dimorphism on the apical spine on the distal endopodal segment of P3 (Fig. 7D, E); vii) well-developed anal operculum (Fig. 5D); viii) integument ornamented with refractile points (Figs. 5A, 8A); and, ix) obtuse setae on distal exopodal segment of P2 and P3 (Fig. 7C, D). Based on the unique combination of some characters, such as the remarkably short terminal accessory seta (VI) and the smooth triangular-shaped anal operculum, we identified the Vietnamese specimens as *B. anninae* (Menzel, 1926). This species was originally described from Java, Indonesia, and later recorded from Guam, Hawaii, and Vanuatu (Menzel, 1926; Lindberg, 1954; Eldredge & Miller, 1997; Reid, 1999).

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