

A NEW *BRYOCYCLOPS* KIEFER (CRUSTACEA: COPEPODA: CYCLOPOIDA) FROM KARSTIC CAVES IN THAILAND

Santi Watiroyram

Applied Taxonomic Research Center, Department of Biology, Faculty of Science
Khon Kaen University, Khon Kaen 40002, Thailand
Email: santikku@hotmail.com

Anton Brancelj

National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
Email: anton.brancelj@nib.si

La-orsri Sanoamuang

Applied Taxonomic Research Center, Faculty of Science, Khon Kaen University, Khon Kaen 40002
and Faculty of Science, Mahasarakham University, Thailand
Email: la_orsri@kku.ac.th (Corresponding author)

ABSTRACT. — *Bryocyclops maewaensis*, new species, collected from a karstic cave in Lampang Province, (northern Thailand), is described. The new species can be distinguished from its congeners by several morphological characters, which include body ornamentation, the shape of anal operculum, three reduced-setae on P4 endopod-2 of the female, and particularly the modified spine on P3 endopod-2 of the male. It is most similar to *B. anninae* (Menzel, 1926), originally described from moist moss in Java. This is the first record of the genus in Thailand and the second representative of cave-dwelling Copepoda there. This species has so far been found only from pools filled with percolating water from the unsaturated zone of a karstic aquifer.

KEY WORDS. — *Bryocyclops maewaensis*, freshwater, subterranean habitats, stygobiont, cave-dwelling copepods

INTRODUCTION

There has been a significant increase in information on freshwater copepods in Thailand but mainly about the order Calanoida (Sanoamuang, 2001a, 2001b, 2001c; Sanoamuang & Teeramaethee, 2006; Proongkiat & Sanoamuang, 2008) thus, members of the Harpacticoida and Cyclopoida are less well known. Only few publications exist about Thailand cyclopoids (Pholpunthin, 1997; Sanoamuang, 1999; Alekseev & Sanoamuang, 2006) and most of the research on this group has been focused on limnetic waters. Other habitats, including subterranean habitats (i.e., porous and karstic aquifers) remain unstudied. Caves harbour especially rich fauna which is unique to the subterranean environment and several new species to science have been discovered there (Culver et al., 2004). Particularly, the unsaturated zone in karstic caves (epikarst included) appears to harbour unique species, frequently endemic, and restricted to a reduced area or even to a single site only (Brancelj & Culver, 2005; Brancelj, 2006, 2009).

A sampling campaign to collect cave-dwelling animals in Thailand was started in 2007. Results of this initiative include the discovery of the harpacticoid copepod *Elaphoidella namnaoensis* Brancelj, Watiroyram & Sanoamuang, 2010, from Tham Yai Nam Nao cave in Nam Nao National Park (Phetchabun Province) (Brancelj et al., 2010). Several other species, including some new to science, have been collected there so far.

Currently, the genus *Bryocyclops* Kiefer, 1927, contains 21 valid species, distributed across Africa, South and North America, and Asia (Reid, 1999; Dussart & Defaye, 2006). The genus *Bryocyclops* was first recorded in Southeast Asia by Menzel (1926), who described three new species from Java and Sumatra (Indonesia). Kiefer (1928) described an additional species from Java. After that no new information on *Bryocyclops* has been reported from Southeast Asia and it has not been hitherto recorded in Thailand. In this contribution we describe a new species of the genus from cave habitats in Thailand.

MATERIAL AND METHODS

Samples were collected between Mar.2008 and Oct.2010 from two caves in Lampang Province in northern Thailand (Tham Nam Phar Ngam cave in Mae Wa National Park and Tham Phar Ngam cave in Jae Sorn National Park) and one cave in the Kanjanaburi Province in central Thailand (Tham Pratoon cave) (Fig. 1). All samples were collected in an unsaturated zone, where water dripping from the ceiling forms pools on the floor. The pools are in a permanently dark section. Water from those water-bodies (volume of up to 10 L) was collected and filtered by means of special sampling equipment (Brancelj & Culver, 2005) with a mesh size of 60 μm .

Samples from individual pools were stored in plastic bottles immediately after sampling, and formaldehyde was added to a final concentration of 4%. Animals were sorted under a stereomicroscope and stored in 70% ethanol. Before dissection, specimens were placed in a mixture of glycerol and 70% alcohol (ratio ~ 1:10 v/v), which was replaced within one hour by pure glycerol. They were dissected at 100 \times magnification under an Olympus SZH-2 stereomicroscope. All appendages and body ornamentation were examined under a magnification of 1000 \times . All drawings, except for the female/male habitus, were made at the same magnification (1000 \times) with the aid of a drawing tube mounted on an Olympus compound microscope (BHS 40). The final version of the drawings was made using the CorelDRAW® 12 graphic



Fig.1. Sampled locality of *Bryocyclops maewaensis*, new species, from northern and central Thailand. 1, Tham Nam Pha Ngam cave; 2, Tham Phar Ngam cave; and 3, Tham Pratoon cave (1 & 2, Lampang Province; 3, Kanjanaburi Province).

program. For permanent slides, all body parts were mounted in glycerol and sealed with nail varnish.

Material examined are deposited in the Science Museum of Khon Kaen University, Thailand (KKU) and the Natural History Museum, London (NHM). The following abbreviations are used throughout the text and figures: Endp = endopod; Exp = exopod; Exp/Endp-1 = proximal segment; Exp/Endp-2 = middle segment; Exp/Endp-3 = distal segment; P1–P6 = thoracic appendages. The nomenclature and descriptive terminology follows that of Huys & Boxshall (1991).

TAXONOMY

Bryocyclops maewaensis, new species

(Figs. 2–6)

Type locality. — Tham Nam Phar Ngam cave is located in Mae Wa National Park (Lampang Province, northern Thailand) (17°28'46.4"N, 99°10'03.2"E). The cave is about 300 m long, with horizontal galleries only and 1–3 m high. The entrance is under a high cliff, at an elevation of 260 m a.s.l. In a permanently dark section, there are three Buddha statues, each of them with a concrete-pool (volume of about 5 to 10 L) in front of them. Pools are filled with water dripping from the cave roof. Those pools were designed as the type locality. The largest pool on the floor was 20 cm deep, 40 cm wide, and 60 cm long, with a water temperature of 20.1°C, pH 8.9, and a conductivity of 685 $\mu\text{S cm}^{-1}$.

Material examined. — Holotype: adult female, completely dissected, semi-permanent slide in glycerol, sealed with nail varnish (cat. no.: NHM 2011.2072). Collected 20 Mar.2008.

Allotype: adult male, completely dissected and mounted on a slide in glycerol and sealed with nail varnish (cat. no.: NHM 2011.2073). Collected 20 Mar.2008. Paratypes: three females and three males (stored in 70% ethanol), same locality and date (cat. no.: NHM 2011.2074–2079); two females and two males (cat. no.: KKU-COP-2008, 001–004). Additional material: specimens from pool on muddy floor (12 females and 7 males; cat. no.: KKU-COP-2009, 001–019) and concrete-pool (21 females and 13 males; cat. no.: KKU-COP-2009, 020–054) on type locality collected on 5 Oct.2009; 7 females and 5 males (cat. no.: KKU-COP-2009, 055–067) from pool on muddy floor in Tham Phar Ngam cave collected on 6 Oct.2009. All specimens collected by first author (SW).

Description. — Female (Figs. 2–4), body length, measured from tip of rostrum to posterior margin of caudal rami, 350–470 μm , (mean: 420 μm ; n = 10), colourless. Prosome moderately slender, dorso-ventrally flattened; cephalothorax wider than rest of body (Fig. 2A). Naupliar eye not discernible. Cephalosome (incl. rostrum), pedigers 2–5, genital double-somite, urosomites, and anal somite (incl. anal operculum) covered with light spots. Cephalosome with dorsal cuticular pores postero-laterally. Posterior margins of cephalosome and pedigers 2–4 with dorsally irregularly serrated hyaline frills. Genital double-somite (Fig. 2B–E) symmetrical, slightly expanded anteriorly, about 1.2 \times as wide as long, with irregularly serrated posterior fringes on dorso-ventral margin, latter ornamentation also on two subsequent urosomites. Genital double-somite with pair of dorsal sclerotized and rounded structures accompanied by

three minute spinules on inner margin; single copulatory pore ventrally. Anal somite (Fig. 2B, C, E) with transverse row of spinules along posterior margin on dorso-ventral surface.

Anal operculum (Fig. 2A, B, E) prominent, ovate, irregularly serrated along distal margin, overreaching posteriorly to approximately 1/2 of caudal rami length. Caudal rami (Fig. 2B, C) asymmetrically conical, each about twice as long as wide, with dorsal longitudinal keel along distal 1/3 of its length. Inner margin smooth. Lateral seta (II) bare, slightly shorter than caudal ramus, inserted at about the middle of its length. Dorsal seta (VII) bare, longer than caudal ramus, inserted at distal end of the keel. Outermost terminal seta (III) pinnate, slightly longer than dorsal seta, with spinules at insertion point on ventral side. Outer terminal seta (IV) about 3× as long as caudal ramus, about distal two-thirds with hairs on both margins, breaking plane not visible. Inner terminal seta (V) approximately 5× as long as caudal ramus, distal two-thirds with same arrangement of hair-like structures as on outer terminal seta, with no discernible breaking plane. Innermost terminal seta (VI) bare; relatively long, reaching 1/2 length of caudal ramus.

Rostrum (Fig. 3F) fused to cephalothorax, as long as width in frontal view, with parallel margins, blunt, anteriorly rounded; with pair of circular pores; dorsal surface smooth.

Antennule (Fig. 3A) relatively short, 11-segmented, not reaching posterior margin of cephalothorax; with refractile points on external surface (as on somites). Setal formula from base to tip as follows: 6.2.5.2.0.2.3.1+A.2.1+A.7+A. All setae slender.

Antenna (Fig. 3B) 4-segmented, comprising coxobasis and three-segmented endopodite. Exp absent. Endp-1 laterally with row of spinules; one inner seta at 2/3 of its length. Endp-2 with five smooth setae (three lateral, one subapical and one apical); with a row of spinules on outer margin. Endp-3 with seven terminal setae; with a row of spinules on outer margin.

Mandible (Fig. 3E) short, robust, with three strongly chitinized teeth on gnathobase; with short, smooth dorsal seta. Mandibular palp reduced to one bare and short seta inserted directly on gnathobase.

Maxillule (Fig. 3D) with robust praecoxa, coxobasis and 1-segmented Endp. Praecoxal arthrite with three strong apical spines fused to arthrite base; with six armature elements along inner margin, proximally one pinnate, others smooth. Coxobasis with two setae distally on basal endite: one slender and smooth, other one strong and pinnate. Exp represented by single smooth seta on outer surface of coxobasis. Endp with three slender and smooth setae.

Maxilla (Fig. 3C) 5-segmented, with praecoxa, coxa, basis, and 2-segmented Endp. Praecoxal endite with two plumose setae. Proximal coxal endite with single plumose seta; distal coxal endite highly mobile, with one smooth and one plumose setae. Basis with typically two claw-shaped expansion, densely serrate along concave margin; with one small seta at base of distal claw. Endp-1 with one smooth seta. Endp-2 with one smooth seta and two strong setae.

Maxilliped (Fig. 3G) 4-segmented, composed of syncoxa, basis and 2-segmented Endp. Syncoxa and basis with a row of spinules on outer margin. Syncoxa with one pinnate and one smooth seta. Basis with one unipinnate seta, accompanied with a row of long spinules at proximal inner margin. Endp-1 with one unipinnate seta at inner margin. End-2 with small segment plus two smooth setae.

P1 (Fig. 4A) with 2-segmented Exp and 2-segmented Endp. Endp smaller than Exp. Intercoxal sclerite with acute projection on distal margin. Coxa with one slender inner seta; with row of spinules laterally and pointed process latero-distally. Basis with slender outer seta and strong, robust inner spine with cluster of spinules at insertion point; setules on distal inner corner. Inner and outer margin of Exp and Endp, respectively, with setules. Exp-1 smaller than Exp-2; with outer spine. Exp-2 with two spines along outer margin, plus one spine and one seta apically, four setae along inner margin. Endp-1 and Endp-2 with row of long spinules along outer margin. Endp-1 with one seta on inner margin. Endp-2 with one seta on inner margin, one spine and one seta terminally, one seta on outer margin.

P2 (Fig. 4B) similar to P1, but coxa without inner seta; basis without inner spine. Intercoxal sclerite with acute projection on distal margin. Exp and Endp-1 similar to those in P1. Endp-2 with one seta on outer margin, one spine and one seta terminally, two setae along inner margin; row of long spinules along outer margin.

P3 (Fig. 4C) coxa without inner seta, basis with slender outer seta. Intercoxal sclerite with acute projection on distal margin. Inner and outer margin of Exp and Endp, respectively, with setules. Exp similar to those in P2. Two distal-most setae on inner margin of Exp-2 blunt. Endp-1 similar to those in P2. Endp-2 with one seta on outer margin, one spine and one seta terminally, three setae along inner margin.

P4 (Fig. 4D) coxa without inner seta; basis with slender outer seta. Intercoxal sclerite with acute projection on distal margin. 2-segmented Exp and Endp. Endp smaller than Exp. Exp-1 and Endp-1 similar to that of P3. Exp-2 with two outer spines, one spine and one seta terminally and three setae along inner margin; inner margin with a row of long spinules. Endp-2 slightly larger than Endp-1, with spiniform seta on outer margin, one spine and one spiniform seta terminally, spiniform seta on inner margin.

P5 (Fig. 2F) with basal segment completely fused to fifth thoracic somite; with three slender setae. Proximal segment represented by single slender naked seta on low prominence. Distal segment with two naked setae; inner seta about twice as long as outer one.

P6 (Fig. 2D) reduced, fused, forming simple cuticular plate; inserted latero-dorsally on genital double-somite, with two minute spines ventrally and one short seta dorsally. Adult female with a pair of egg sacs, each contains two large eggs.

Male (Figs. 5, 6), slightly smaller than female; body length, excluding caudal setae, 340–450 μm (mean: 380; $n = 10$). Body shape generally similar to female, except for genital section (Fig. 5A). Antennae, mouthparts, P1 (Fig. 6A), P2 (Fig. 6B), P3–P4 exopods (Fig. 6C, D) as in female. Anal operculum (Fig. 5A, B) slightly longer than in female, more or less triangular shape with rounded tip. Caudal rami similar to female, but lacking dorsal keel.

Antennule (Fig. 5E) 15-segments. Setal formula as follows: 7+2A.4.2.4+A.4.1+A.1.1+A.3.1.1.1+A.1.4.6+A. Seta on segment 9 stout. Segment 12 with strong spinules distally. All setae smooth, segments 9 and 12 with one and two unipinnate setae, respectively.

P3 (Fig. 6C) Endp 2-segmented; proximal segment smaller than terminal one. Outer margin of Endp-1 & Endp-2 setulated. Endp-1 with inner seta. Endp-2 with spiniform terminal seta; two plumose setae along inner margin. Terminal spine modified, with subapical, bulbous, smooth expansion and acute, hooked end. Distance between hooked tip of modified spine and its subterminal bulbous on Endp-2 very short.

P4 (Fig. 6D) Endp 2-segmented; proximal segment slightly shorter than wide. Outer margin of Endp-1 & Endp-2 with setules. Endp-1 with seta on inner margin. Endp-2 with one seta on inner and outer margin; terminally one spine and one seta. Setae soft and longer than in female.

P5 as in female.

P6 (Fig. 5B–D) reduced to simple plate, represented by three bare setae, longer than in female, ventral-most the longest and dorsal-most the shortest.

Etymology. — The new species is named after Mae Wa National Park, the place where it was found for the first time. The name is an adjective agreeing in gender with the (feminine) generic name.

Differential diagnosis and remarks. — With fused P5 to somite bearing it, basis of P2–P4 without inner seta, and with a modified apical spine on the male P3 Endp-2, *B. maewaensis*, new species, clearly fits in the genus *Bryocyclops* Kiefer, 1927, s. str. The armature of the female P1 and P4, the male P3, the setal and spine formula of swimming legs exopods (5.5.5.4. and 3.3.3.3., respectively) allowed us to assign this species to Lindberg's (1954) group I. This group includes 6 species: *B. anninae* (Menzel, 1926), *B. chappuisi* Kiefer, 1928, *B. apertus* Kiefer, 1935, *B. difficilis* Kiefer, 1935, *B. elachistus* Kiefer, 1935, and *B. phyllopus* Kiefer, 1935.

Following the key by Monchenko (1972), with an anal operculum with serrated margin, about 1/2 the caudal rami length, the new species is clearly distinguished from *B. apertus* from Kenya. The other remarkable character is the distance between the hooked tip of the modified spine and its subterminal bulbous on the male P3 Endp-2. The distance in *B. maewaensis*, new species, is very short compared to that

in the males of *B. apertus* (for details see Fig. 6C; Kiefer, 1939: Fig. 105).

The females of the new species also differs from the African *B. difficilis*, which has a relatively small anal operculum and the innermost terminal seta (VI) of caudal ramus is very short and spiniform. The caudal ramus of *B. difficilis* has long dorsal seta, bare outermost terminal seta (III), and dorsal keel well developed compared to those in the female of *B. maewaensis*, new species. Additional differences between both species are also in the armature of P4 Endp-2 in females, setae are relatively longer in *B. difficilis* (for details see Figs. 2A, B, 4D; Kiefer, 1939: Figs. 91, 92).

Bryocyclops maewaensis, new species, differs from *B. elachistus*, another species from Kenya, too. The caudal rami are about twice as long as they are wide in *B. maewaensis*, new species, but less than 1.7 as long as wide in *B. elachistus*. Furthermore, in *B. elachistus* the female P4 Endp-2 bears three long setae and one short spine, vs. three short spiniform setae in the new species. The shape of the anal operculum is triangular in *B. elachistus* but ovate in the new species (for details see Figs. 2A, B, 4D; Kiefer, 1939: Figs. 85, 86).

There are also differences between the new species and *B. chappuisi* Kiefer, 1928, from Java. *Bryocyclops chappuisi* is one of five south-east Asian species but only three (*B. chappuisi*, *B. anninae*, and *B. maewaensis*, new species) belong to group I sensu Lindberg (1954). Monchenko (1972) recognised *B. chappuisi* by having an anal operculum which is triangular and its lateral seta (II) on caudal ramus inserted toward the terminal part, whereas *B. maewaensis* has a more rounded anal operculum, with lateral seta (II) attached at about 1/2 length of the caudal rami.

The new species closely resembles *B. anninae* in habitus and cuticular ornamentation, with similar refractile points on prosome and urosome but the pattern is distinctively weaker on the urosome of the new species. The cephalosome has transverse scars indicating fusion line of cephalon and pediger 1; these characters are absent in *B. anninae*. The new species' anal operculum differs markedly from *B. anninae*, which is irregularly acuminate and the free margin is smooth. In the new species pedigers 1–4 have irregularly serrated hyaline frills, present only on pedigers 2–4 in *B. anninae*. The setation of the caudal ramus is also different between *B. anninae* and *B. maewaensis*; dorsal seta (VII) plumose and very short innermost seta (VI) in *B. anninae* but dorsal seta (VII) is naked in the new species, and with long innermost seta (VI). The two species differ also in the presence of a discernible fracture plane of the outer terminal seta (IV) of the caudal ramus in *B. anninae*, which is completely absent in *B. maewaensis*, new species. P2 and P3 of both species have two blunt setae on Exp-2 but with two additional blunt setae on P4 Exp-2 in *B. anninae* (for details see Fig. 4B–D; Reid, 1999: Figs. 3F–H). P4 Endp-2 of female in *B. maewaensis*, new species, carries three short spiniform setae, similar in length to the apical spine, whereas in *B. anninae* there are three long setae. Those three spiniform setae are the most obvious discernible character of the new species.

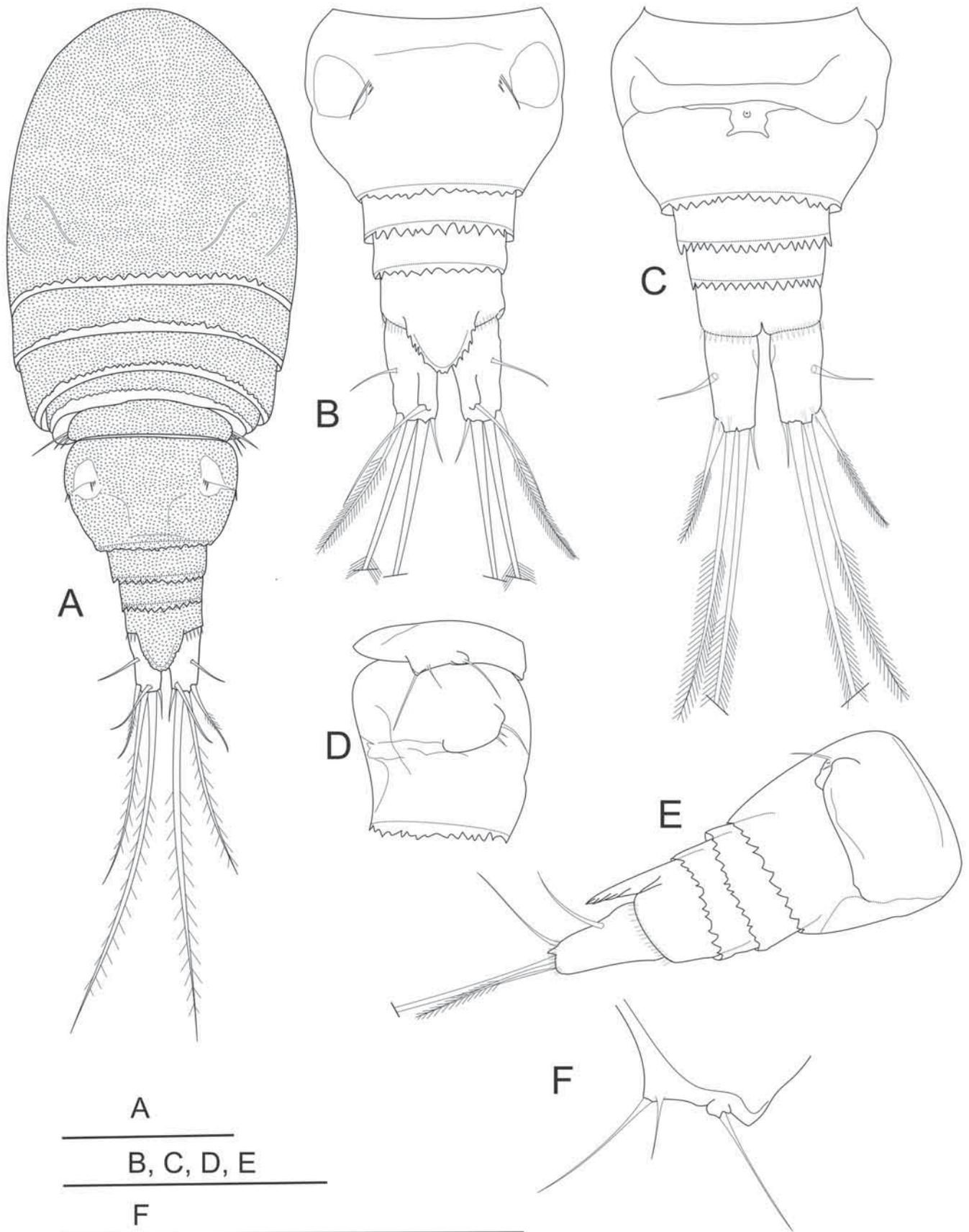


Fig. 2. *Bryocyclops maewaensis*, new species, adult female (NHM 2011.2072, holotype), from Tham Nam Phar Ngam cave, Lampang province, Thailand. A, habitus – dorsal view; B, double genital somite, urosomites, and anal somite – dorsal view; C, genital double-somite, urosomites and anal somite – ventral view; D, pediger 5 and genital double-somite – lateral view; E, genital double-somite, abdominal somites and anal somite – lateral view; F, P5. Scale bars = 100 μ m.

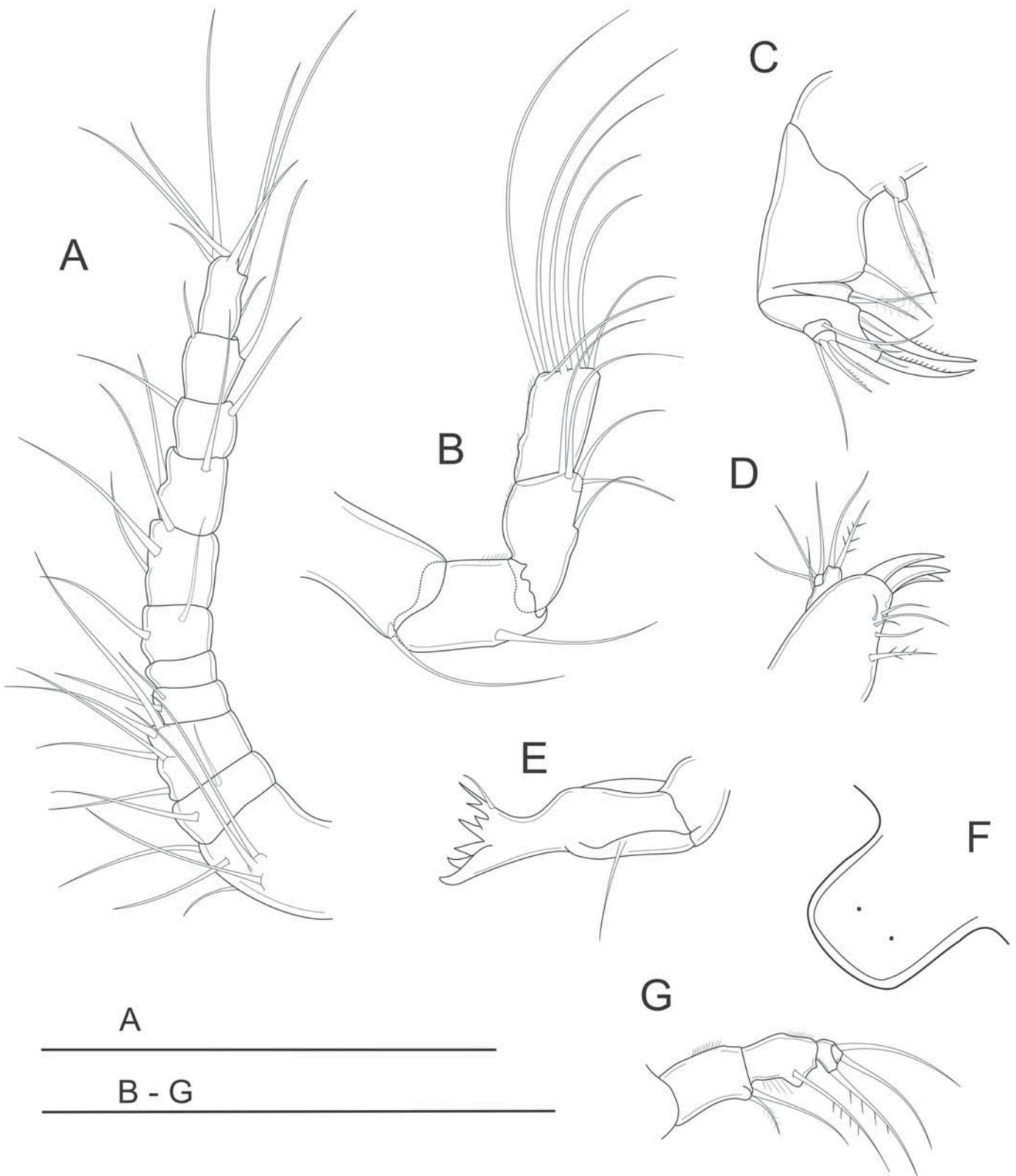


Fig. 3. *Bryocyclops maewaensis*, new species, adult female (NHM 2011.2072, holotype), from Tham Nam Phar Ngam cave, Lampang province, Thailand. A, antennule; B, antenna; C, maxilla; D, maxillule; E, mandible; F, rostrum; G, maxilliped. Scale bars = 100 μ m.

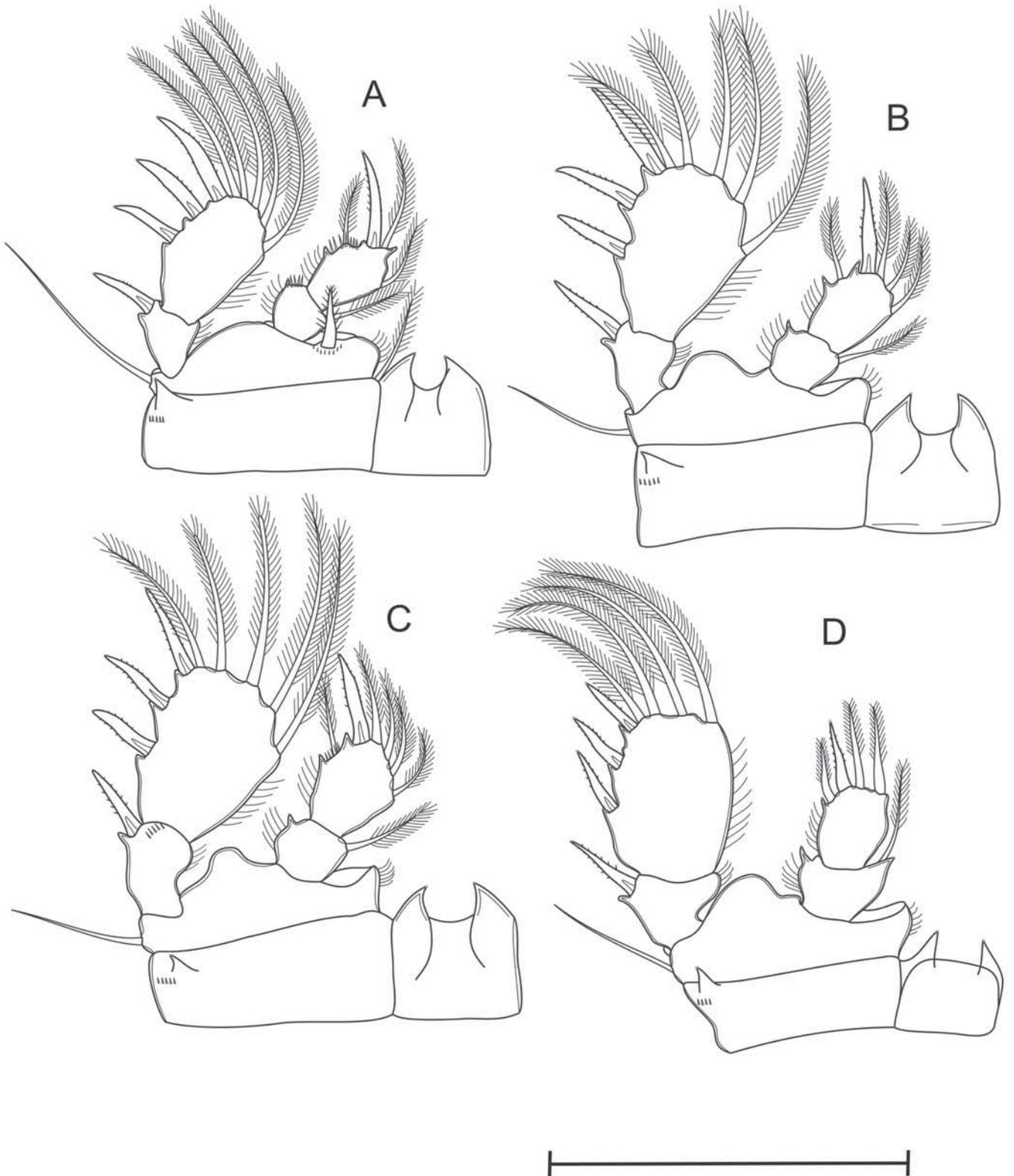


Fig. 4. *Bryocyclops maewaensis*, new species, adult female (NHM 2011.2072, holotype), from Tham Nam Phar Ngam cave, Lampang province, Thailand. A, P1; B, P2; C, P3; D, P4. Scale bar = 100 μ m.

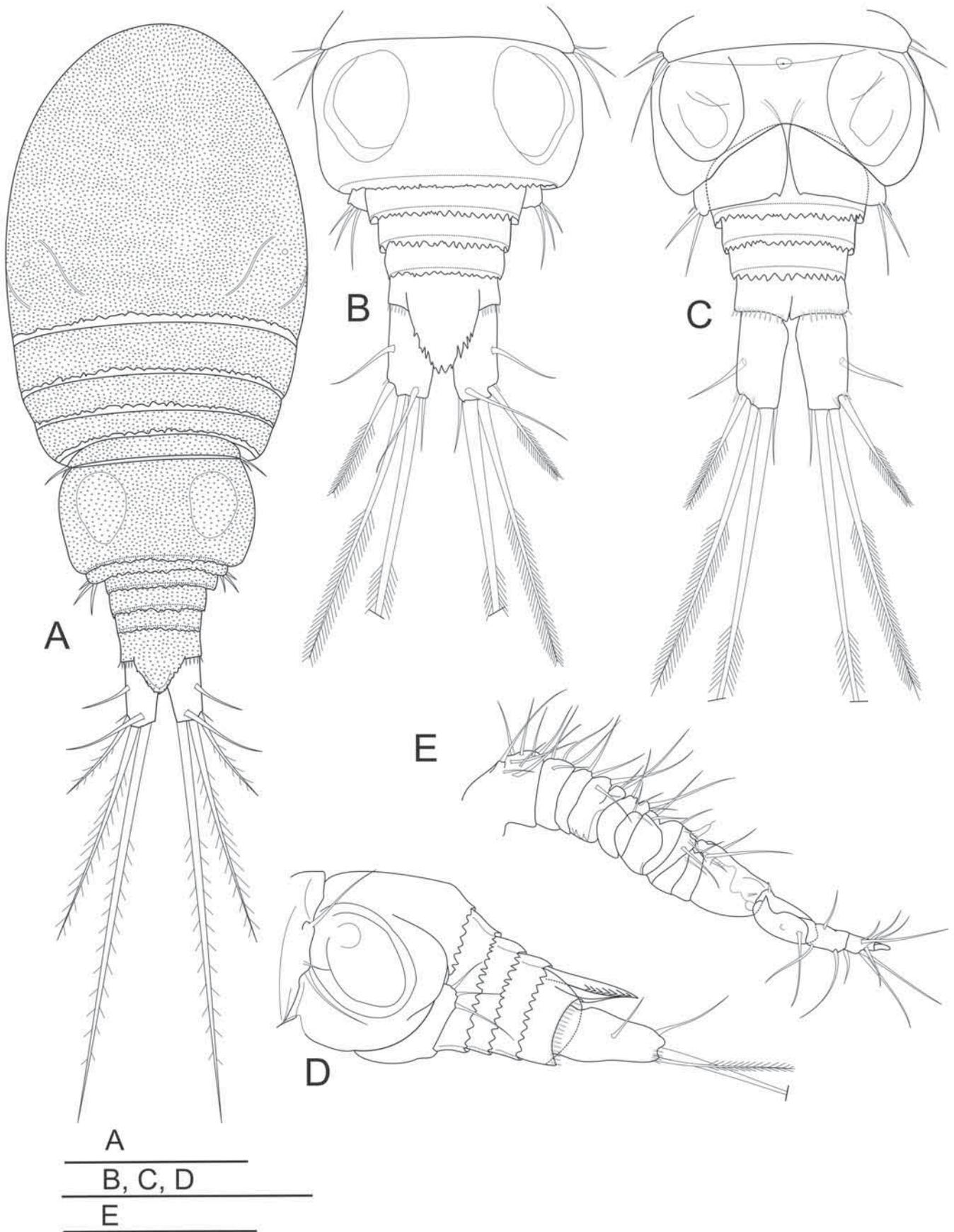


Fig. 5. *Bryocyclops maewaensis*, new species, adult male (NHM 2011.2073, allotype), from Tham Nam Phar Ngam cave, Lampang province, Thailand. A, habitus – dorsal view; B, double genital somite, urosomites, and anal somite – dorsal view; C, genital double-somite, urosomites, and anal somite – ventral view; D, pediger 5, genital double-somite, and urosome – lateral view; E, antennule. Scale bars = 100 μ m.

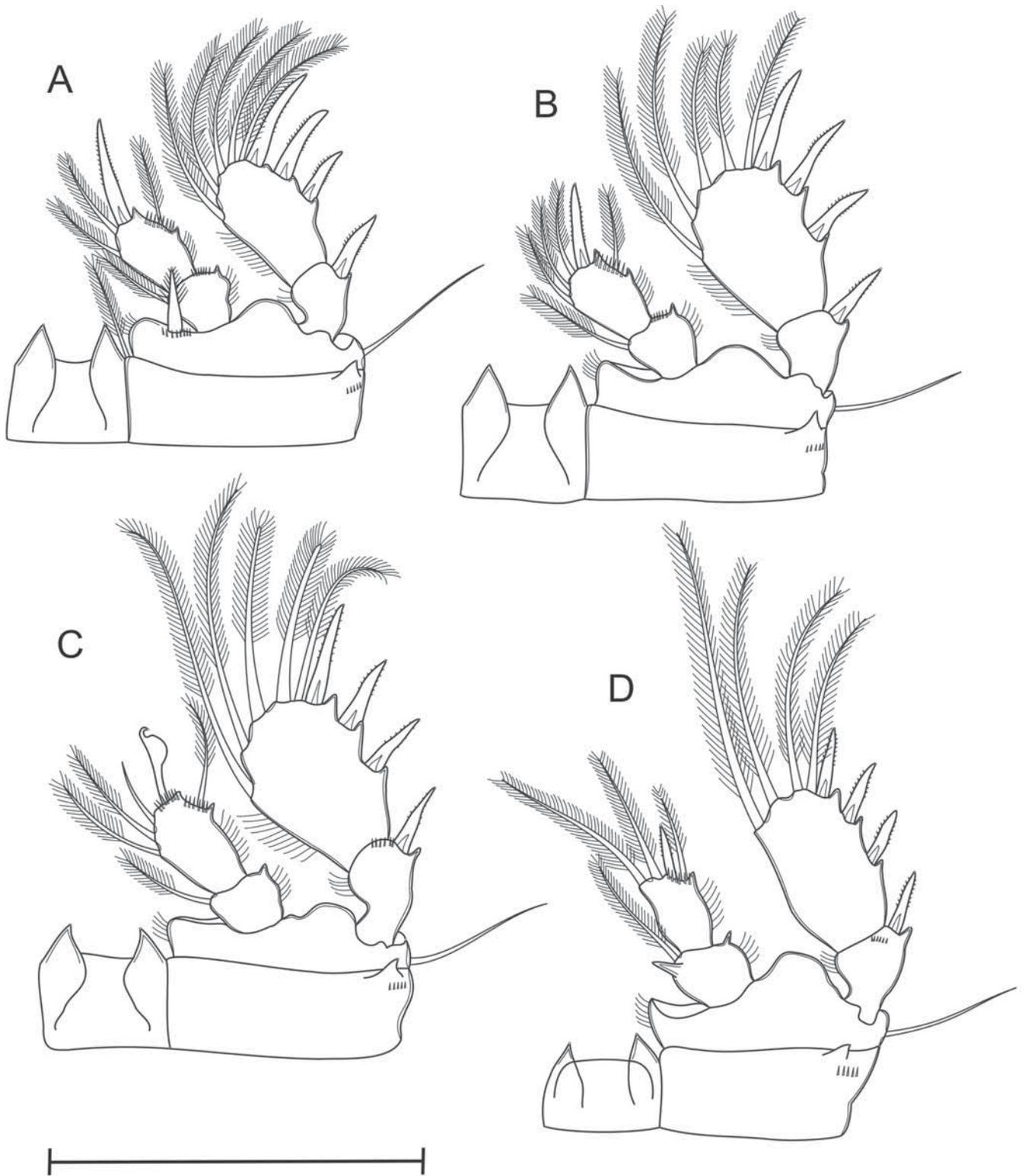


Fig. 6. *Bryocyclops maewaensis*, new species, adult male (NHM 2011.2073, allotype), from Tham Nam Phar Ngam cave, Lampang province, Thailand. A, P1; B, P2; C, P3; D, P4. Scale bar = 100 μ m.

Table 1. List of species of the genus *Bryocyclops* Kiefer, 1927 and their habitats.

<i>Bryocyclops</i> species	Habitat
<i>B. absalomi</i> Por, 1981	dripping pools in caves
<i>B. africanus</i> Kiefer, 1932	wet moss near spring
<i>B. ankaratranus</i> Kiefer, 1955	wet moss near waterfall
<i>B. anninae</i> (Menzel, 1926)	phytothelmata and wet moss
<i>B. apertus</i> Kiefer, 1935	wet moss near small spring
<i>B. arenicolous</i> (Fryer, 1956)	interstitial habitats / alluvium
<i>B. bogoriensis</i> (Menzel, 1927)	phytothelmata wet leaf-litter
<i>B. campaneri</i> Rocha & Bjornberg, 1987	interstitial habitats
<i>B. caroli</i> Bjornberg, 1985	phytothelmata and wet leaf-litter
<i>B. chappuisi</i> Kiefer, 1928	wet leaf-litter
<i>B. constrictus</i> Lindberg, 1947	wet moss
<i>B. difficilis</i> Kiefer, 1935	spring and wet moss
<i>B. elachistus</i> Kiefer, 1935	wet moss near waterfall
<i>B. feci</i> Shen, 1956	semiterrestrial habitats
<i>B. fidjiensis</i> Lindberg, 1954	phytothelmata, tree-holes
<i>B. jankowskajae</i> Monchenko, 1972	groundwater (well)
<i>B. mandrakanus</i> Kiefer, 1955	wet moss near waterfall
<i>B. muscicola</i> (Menzel, 1926)	wet moss
<i>B. phyllopus</i> Kiefer, 1935	spring and wet moss
<i>B. soqotraensis</i> Mirabdullayev, Van Damme & Dumont, 2002	dripping pools in caves
<i>B. travancoricus</i> Lindberg, 1947	wet moss
<i>B. maewaensis</i> , new species	dripping pools in caves

The difference in males between both species is in the anal operculum, which is, according to the original description, very small in *B. anninae* (Menzel, 1926), whereas in the new species, it is considerably larger. The innermost seta (VI) is also very short in the male of *B. anninae* compared to those in our specimens.

Variability. — No variability was observed, except in body length.

Distribution and ecology. — *Bryocyclops maewaensis* was found in North (type locality and Tham Phar Nagm cave in Jae Sorn National Park, Lampang Province) and Central Thailand (Tham Pratoon cave, Kanjanaburi Province). This species is at the moment known only from the vadose part with percolating water (e.g., from pools on muddy floor and from different types of water containers collecting exclusively dripping water) indicating their origin from the epikarstic zone of the cave.

Members of genus *Bryocyclops* are either epigeal species, known from several types of semi-terrestrial habitats such as moist mosses, wet leaf litter, bromeliad and other plants' leaf arm pits (Phytothelmata) or they are subterranean, usually collected from groundwater in alluvium or karstic caves (see Table 1). So far, four species (including the new one) have been described from subterranean habitats; one from well (interstitial aquifers) and three from caves (karstic aquifers). In karstic caves the following species have been found: *B. absalomi* Por, 1981, from Soreq cave (Israel); and

B. soqotraensis Mirabdullayev, Van Damme & Dumont, 2002, from Hoq cave (Socotra Isl., Yemen). Thus, *B. maewaensis* is the third cave-dwelling species of the genus and the first one found in Thai cave.

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