

A new representative of the genus *Bryocyclops* Kiefer, 1927 from a karst cave in north-eastern Thailand (Copepoda, Cyclopoida, Cyclopidae) and comments on the generic affinities

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Abstract

The seventh Thai species of *Bryocyclops* Kiefer, 1927 – *Bryocyclops jayabhumi* **sp. nov.** – was found in a karst cave in the Chaiyaphum Province of north-eastern Thailand. The new species differs from all previously-known species by the absence of an inner seta on the proximal endopod of the first four swimming legs. *Bryocyclops jayabhumi* **sp. nov.** is most similar to *B. maholarnensis* Watiroyram, Brancelj & Sanoamuang, 2015 – the monotypic species of Group VII, which was previously described from Thailand. However, the new species differs from *B. maholarnensis* by having the following characteristics: i) posterior margin of urosomites serrated; ii) anal operculum triangular with acute-tip; iii) P1–P4 Enp-1 without an inner seta; iv) armature on the female P2–P3 Enp-2 and P4 Enp; v) a transformed spine on the male P3 Enp-2. In this study, the generic affinity of the genus *Bryocyclops* Kiefer, 1927 is discussed and redefined, based on the available literature concerning its principle morphology to fill the present knowledge gap about the characteristics of the genus.

Key Words

cave fauna, epikarst, groundwater, Southeast Asia, Thailand

Introduction

North-eastern Thailand (locally called ‘Isan’) is located on the Khorat Plateau and encompasses approximately 200,000 km² or one-third of the country (Smith and Stokes 1997). Its major geographic features are the plains and mountain ranges along the region’s western edge (i.e. Phetchabun Mountain), especially in the Loei, Nong Bua Lam Phu and Chaiyaphum Provinces. Two faunal groups (snails and geckos) have been intensively researched in the caves in this area (Ellis and Pauwells 2012; Pauwells et al. 2014; Tumpeesuwan and Tumpeesuwan 2014; Tanmuangpak 2015), but few studies have focused on aquatic invertebrates, though the region boasts highly diverse, cave-dwelling fauna – especially Copepoda (Watiroyram et al. 2015, 2017). To date, four cave-dwelling copepods have been reported in north-eastern Thailand,

including *Bryocyclops maholarnensis* Watiroyram, Brancelj & Sanoamuang, 2015; *B. muscicola* (Menzel, 1926); *Elaphoidella bidens decorata* (Daday, 1901); and *E. namnaoensis* Brancelj, Watiroyram & Sanoamuang, 2010 (Watiroyram et al. 2017; Watiroyram 2018).

The genus has, so far, been divided into seven species groups (I–VII) and four subgenera: *Bryocyclops* s. str.; *Haplocyclops* Kiefer, 1952; *Palaeocyclops* Monchenko, 1972; and *Rybocyclops* Dussart, 1982. The latter three have been further split into the three different genera (Kiefer 1927; Lindberg 1953, 1956; Monchenko 1972; Dussart 1982; Rocha and Bjornberg 1987; Ranga Reddy and Defaye 2008; Watiroyram et al. 2015; Fiers and Van Damme 2017). Recently, a species of Group II – *B. soqotraensis* Mirabdullayev, Van Damme & Dumont, 2002 – was re-evaluated and given to a new genus: *Thalamocyclops* Fiers & Van Damme, 2017 (Rocha et al. 1998; Fiers 2002;

Fiers and Van Damme 2017). In addition, Fiers and Van Damme (2017) note that the species of Groups I, II and VII (Lindberg 1953; Watiroyram et al. 2015) share generic affinities and a common lineage, but species of Group III (*B. constrictus* Lindberg, 1947; *B. travancoricus* Lindberg, 1947) and Group IV (*B. africanus* Kiefer, 1932); *Bryocyclops* (*Palaeocyclops*) *jankowskajae* Monchenko, 1972 present different lineages that are clearly unrelated to *Bryocyclops* s. str. (Reid and Spooner 1998; Fiers 2012). Based on the available literature and the discovery of the new species described herein, this study discusses and updates the generic affinity of *Bryocyclops* Kiefer, 1927.

Materials and methods

Site description

Chaiyaphum Province sits in the westernmost edge of this plateau, mostly covered by Triassic-Tertiary sedimentary rocks and Permian limestone (Singtuen and Won-In 2018). The Prakai Phet cave of Chaiyaphum Province, located in the Phetchabun Mountain Range, is made of dolomitic limestone karst formed in the Permian Period and it served as a refuge for several Pleistocene mammals (Filoux et al. 2014). This cave is about 1.5 km long, ending in a 45 m-deep abyss and only 15 m of the cave's anterior is open to tourists. The present author visited the cave in the rainy season once a year – September and October 2017–2019 – and a single population of the new copepod on the floor and stalagmites, which are formed by dripping water, was collected at about 4–5 m from the cave's entrance (Fig. 1).

Sampling and specimen preparation

Samples were collected using a hand net (60 μ m) from drip pools on the stalagmites and the cave floor and then fixed immediately in ca. 70% ethanol. Adult animals were picked out and preserved with 70% ethanol in 1.5 ml microtube. Adult specimens were dissected under an Olympus SZ51 stereomicroscope in a mixture of glycerol and 70% ethanol (ratio ~ 1:10 v/v). Dissected specimens were mounted in pure glycerol and sealed with transparent nail polish. Permanent slides with dissected animals were examined with an Olympus compound microscope (CX31) at 1000 \times magnification. Pencil drawings were made with a drawing tube (an Olympus U-Da) mounted on a compound microscope, then the final drawings were scanned for correction in the CORELDRAW 12.0 graphic programme. Specimens for scanning electron microscopy (SEM) were dehydrated in progressive ethanol concentrations (70%, 80%, 90%, 95%, 100% and 100% absolute ethanol) for 15 min each concentration. Specimens were dried in a critical point dryer using liquid carbon dioxide as the exchange medium. Dried specimens were mounted on stubs using adhesive tape under the stereomicroscope. Specimens were coated with gold in a sputter-coater. The SEM photographs were made using a scanning electron microscope (LEO 1450 VP).

The morphological terminology follows Huys and Boxshall (1991). Specimens were deposited at the Natural History Museum, London, United Kingdom (NHMUK) and the Nakhon Phanom University, Faculty of Science, Thailand (NPU).

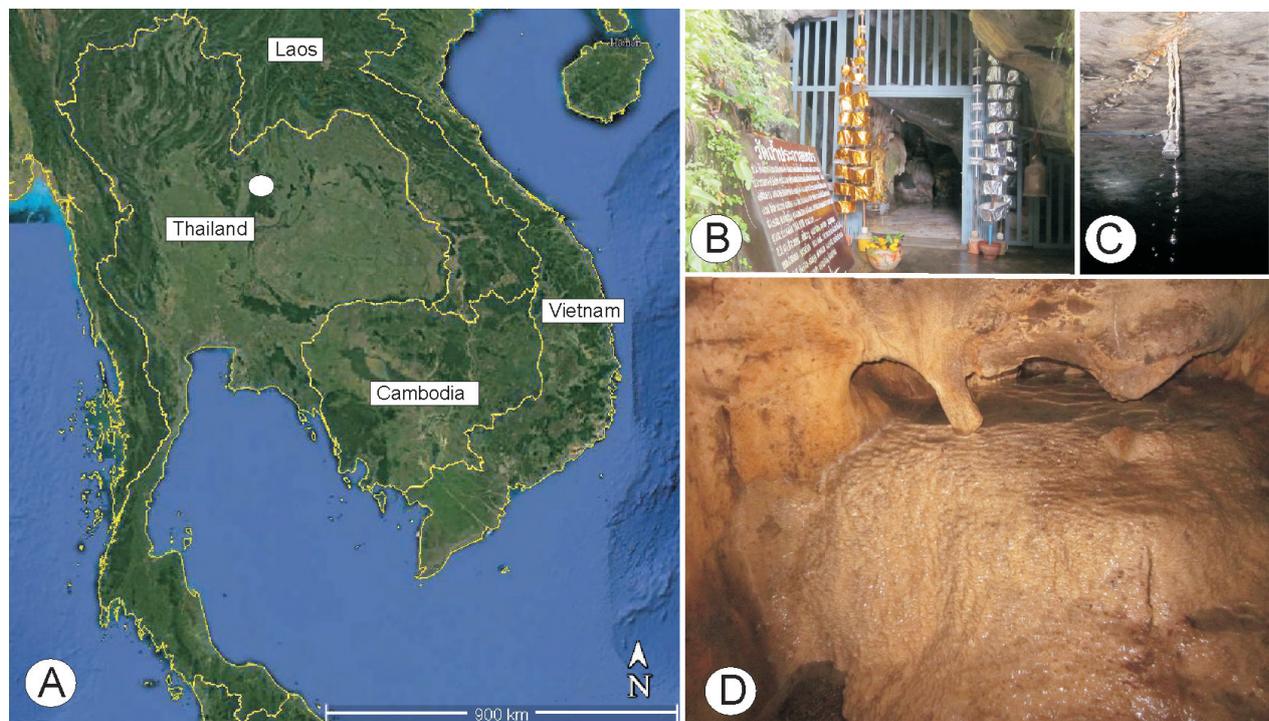


Figure 1. The sampling site and habitat of *B. jayabhumi* sp. nov.: **A.** Sampling site indicated with white circle (○); **B.** The entrance of the cave; **C.** Dripping water from the plant roots penetrating from the surface; **D.** pool on stalagmite.

Abbreviations

The following abbreviations are used throughout the text and figures:

A	aesthetasc;
Enp	endopod;
Exp	exopod;
Exp/Enp-n	exopodal segment n/endopodal segment n;
P1–P6	swimming legs 1–6;
Sp	spine/spines.

Taxonomic section

Order Cyclopoida Rafinesque, 1815

Family Cyclopidae Rafinesque, 1815

Genus *Bryocyclops* Kiefer, 1927

Bryocyclops jayabhumii sp. nov.

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Figs 2–6

Type locality. A rimstone pool that is close to an entrance (see site description) in the Prakai Phet Cave (Fig. 1), Thung Luilai Subdistrict, Khon San District, Chaiyaphum Province, north-eastern Thailand; coordinates of cave entrance: 16°29'03"N, 101°47'05"E, altitude: 573 m above sea level.

Material examined. *Holotype*: one adult female dissected and mounted on one slide (NHMUK 2020.48); *allotype*: one adult male dissected and mounted on one slide (NHMUK 2020.49); *paratypes*: one adult female dissected and mounted on one slide (NPU 2020–001), three adult females and three adult males preserved in 70% ethanol (NHMUK 2020.50–55), three adult females and three adult males preserved in 70% ethanol (NPU 2020–002). All specimens were collected from the type locality on 9 October 2017 by the author.

Etymology. The species name is taken from the Sanskrit words 'jaya' and 'bhumi', meaning 'land of victory' or 'Chaiyaphum' in Thai, referring to the Chaiyaphum Province, where the new species was collected.

Description. Adult female. Habitus (Fig. 2A) cyclopiform ($n = 5$). Body length, excluding caudal setae, 340 μm , with prosome/urosomes ratio of 1.6. Body surface ornamented with refractile points (not figured). Nauplius eye indiscernible. Prosome with length/width ratio of 1.9. Posterior margins of prosomites with smooth hyaline fringe. Cephalothorax completely fused, with several pairs of sensilla scattered dorsally on surface; pedigers 2–3 with pair of sensilla dorsally; greatest width at anterior part of cephalothorax (Fig. 2B, C). Urosome length 130–151 μm ; length/width ratio of 2.2 ($n = 5$). Genital double-somite (Fig. 2C, D) enlarged; anterior slightly wider than posterior; 1.3 times as wide as long ($n = 5$), with transverse sclerotised suture indicating ancestral segmentation; with pair of dorsal sclerotised rounded

structures and P6 dorsolaterally; posterior margin with slightly irregular serrated hyaline fringe. Copulatory pore (Fig. 3I) behind one-half length of segment; copulatory duct narrow, short, strongly sclerotised. Seminal receptacle with anterior expansion at about one-half length of anterior portion; lateral arm narrow, slightly posteriorly curved. Urosomites 3–4 (Fig. 2E, G) shorter than wide, 1.6 times as long as wide ($n = 5$); ornamented with transverse row of shallow pits dorsolaterally; narrow serrated hyaline fringe. Anal somite (Fig. 2E, G) short, 1.6 times as long as wide ($n = 5$); two dorsal sensilla at base of anal operculum; transverse row of spinules distally on ventral and dorsolateral side. Anal operculum (Fig. 2E) well developed, extended to tip of caudal ramus; triangular, acute tip; free margin smooth. Caudal ramus (Fig. 2E, G) asymmetrically conical, about 1.5 times as long as wide, with dorsal longitudinal keel. Lateral seta (II) bare, slightly shorter than caudal ramus, inserted at one-half of caudal ramus length. Outermost terminal seta (III) bipinnate, longer than caudal ramus, with spinules at insertion point on ventrolateral side. Outer terminal seta (IV) bipinnate, about 4.0 times as long as caudal ramus, with fracture plane. Inner terminal seta (V) bipinnate, about 6.0 times as long as caudal ramus, with fracture plane. Innermost terminal seta (VI) bare; short. Dorsal seta (VII) bipinnate, about 2.0 times as long as caudal ramus, inserted at distal end of longitudinal keel.

Antennule (Fig. 3A). Eleven-segmented, not reaching posterior margin of cephalothorax, ornamented with refractile points. Armature formula as follows: 6.2.5.2.0.2.3.1+A.2.2.6+A; all setae smooth; aesthetascs slender, fused basally with seta as acrothek.

Antenna (Fig. 3B). Four-segmented, coxobasis with one distomedial seta. Enp-1 unarmed. Enp-2 with five distomedial setae. Enp-3 with seven apical setae, both ornamented with spinular row along lateral margin. All setae smooth.

Mandible (Fig. 3C) with six strongly-chitinised teeth; dorsal seta on gnathobase. Palp reduced to one bare seta.

Maxillule (Fig. 3D) with three strongly-chitinised teeth on precoxal arthrite; four bare setae and one pinnate seta on inner margin. Coxobasis with three bare setae distally. Exp reduced to one bare seta. Enp with three bare setae.

Maxilla (Fig. 3E) with precoxal endite with two pinnate setae. Coxa with two endites: proximal endite with one bare seta; distal endite with two bare setae. Basis with two strong claw-like expansions, bare seta close to its base. Two-segmented Enp: Enp-1 with one bare seta; Enp-2 with three bare setae.

Maxilliped (Fig. 3F). Four-segmented; syncoxa with transverse row of spinules and two pinnate setae. Basis and Enp-1–2 with one, one and two smooth setae, respectively.

P1–P4 (Fig. 4A–D). P1–P3 with two-segmented Exp and Enp; P4 with two-segmented Exp and one-segmented Enp. Armature formula (seta in Arabic numerals and spine in Roman numerals from outer-inner or outer-apical-inner margins) as follows:

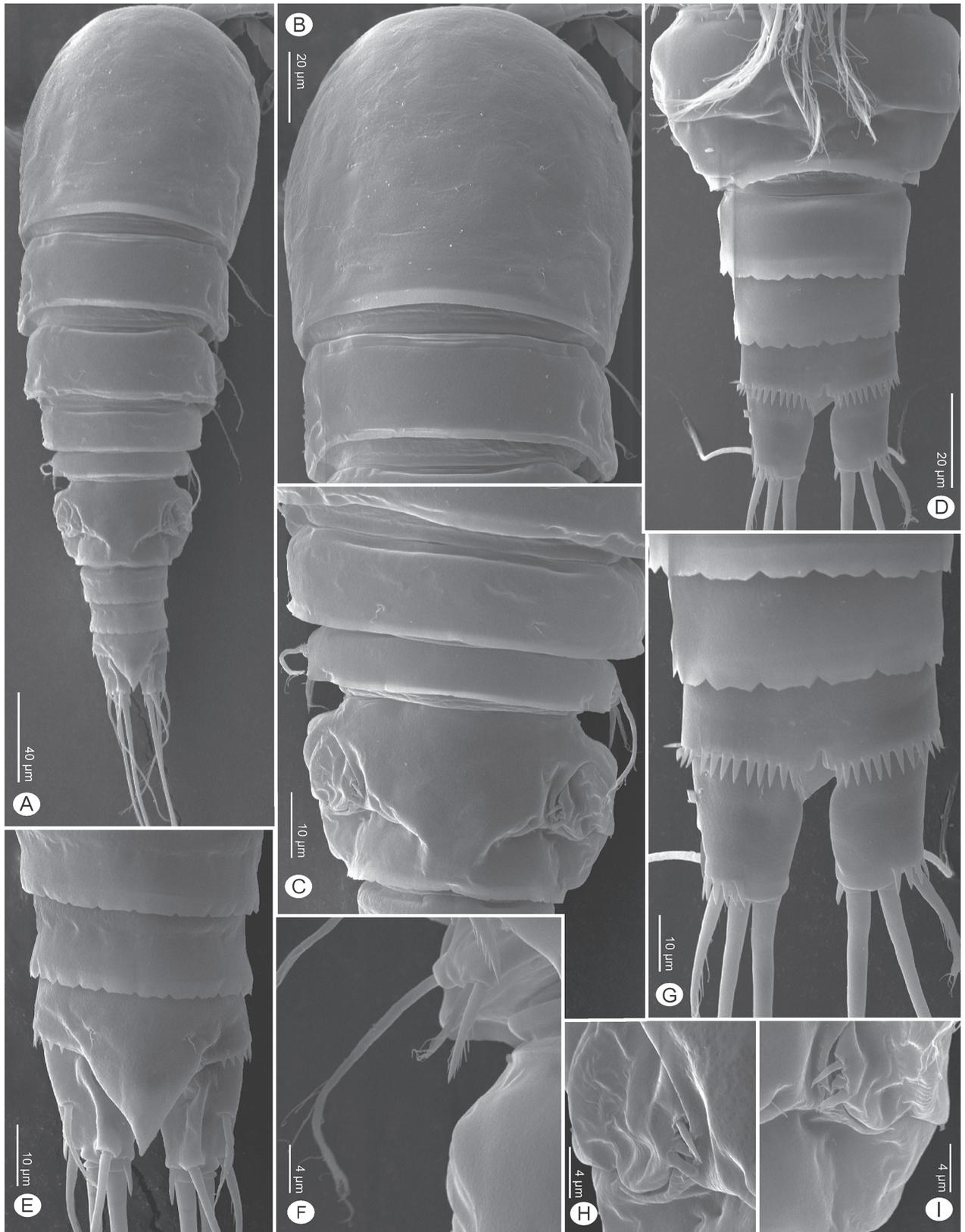


Figure 2. *Bryocyclops jayabhumi* sp. nov., SEM photographs of adult female: **A.** Habitus, dorsal view; **B.** Cephalothorax and pediger 2, dorsal view; **C.** Pediger 4–5 and genital double-somite, dorsal view; **D.** Urosome (without pediger 5), ventral view; **E.** Urosome 3–5 and caudal rami, dorsal view; **F.** P5 on pediger 5, dorsal view; **G.** Urosome 4–5 and caudal rami, ventral view; **H–I.** Left and right P6 on genital double-somite, dorsal view.

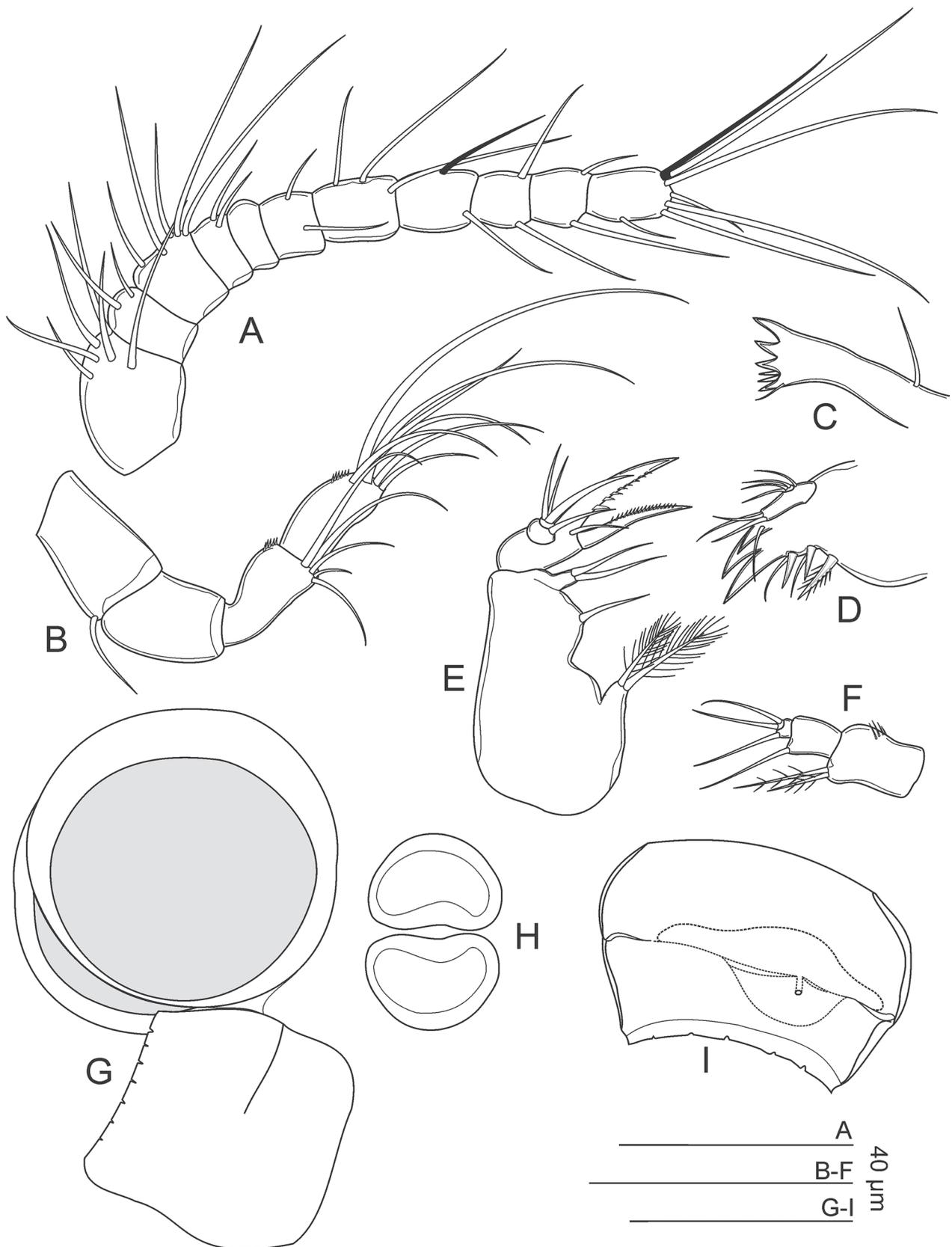


Figure 3. *Bryocyclops jayabhumi* sp. nov., adult female: **A.** Antennule; **B.** Antenna; **C.** Mandible; **D.** Maxillule; **E.** Maxilla; **F.** Maxilliped; **G.** Egg sacs on genital double-somite; **H.** Pair of spermatophore; **I.** Genital double-somite, ventral view.

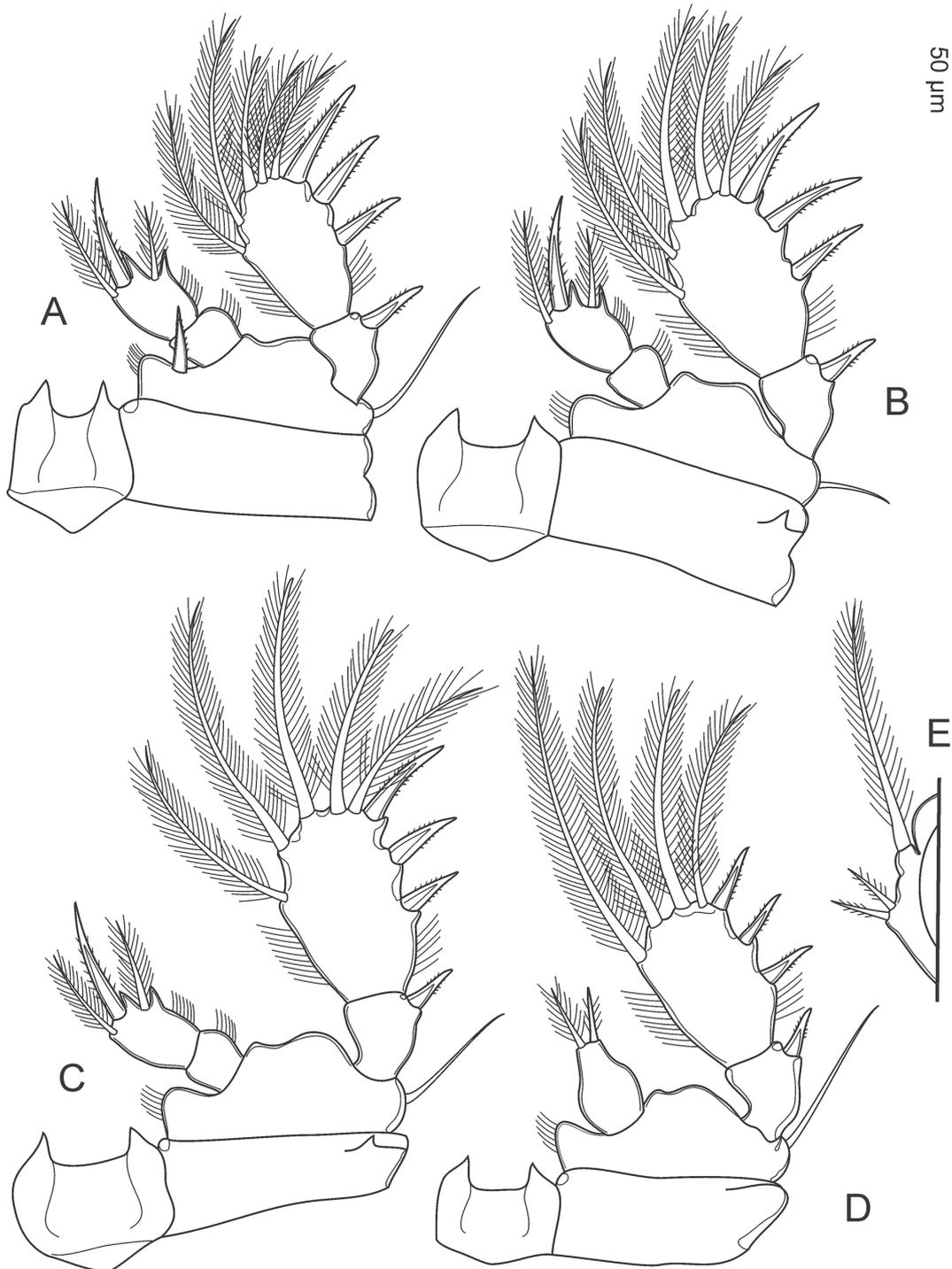


Figure 4. *Bryocyclops jayabhumi* sp. nov., adult female: A. P1; B. P2; C. P3; D. P4; E. P5.

	Coxa	Basis	Exp	Enp
P1	0-0	1-1	1-0; III-2-3	0-0; 1-1+1-0
P2	0-0	1-0	1-0; III-2-3	0-0; 1-1+1-0
P3	0-0	1-0	1-0; III-2-3	0-0; 1-1+1-0
P4	0-0	1-0	1-0; III-2-2	0-1+1-0

P1 (Fig. 4A). Intercoxal sclerite with acute distal margins. Coxa without inner seta. Basis with bare, slender outer seta and robust inner spine; setules on inner distal corner. Exp-1 with outer spine. Exp-2 with three spines on outer margin; two apical setae; blunt seta and two normal setae on inner margin. Enp-1 without seta on inner

margin. Enp-2 with apical seta and spine; additional seta on outer margin.

P2–P3 (Fig. 4B, C). Intercoxal sclerite, coxa, basis similar to P1, but basis without inner spine. Exp-1 with outer spine. Exp-2 with three outer spines, apical normal seta and blunt seta, inner blunt seta and two normal setae. Enp-1 without inner seta. Enp-2 with apical seta and spine; additional seta on the outer margin.

P4 (Fig. 4D). Intercoxal sclerite with acute distal margins. Coxa without inner seta. Basis with slender outer seta. Two-segmented Exp. Exp-1 with outer spine.

Exp-2 with three outer spines, apical normal seta and blunt seta, inner blunt seta and normal seta. One-segmented Enp, oval; with two apical pinnate setae, both shorter than segment.

P5 (Figs 2F, 4E). Completely fused to somite ventrolaterally, with one long and two short pinnate setae. Proximal (dorsal) seta arising from lateral prominence; slender, longer than 4.0 times of the remaining setae; distal (ventral) setae strong, subequal in length.

P6 (Fig. 2H, I). Reduced to semi-circular plate, with three short elements: anterior seta articulated, two poste-

rior setae fused to plate. Posterior seta as long as anterior one. Middle seta spiniform, shortest.

Egg sac (Fig. 3G). Two large eggs attached ventrally to segment, with an average egg diameter of 60 μm .

Spermatophore (Fig. 3H). The spermatophores are generally bean-shaped.

Adult male. Body length (Fig. 5A), excluding caudal rami, 320–335 μm (mean 330 μm , $n = 5$); smaller than female. General segmentation and ornamentation (Fig. 5A–D) similar to female. Genital somite and urosomite 3 not fused as in female, 1.8 times as long as

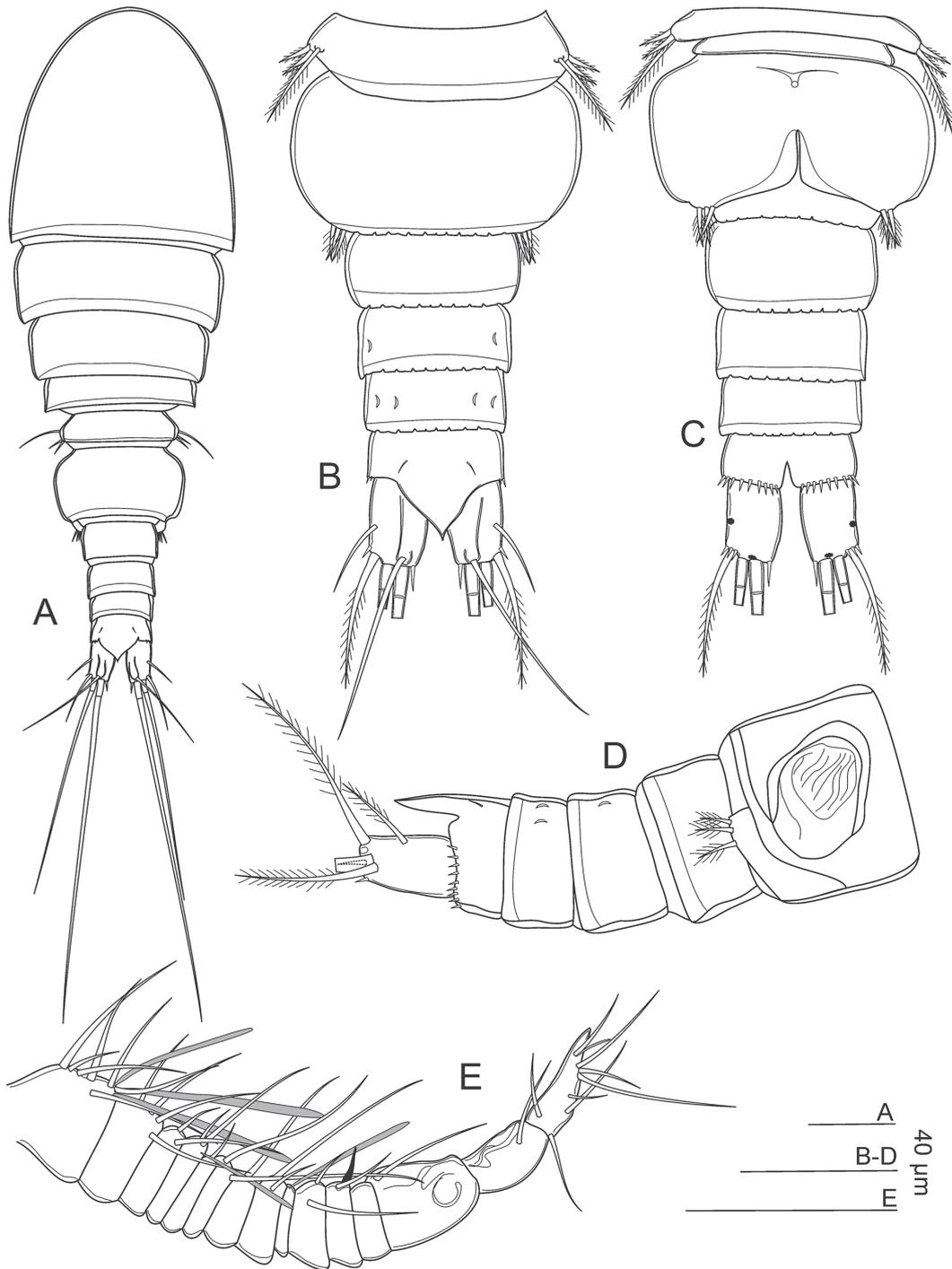


Figure 5. *Bryocyclops jayabhumi* sp. nov., adult male: **A.** Habitus, dorsal view; **B.** Urosome, dorsal view; **C.** Urosome, ventral view; **D.** Urosome (without pediger 5), lateral view; **E.** Antennule.

wide ($n = 5$). Anal operculum (Fig. 5B, D) as in female, but shorter.

Antennule (Fig. 5E). Fifteen-segmented, geniculate. Armature formula as follows: 7+3A.4.2.2+A.1.2.1.2.A.2.1+Sp.0.1.1.8+A.

Antenna, mouthparts, P1 (Fig. 6A) and P5 similar to those in female. Sexual dimorphism is observed on P2–P4 and P6 as follows:

P2 (Fig. 6B). Intercoxal sclerite with acute distal margins. Coxa without inner seta. Basis with bare, slender outer seta; setules on inner distal corner. Two-segmented Exp, Exp-1 with outer spine. Exp-2 with three outer spines, apical normal seta and blunt seta, inner blunt seta and two normal setae. Two-segmented Enp, Enp-1

without inner seta. Enp-2 with one spine and three pinnate setae (inner, apical and outer setae).

P3 (Fig. 6C). Intercoxal sclerite, coxa, basis, Exp similar to P2. with outer spine. Enp-1 without inner seta. Enp-2 with one transformed spine and two pinnate setae (apical and outer setae). Apical transformed spine with hook-like tip bent outwards, medial part slightly swollen, smooth.

P4 (Fig. 6D). Intercoxal sclerite with acute distal margins. Coxa without inner seta. Basis with slender outer seta. Two-segmented Exp. Exp-1 with outer spine. Exp-2 with three outer spines, two apical and two inner normal setae. Two-segmented Enp. Enp-1 without inner seta. Enp-2 with two apical pinnate setae.

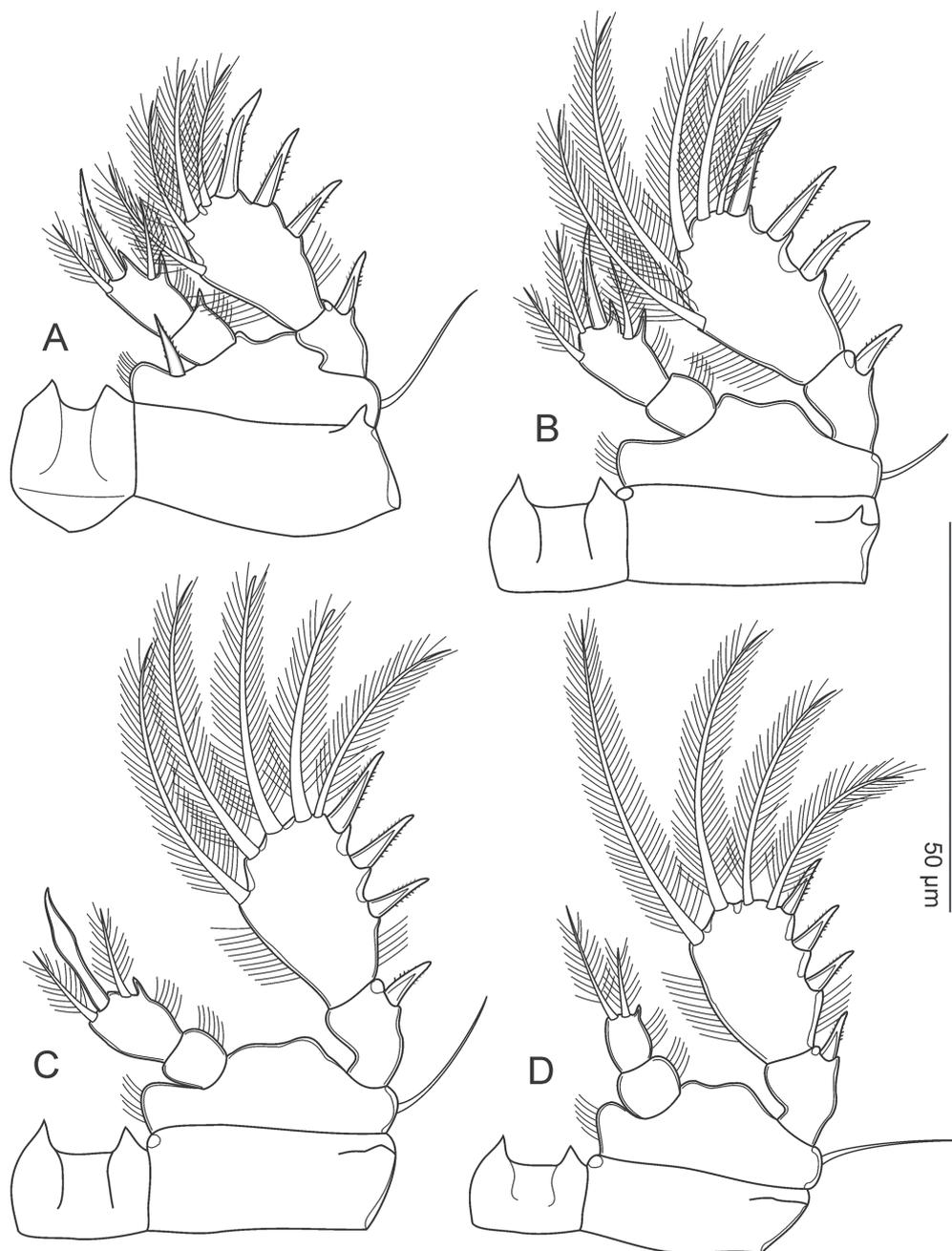


Figure 6. *Bryocyclops jayabumi* sp. nov., adult male: **A.** P1; **B.** P2; **C.** P3; **D.** P4.

P6 (Fig. 5B–D). Reduced to simple plate, represented by three subequal pinnate setae.

Differential diagnosis. *Bryocyclops jayabhumii* sp. nov. can be assigned to the genus *Bryocyclops* s. str. as it exhibits the following characteristics: i) P5 completely fused to somite, with three elements inserted directly on the thoracic somite; ii) P1–P4 with two-segmented Exp and Enp, except female P4 with one-segmented Enp; iii) P1–P4 intercoxal sclerites with acute free distal margins; iv) P1–P4 Exp-2 with spine and setal formula 3.3.3.3 and 5.5.5.4, respectively; and v) sexual dimorphisms on P3–P4, with transformed spine on male P3 Enp-2. The new species, therefore, fits into Group VII sensu Watiroyram et al. (2015), because it has two elements on the P4 Enp and P1 coxa without inner seta.

Group VII contains two species collected from Thailand – *B. jayabhumii* sp. nov. and *B. maholarnensis* Watiroyram, Brancelj & Sanoamuang, 2015. Both species are obviously different from other species due to their P4 Enp, which terminates in two elements instead of five elements (when one-segmented) or four elements (when two-segmented). These species could, however, be differentiated from each other by the following characteristics: i) posterior margin of urosomites serrated (smooth in *B. maholarnensis*); ii) anal operculum triangular with acute-tip (round in *B. maholarnensis*); iii) inner setae on medial margin of P1–P4 Enp-1 absent (present in *B. maholarnensis*); iv) armature on female P2–P3 (each with three elements in the new species, versus four and two elements, respectively, in *B. maholarnensis*); v) armature on female P4 Enp (two apical elements in the new species versus one apical and one inner seta in *B. maholarnensis*). The male P3 Enp-2 of the new species has a well-developed, transformed spine similar to those in *B. maholarnensis*, but with a swollen medial portion.

The new species shows two remarkable characteristics on its swimming legs and in the urosomal serration in both sexes, which have never been seen in other examples of *Bryocyclops* s. str. The new species lacks inner distal setae on P1–P4 Enp-1 and has a different serrated pattern on urosomites 1–4. The posterior margin on the somites of its congeners have normal serration, with lobes on free margin of hyaline fringe (for example, *B. anninae* (Menzel, 1926); *B. aetus* Watiroyram, 2018; *B. maewaensis* Watiroyram, Brancelj & Sanoamuang, 2012; *B. muscicola* (Menzel, 1926); *B. muscicoloides* Watiroyram, 2018; *B. trangensis* Watiroyram, 2018), versus the new species, which has sparsely indented and a completely smooth hyaline fringe in *B. maholarnensis*.

Ecology and distribution. The new species has been found in only one locality, about 140 km away from its most similar species (*B. maholarnensis*). *Bryocyclops jayabhumii* sp. nov. is so far confined to this locality, while *B. maholarnensis* has a wide distribution range across Loei and Nong Bua Lam Phu Provinces. Although the salinity of water containing *B. jayabhumii* sp. nov. was not measured, it is evidently a freshwater species, as its locality is 573 m above sea level and approximately 450 km from the nearest sea. The sampling site, Prakai Phet Cave,

is not connected to running water or other groundwater; it is only fed water from the stalactites and tree roots penetrating its ceiling (Fig. 1C). No other copepods were found during the study period.

Discussion

The genus *Bryocyclops* Kiefer, 1927 is polyphyletic. Variable characteristics determine species in this genus and, as it contains such a complex array of species, it will likely be divided into distinct genera (Dussart and Defaye 2006; Fiers and Van Damme 2017). The diagnostic characteristics for *Bryocyclops* are incompletely defined because there are deficiencies in the descriptions of most species, especially their antennae, mouthparts, first three swimming legs, integument ornamentations and genital fields. Different authors use different criteria to characterise the genus – except for the male P3 Enp-2, which is uniformly described as having an apical transformed spine, as well as the P4 coxa, which has no inner seta (Rocha and Bjornberg 1987).

The original generic affinities

Kiefer (1927) separated three Java species from the genus *Cyclops* Müller O.F., 1785: *C. anninae* Menzel, 1926; *C. bogoriensis* Menzel, 1926; and *C. muscicola* Menzel, 1926. From these, he erected the genus *Bryocyclops* Kiefer, 1927, the species of which were characterised by P5 being completely fused to the thoracic somite, having three setae. Earlier species, described before 1937 and having this reduced P5, were considered members of *Bryocyclops*.

In 1937, Kiefer provided the additional four characteristics of the genus: 1) a genital segment that is wider than it is long, 2) a P4 coxa without inner seta, 3) a male P3 with a transformed spine and 4) an acute distal margin of the intercoxal sclerite of the legs (unspecified legs, but probably P1–P4).

Later, Lindberg (1953–1954) revised the genus and lumped all species into *Bryocyclops* s. lato by using the following characteristics: 1) reduced P5, 2) sexual dimorphism of P3–P4 (male P3 with/without a transformed spine), 3) P4 coxa without inner seta, 4) P1 basis with/without inner spine, 5) round or acute distal margin of the intercoxal sclerite of P4, 6) variable spine and setae formulae for P1–P4 Exp-2, 7) variable armature on P4 Enp, 8) male P6 with 2–3 elements and 9) bulging anterior receptaculum seminis, which is more developed than the posterior receptaculum seminis. At the same time, Lindberg also divided *Bryocyclops* s. lato. into six groups (Groups I–VI).

The diagnosis of the genus *Bryocyclops* s. lato, as well as the status of the groups comprised in it, has been much debated recently and its members have been separated into five genera: *Bryocyclops* s. str.; *Haplocyclops* Kiefer, 1952; *Palaeocyclops* Monchenko, 1972; *Rybocyclops* Dussart, 1982; and *Thalamocyclops* Fiers & Van Damme, 2017.

Although some authors believe that the *Palaeocyclops* genus should have remained the subgenus *Bryocyclops* (*Palaeocyclops*) Monchenko, 1972, the status of the genus *Palaeocyclops* is indubitable when considering the possibility of convergences and the morphological differences (Fiers 2002; Dussart and Defaye 2006; Ranga Reddy and Defaye 2008; Fiers and Van Damme 2017).

Remarks on some generic characteristics

Members of *Bryocyclops* s. str. have acute intercoxal sclerites on their free distal margins, at least on P4. The swimming legs of many early known species (before 1972) are incompletely illustrated and described, except for P4. Regarding the available information, all species of Groups III–VI are characterised by round distal margins in all legs (P1–P4) versus the acute forms on P4 in Groups I, II and VII. In addition, amongst Groups I, II and VII, the anterior legs tend to have rounder distal margins compared to the posterior legs. For example, P1 is round in the females of *B. anninae* (Menzel, 1926) and *B. aetus* Watiroyram, 2018 (Watiroyram 2018a; Sanoamuang et al. 2019).

The presence of an inner coxal seta is also, so far, unified for the system of cyclopine genera (Reid and Spooner 1998). This seta and a medial spine at the basis are present on P1, but lacking on other legs in all known *Bryocyclops* s. str. species and both of these characteristics are identical in the *Bryocyclops* s. str., *Palaeocyclops* and *Thalamocyclops* genera. However, the inner coxal seta is absent on the P1 of some *Bryocyclops* species, such as *B. absalomi* Por, 1981, *B. aetus*, *B. maholarnensis* and *B. jayabhumi* sp. nov. Thus, the presence of the inner coxal seta on P1 is a non-significant characteristic for generic diagnosis of *Bryocyclops* s. str.

All *Bryocyclops* s. str. species exhibit specialised sexual dimorphism on P3 and P4, which other cyclopids usually express only on the urosomal segmentation, antennules, P5 and P6 (Fiers 2002). Rocha and Bjornberg (1987) note that the transformed spine of the male P3 Enp-2 is traditionally used to determine genus because it is present amongst all *Bryocyclops* s. str. Additionally, the discovery of new species has confirmed that the apical spine of the male P3 Enp-2 varies in the degree of transformation (its tip, position and ornamentation of medial swelling). For example, *B. maewaensis* has the greatest medial swelling at the distal half of the spine, produced in the outer margin, similar to most of its congeners; *B. jayabhumi* sp. nov. has less medial swelling at about one-half of the spine length and is enlarged in both margins; and *B. maholarnensis* presents the lowest level of transformation. These three species have no ornamentation (spinules) on the surface of their expended parts, but these spinules are present in *B. aetus*, *B. muscicola*, *B. muscicoloides* and *B. trangensis*. This transformed spine on the male P3 Enp-2 has also been reported in the *Thalamocyclops*, *Palaeocyclops* and *Siamcyclops* genera

(Monchenko 1972; Fiers and Van Damme 2017; Boonyanusith et al. 2018). This characteristic has traditionally been used to recognise *Bryocyclops* and to reasonably conclude that the genera *Thalamocyclops*, *Palaeocyclops* and *Siamcyclops* are more closely related to *Bryocyclops* s. str. than are *Haplocyclops* and *Rybocyclops*. Reid and Spooner (1998) propose that the female P4 Enp of *Bryocyclops*, which has five elements in most previously-described species (e.g. *B. bogoriensis*, *B. campaneri*, *B. caroli*, *B. muscicola*, *B. muscicoloides*, *B. trangensis* etc.) is considered plesiomorphic. Thus, the presence of two instead of five elements on the P4 Enp in *B. maholarnensis* and *B. jayabhumi* sp. nov. (Group VII) is probably apomorphic and new to the genus.

The male P6 of the new species and of all *Bryocyclops* s. str. (except *B. africanus*, Group III) and *Thalamocyclops* species, has three setae. These are considered to be plesiomorphic formations in cyclopines, which have been reduced to two setae in closely-related genera, including *Palaeocyclops*, *Rybocyclops* and *Haplocyclops*. The female P6 of *Bryocyclops* s. str. and *Thalamocyclops* are also similar, but they show more reduction than the male forms; the P6 vestiges have three elements in spiniform and setiform, but they always appear in *Haplocyclops* and *Rybocyclops* with setae (unknown amongst *Palaeocyclops*). Amongst seven species collected in Thailand, the P6 of *B. trangensis* is most similar to *Thalamocyclops* (but *B. trangensis* and other *Bryocyclops* are easily distinguished from *Thalamocyclops* by the structure of P5), having two setae and one spinule, versus six other species armed with one seta and two spinules (*B. jayabhumi* sp. nov. with shorter and stronger setae than *B. aetus*, *B. maewaensis*, *B. maholarnensis*, *B. muscicola* and *B. muscicoloides*).

The new species, depicted in this study, is ornamented with refractile points or pits on the body surface, which is another characteristic presented mostly in *Bryocyclops* s. str., except for *B. aetus* (Watiroyram 2018a). Although the possession of refractile points is not confined only to *Bryocyclops*, it is useful for separating *Bryocyclops* species from those in closely-related genera, which lack this ornamentation (J. Reid, personal communication), including *Rybocyclops* (= *B. pauliani*), *Haplocyclops* (= *B. correctus*, *B. neuter*) and *Thalamocyclops* (= *B. soqotraensis*) (Reid 1999; Fiers 2002; Fiers and Van Damme 2017; Watiroyram 2018b). Actually, this characteristic is either present or absent amongst copepod species of the same genus in both harpacticoid and cyclopoid copepods, especially those living in groundwater, such as *Halicyclops* Norman, 1903 (*H. maculatus* Rocha & Hakenkamp, 1993, from wells); *Speocyclops* Kiefer, 1937 (*S. orcinus* Kiefer, 1937, from caves); *Elaphoidella* Chappuis, 1929 (*E. bromeliaecola* (Chappuis, 1928), from caves and phytotelmata); *Moraria* T. & A. Scott 1893 (*M. affinis* Chappuis, 1927, from moss; *M. cristata* Chappuis, 1929, from caves; and *M. laurentica* Willey, 1927, from litter) (Rocha and Hakenkamp 1993; Fiers and Moldovan 2012; Watiroyram 2018a). The posses-

sion of refraction points is also probably associated with weak swimmers in groundwater, scleractinian and parasitic, rather than true planktons; it may also depend on the species lineage, rather than on convergent evolution forced from dependency in the same habitats. This idea is supported by the presence of two cyclopinines in the same cave from Vietnam: *Pseudograeteriella longiaesthetascus* Sanoamuang, Boonyanusith & Brancelj, 2019, which does not have this characteristic and *Bryocyclops anninae*, which possesses the characteristic and has been found outside the cave in phytothelmatata and wet moss. This characteristic is also lacking in the other stygobitic copepods recently reported in various Thailand caves: *Boholina laorsriae* Boonyanusith, Wongkamhaeng & Athibai, 2020; *Fierscyclops tanaosriensis* Boonyanusith, Brancelj & Sanoamuang, 2013; *F. solaris* Boonyanusith, Brancelj & Sanoamuang, 2013; *Metacyclops thailandicus* Boonyanusith, Sanoamuang & Brancelj, 2018; and *Siamcyclops cavernicolus* Boonyanusith, Sanoamuang & Brancelj, 2018. Likewise, the blunt or obtuse setae on P2–P3 Exp-2 (and, rarely, on P1) were always present in the early observations of *Bryocyclops* s. str. compared to other cyclopinines (Reid 1999; Sanoamuang et al. 2019). However, the *Bryocyclops* species collected recently in Thailand differ from previous reports in that *B. asetus*, *B. muscicoloides* and *B. trangensis* lack blunt setae (Watiroyram 2018a, b).

The receptaculum seminis is also useful for determining species and genera in the *Bryocyclops* s. lato. Although this characteristic is still unknown for many precise species, it seems that *Bryocyclops* species have a developed anterior part of the receptaculum seminis, as do *Haplocyclops*, *Thalamocyclops* species: *B. anninae*; *B. caroli* Bjornberg, 1985; *B. campaneri* Rocha & Bjornberg, 1987; and *B. jayabhumi* sp. nov. (Fiers and Van Damme 2017; Sanoamuang et al. 2019; the present study). On the contrary, both the anterior and posterior portions of this structure are developed in *Palaeocyclops* and *Rybocyclops* species (Dussart and Defaye 2001; Ranga Reddy and Defaye 2008). The shape of the spermatophore has, therefore, been used as a generic characteristic to differentiate *Siamcyclops* from *Bryocyclops* s. str. *Siamcyclops* have L-shaped spermatophore structures versus the kidney or bean-shaped spermatophore structures found in most *Bryocyclops* species (Boonyanusith et al. 2018). However, this characteristic does not fit *B. trangensis* and *B. asetus*, which present two other shapes (see Watiroyram 2018a, fig. 3C; Watiroyram 2018b, figs 8C, 9A). It seems, therefore, that the spermatophore structure takes a unique form in *Bryocyclops* s. str. and, thus, it must be re-observed amongst the earlier discovered species, whose descriptions are lacking this information. The shapes of spermatophore structures vary more amongst cyclopoids than do those in harpacticoids and calanoids, which are generally cylindrical (Williamson and Reid 2009); in this way, spermatophore shapes may be used to determine what species belong to the *Bryocyclops* s. str. genus.

Conclusion

Bryocyclops jayabhumi sp. nov. is undoubtedly a new species of *Bryocyclops* s. str. It shares common characteristics with Group VII, showing sexual dimorphism on P3–P4 and the armature of P4. The new species is easily distinguished from its congeners by lacking an inner seta on the Enp-1 of P1–P4. This study asserts that the characteristics of Group VII, sensu Watiroyram et al. (2015) may, therefore, be amended from ‘the male P3 Enp-2 without a transformed spine’ to ‘the male P3 Enp-2 with a transformed spine’. Based on the available information concerning the common characteristics in all described species, a generic emendation of *Bryocyclops* s. str. is proposed as follows: i) 11-segmented antennule in female; ii) well developed anal operculum; iii) genital-double somite with length/width ratio less than 1; iv) P1–P4 with two-segmented rami or one-segmented rami; v) P1 basis with inner spine; vi) male P3 Enp-2 with an apical transformed spine; vii) P4 intercoxal plate with acute distal margins; viii) P4 coxa without inner seta; ix) spine and setal formula for P1–P4 Exp-2 being 3.3.3.3 and 5.5.5.4, respectively; x) P5 with three elements and without segment; and xi) male P6 with three elements. According to this view, the genus *Bryocyclops* s. str. contains currently 21 species. Excluded species are as follows: *B. constrictus* Lindberg, 1947; *B. travancoricus* Lindberg, 1947; and *B. africanus* Kiefer, 1932.

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