A new cave-dwelling copepod from northeastern Thailand (Cyclopoida: Cyclopidae)

Santi Watiroyram1*, Anton Brancelj2,4 & La-Orsri Sanoamuang1

Abstract. Bryocyclops maholarnensis, new species, is a stygobiotic copepod found in four caves in northeastern Thailand. It is described and figured hereafter. The new species shares some characteristics with those of species from the Group II sensu Lindberg (1954). However, it stands out by having the following distinctive features: no modified spine in the male P3 endopod-2; P1 with an inner basal spine but no inner coxal seta; intercoxal plate on P1–P4 with acute protuberances; a setal and spine formula of P1–P4 exopod-2: 5.5.5.4 and 3.3.3.3. It also has a reduced number of armature elements on the P4 endopod. The new species has two elements on the straight endopod in both sexes, whereas the Group II has five in females and four in males. Therefore, a new group (Group VII) is proposed for this new species. The new species can be distinguished from B. muscicola (Menzel, 1926), a closely resembling member of the Group II, by the following features: P1 without inner coxal seta; setal formula of P1–P4 distal endopod in female and male is 2.3.1.2 and 2.3.2.2, (the spine formula of P1–P4 distal endopod is 1.1.1.0 in both sexes in the new species). Additionally, the anal operculum and innermost seta on the caudal rami are shorter than those in B. muscicola.

Key words. Copepoda, groundwater, Southeast Asia, stygobiont, subterranean

INTRODUCTION

A recent review of aquatic fauna in inland habitats revealed that 607 stygobiotic copepods are known worldwide (Boxshall & Defaye, 2008), of which only 20 species have been found in Southeast Asia (Brancelj et al., 2013). Among those 20 species, 17 are harpacticoids, two are cyclopoids, while only one is a calanoid. To date, the number of stygobiotic copepod species recorded in Southeast Asia is the highest in Thailand (10 species), followed by the Philippines (six species), Vietnam (five species), Indonesia (two species), and Malaysia (one species) (Brancelj et al., 2013).

In Thailand, when the survey of copepods from subterranean waters started in 1985, Elaphoidella margaritae Pesce & Apostolov, 1985 was described from fresh and brackish-water wells in Phuket Province (Pesce & Apostolov, 1985). Twenty five years later, the first cave-dwelling copepod, Elaphoidella namnaoensis Brancelj, Watiroyram & Sanoamuang, 2010 was reported from a cave in Nam Nao National Park, Petchabun Province, northern Thailand (Brancelj et al., 2010). In the same year, Asiacaris dispar Cottarelli, Bruno & Berera, 2010 was described from a hyporheic habitat in the Than Sadet River, Pha-Ngan Island. In 2012, Bryocyclops maewaensis Watiroyram, Brancelj & Sanoamuang, 2012 collected from a karstic cave in Lampang Province, northern Thailand was described (Watiroyram et al., 2012).

Two new species of the genus Fierscyclops Karanovic, 2004, F. tanaosriensis Boonyanusith, Brancelj & Sanoamuang, 2013 and F. solaris Boonyanusith, Brancelj & Sanoamuang, 2013 were described from western Thailand (Boonyanusith et al., 2013). In 2015, Kinnekaris iulianae Bruno & Cottarelli, 2015 was described from an hyporheic habitat in Pha-Ngan Island (Bruno & Cottarelli, 2015). Additionally, Elaphoidella thailandensis Watiroyram, Brancelj & Sanoamuang, 2015 and E. jaesornensis Watiroyram, Brancelj & Sanoamuang, 2015 were found in pools filled by percolating water in karstic caves in Phitsanuloke and Lampang Provinces, northern Thailand (Watiroyram et al., 2015). Most of the stygobiotic copepods in Thailand were found in karstic caves (Brancelj et al., 2010; Watiroyram et al., 2012; Boonyanusith et al., 2013). During the ongoing study of cave-dwelling copepods in northeastern Thailand, another new species of cyclopoid copepod was collected from four caves in northeastern Thailand, and described as Bryocyclops maholarnensis.

MATERIAL AND METHODS

Samples were collected between August 2013 and October 2014 in northeastern Thailand. They were collected from the pools on the floor of cave galleries filled with dripping water from the ceiling. Pools were either at the entrance (semi-illuminated zone) or cave interior (completely dark zone).
Water from the pools (volume of up to 20 L) was collected by means of a plastic cup and filtered with filtering bottle having a mesh size of 60 μm (Brancelj, 2004). Samples from individual pools were stored in plastic bottles containing 70% alcohol. Animals were sorted under a stereomicroscope, transferred into plastic vials and stored in 70% alcohol.

Before dissection, specimens were placed in a mixture of glycerol and 70% alcohol (ratio ~1:10 v/v), which was replaced after one hour with pure glycerol. Body length was measured from tip of the rostrum to the posterior margin of the caudal rami. They were dissected at 100× magnification under an Olympus SZ51 stereomicroscope. All appendages and body ornamentation were examined under a magnification of 1000×. Drawings were made at the same magnification with the aid of a drawing tube mounted on an Olympus compound microscope (CX31). The final version of the drawings was made using CorelDRAW® 12. For permanent slides, all body parts were mounted in glycerol and sealed with nail polish.

Material is deposited at the Faculty of Science, Nakhon Phanom University, Thailand (NPU) and the Natural History Museum, London (NHMUK).

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

**TAXONOMY**

Cyclopoida Sars, 1886

Cyclopidae Burmeister, 1834

*Bryocyclops* Kiefer, 1927

*Bryocyclops maholarnensis*, new species

(Figs. 2–6)

**Type locality.** It is located in Wat Tham Maholarn (Wat = temple, Tham = cave, Maholarn = local name of the cave) in Nonghin District, Loei Province, northeastern Thailand. The coordinates of the entrance are: 17°06’23.04” N, 101°52’48.54” E; altitude 315 m a.s.l. (Fig. 1). The cave is about 220 m long, with several pools filled exclusively with dripping water. A pool on a stalagmite at the entrance zone filled exclusively with water dripping from the ceiling was selected as the locality type. It is in a semi-illuminated zone. The water temperature during sampling was 23.0°C, pH 8.1, and conductivity 405 μS cm⁻¹.

**Material examined.** Holotype: an adult female was dissected and mounted on a slide in glycerol and sealed with nail polish, NHMUK 2015.2782, coll. S. Watiroyram, 21 August 2014. Allotype: an adult male was dissected and mounted...
Fig. 2. *Bryocyclops maholarnensis*, new species, female. A, habitus – dorsal view; B, pediger 5, genital double-somite and urosome – dorsal view; C, pediger 5, genital double-somite and urosome – ventral view; D, pediger 5, genital double-somite and urosome – lateral view. Scale bars = 100 µm.
on a slide in glycerol and sealed with nail polish, NHMUK 2015.2783, coll. S. Watiroyram, 21 August 2014. Paratypes: four females and three males were stored in 70 % alcohol, NHMUK 2015.2784–2790; two females and two males were stored in 70 % alcohol, NPU 2015.001, all specimens were collected at the same place and on the same date as the holotype.

Additional material: 10 females and five males were stored in 70% alcohol, NPU 2015.002, Phra cave, Chiang Kan District, Loei Province, 17°43'38.22" N, 101°45'53.34" E; 10 females and five males were stored in 70% alcohol, NPU 2015.003, Erawan cave, Nawang District, Nong Bua Lamphu Province, 17°20'54.74" N, 102°01'05.59" E; five females and two males were stored in 70% alcohol, NPU 2015.004, Phr Kham cave, Na Duang District, Loei Province, 17°06'23.04" N, 101°52'48.54" E. All specimens were collected between October 2013 and August 2014, coll. S. Watiroyram.

Description. Female (Figs. 2–4): body length: 410–480 μm (mean: 430 μm; n = 10), color of preserved specimens colorless. Cephalosome wider than rest of body, compressed dorsoventrally (Fig. 2A). Naupliar eye not discernible. Cephalosome (incl. rostrum), pedigers 2–5, genital double-somite, urosomites and anal somite (incl. anal operculum) covered with fine refractile points. Posterior dorsal margins of cephalosome, pedigers 2–5, genital double-somite and urosome with smooth hyaline frills. Genital double-somite (Fig. 2B–D) symmetrical, with a slightly expanded anterior part, about 1.4× as wide as long; with a pair of dorsal sclerotised and rounded structures accompanied by three minute spinules of P6 on inner margin; copulatory pore ventrally, single, large, circular, situated at 1/2 of genital segment; copulatory duct narrow, sclerotised. Anal somite (Fig. 2B, C) with a transverse row of spinules along the posterior margin on the ventral surface. Base of anal operculum with small sensillum at each side.

Anal operculum (Fig. 2A, B, D) prominent, semi-circular, irregularly serrated along the distal margin; well overreaching distal margin of anal somite. Caudal rami (Fig. 2B, C) asymmetrically conical, about 1.3× as long as wide, with dorsal longitudinal keel. Lateral seta (II) bare, slightly shorter than the caudal ramus, inserted at 1/2 of its length. Dorsal seta (VII) pinnate, about 2× as long as the caudal ramus, inserted at distal inner margin of the caudal ramus. Outermost terminal seta (III) bipinnate, slightly shorter than dorsal seta, with spinules at insertion point on the ventral side. Outer terminal seta (IV) bipinnate, about 4× as long as the caudal ramus; with fracture plane. Inner terminal seta (V) bipinnate, approximately 6× as long as the caudal ramus, with fracture plane. Innermost terminal seta (VI) bare; very short, spiniform.

Rostrum (Fig. 3A) fused to cephalosome, as long as wide in frontal view, rounded anteriorly.

Antennule (Fig. 3B) were relatively short, 11-segmented, not reaching the posterior margin of the cephalosome; with refractile points on the external surface (as on somites). Setal formula as follows: 6.2.5.2.0.2.3.1+A.2.1+A.7+A.

Antenna (Fig. 3C) 4-segmented, comprising coxobasis and three-segmented Endp. Exp absent. Coxobasis with bare seta on distal inner margin. Endp-1 with row of spinules on outer margin; seta on the inner margin at 2/3 of its length. Endp-2 with five smooth setae on the outer distal margin (one laterally and four terminally); row of spinules on the outer margin. Endp-3 with seven terminal setae.

Mandible (Fig. 3D) was short, robust, with six strongly chitinised teeth on gnathobase; short, smooth dorsal seta. Mandibular palp were reduced and represented by one bare seta.

Maxillule (Fig. 3E) with robust prae Coxoa, coxobasis and one-segmented Endp. Praecoxal arthrite with two strong apical spines fused to arthrite base; with six armature elements along inner margin, proximally one pinnate, others smooth. Coxobasis with three setae distally: one pinnate and two bare. Exp represented by one and Endp with three smooth setae.

Maxilla (Fig. 3F) five-segmented, with prae Coxoa, coxa, basis, and two-segmented Endp. Praecoxal endite with two spiniform setae. Proximal coxal endite with smooth seta; distal coxal endite with two setae, one smooth and one spiniform. Basis with two claw-shaped expansions; smooth seta at the base of distal claw. Endp-1 with smooth seta. Endp-2 with one spiniform and two smooth setae.

Maxillipeds (Fig. 3G) four-segmented, composed of syncoxa, basis and two-segmented Endp. Syncoxa and basis with a row of spinules. Syncoxa with one spiniform and one smooth seta. Basis with spiniform seta. Endp-1 with spiniform seta, Endp-2 with two smooth setae.

P1 (Fig. 4A) two-segmented Exp and two-segmented Endp. Exp shorter than Exp. Intercoxal sclerite with acute projections on distal margin. Caoxa without inner seta; with row of spinules and pointed process on outer margin. Basis with bare, slender outer seta and robust inner spine with a cluster of spinules at insertion point; setules on inner distal corner. Exp-1 with outer spine. Exp-2 twice as long as wide, with two spinules on outer margin; spine and two apical setae; blunt seta and two normal setae along inner margin. Endp-1 with seta on inner margin. Endp-2 with apical seta and spine; additional seta on outer margin.

P2 (Fig. 4B) coxa, basis and intercoxal sclerite similar to P1, but basis without inner spine. Exp-1 with outer spine. Exp-2 with two outer lateral spinules, apical spine and seta, two inner blunt setae and two normal setae. Endp-1 with inner seta. Endp-2 with inner seta, apical seta and spine; additional seta on the outer margin.

P3 (Fig. 4C) coxa without inner seta, basis with slender outer seta. Intercoxal sclerite with acute projections on the distal margin. Exp similar to those in P2. Endp-1 with inner seta. Endp-2 with apical seta and spine.
Fig. 3. *Bryocyclops maholarnensis*, new species, female. A, rostrum; B, antennule; C, antenna; D, mandible; E, maxillule; F, maxilla; G, maxilliped; H, P5. Scale bars = 100 µm.
Fig. 4. *Bryocyclops maholarnensis*, new species, female. A, P1; B, P2; C, P3; D, P4. Scale bar = 100 μm.
Fig. 5. *Bryocyclops maholarnensis*, new species, male. A, habitus – dorsal view; B, genital somite and urosome – dorsal view; C, genital somite and urosome – ventral view; D, pediger 5, genital somite and urosome – lateral view; E, antennule. Scale bars = 100 µm.
Fig. 6. *Bryocyclops maholarnensis*, new species, male. A, P1; B, P2; C, P3; D, P4. Scale bar = 100 µm.
P4 (Fig. 4D) coxa without inner seta; basis with slender outer seta. Intercoxal sclerite with acute projections on distal margin. Two-segmented Exp; Exp-1 with outer spine. Exp-2 with two outer spines, short apical spine and long seta; three normal setae along inner margin. Endp reduced to stout segment; ancestral division between endopodal segments indicated by transverse ridge; with two pinnate setae: inner and apical ones.

P5 (Figs. 2D, 3H) completely fused to fifth thoracic somite; with three slender setae. Proximal segment represented by longest seta on prominence. Distal segment with two setae; inner seta longer than outer one.

P6 (Fig. 2D) reduced, fused, forming simple cuticular plate; inserted laterodorsally on genital double-somite, with two minute spines and short seta, increasing in length ventrally.

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

Male (Figs. 5, 6): slightly smaller than female; body length, excluding caudal setae, 380–460 μm (mean: 410 μm; n = 10). Body shape similar to female, except in genital section (Fig. 5A). Antenna, mouthparts, P1 (Fig. 6A), P2 (Fig. 6B), P3–P4 exopods (Fig. 6C, D) similar to those in female. Anal operculum (Fig. 5C, D) slightly longer than in female. Caudal rami similar to female.

Antennule (Fig. 5E) 15-segments. Setal formula as follows: 7+2A.4.2.2+A.1.3.2.1+A.3.1.1.1+A.0.1.8+A. Seta on segment 11 stout. All setae smooth.

P3 (Fig. 6C) Endp 2-segmented; proximal segment smaller than apical one. Endp-1 with inner seta. Endp-2 with apical spine and seta, equal in length; outer lateral seta slightly shorter than apical one.

P4 (Fig. 6D) Endp two-segmented; proximal segment as long as wide. Endp-1 with inner seta. Endp-2 small, as long as wide, with two apical setae; inner seta about twice as long as outer seta.

P5 (Fig. 5D) as in female.

P6 (Fig. 5B, D) reduced to simple plate, represented by three plumose setae, sub-equal in length.

Variability. P4 Endp-2 of male with one distal seta in two specimens; two setae in 13 specimens.

Etymology. The new species is named after Maholarn cave, the place where it was first found. The name is an adjective having gender agreement (feminine) with the generic name.

Differential diagnosis and remarks. Bryocyclops maholarnensis, new species, is characteristic in 1) the P5 that is equipped with three setae and fused to the fifth thoracic somite, and 2) an intercoxal plate on the P4 has acute protuberances. These condition clearly indicates that the new species is a member of the genus Bryocyclops Kiefer, 1927, s. str. Among six species groups proposed by Lindberg (1954), the new species is similar to members of the Group II in their sexual dimorphism occurring on P3 Endp-2 and P4 Endp-2, P1 basis with an inner spine, intercoxal plate of P1–P4 with an acute protuberances, the setal and spine formula of P1–P4 Exp-2 (5.5.5.4. and 3.3.3.3, respectively), and an articulation pattern on P4. Group II includes five species: B. muscicola (Menzel, 1926), B. bogoriensis (Menzel, 1926), B. fidjiensis Lindberg, 1954, B. caroli Bjornberg, 1985 and B. campaneri Rocha & Bjornberg, 1987. The new species, however, does not fit into Group II completely, as it differs in the absence of a modified spine on P3 Endp-2 of male (present in Group II, but not confirmed in B. fidjiensis), the absence of inner seta from P1 coxa (present in Group II), and the number of setae on distal Endp P4 is reduced to two (three and four setae plus one spine in males and females, respectively, except B. fidjiensis which has three setae in females) (Table 2). Since B. maholarnensis differs significantly from the members of Group II as well as the other five groups proposed by Lindberg (1954), a new group (Group VII) is proposed here. Groups II and VII are, according to observed characters, an intermediate group in terms of evolution toward oligomerization, as they show reductions in armature elements on the swimming legs and number of segment of Endp in females. Group I, with plesiomorphic characteristics by having two-segmented Exp and Endp in both sexes and the primitive spine and seta formula of Exp-2, can be assumed the most primitive group (Huys & Boxshall, 1991). The new species shows the highest reduction in setation on P4 Endp in both sexes (Table 1), thus formation of a new group is justified.

The new species closely resembles B. muscicola in the following combination of morphological characteristics: (a) identical segmentation of swimming legs; two-segmented Endp of P1–P3 in both sexes but one-segmented Endp of P4 in females and two segmented in males; (b) identical armature of allobasis, Endp-2 and 3 of antenna; there are one, five and seven setae, respectively on those segments; (c) the absence of inner seta on bases of swimming legs P2–P4; (d) identical setal and spine formula of P1–P4 Exp-2; 5.5.5.4 and 3.3.3.3, respectively; and (e) serrated free margin of anal operculum. The new species, however, differs from B. muscicola in the following characteristics: (a) the absence of inner seta on coxa of P1 in the new species; (b) the number of setae on P1 and P3 Endp-2; the female of the new species has two and one seta, respectively, but there are three and five setae in B. muscicola; P3 Endp-2 in the male of the new species has two setae, but there are four in B. muscicola; (c) the armature on distal Endp P4; the new species has two setae in both sexes, but B. muscicola has four and three setae plus one spine in females and males, respectively; and (d) the length of anal operculum and innermost seta (VI) is shorter in the new species compared to those in B. muscicola.
Table 1. Comparative details of the characteristics used in group definition by Lindberg (1954); the number in parentheses indicates the data in original species description.

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<th>Group</th>
<th>I</th>
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<th>VI</th>
<th>VII (the new species)</th>
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<td>Characters</td>
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<td>Sexual dimorphism</td>
<td>Endp-2 P3</td>
<td>Endp-2 P3</td>
<td>Endp P4</td>
<td>Exp P4</td>
<td>Endp-2 P3</td>
<td>No?</td>
<td>Endp-2 P3 (but without modified spine) and Endp P4</td>
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<td>P1 with inner seta/spine</td>
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<td>- Basis</td>
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<td>- Coxa</td>
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<td>Intercocular plate of P1-P4</td>
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<td>Setal and spine formula of Exp-2 P1-P4</td>
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<td>5(4).5.5.4</td>
<td>5.4.4.4</td>
<td>5.4.4.4</td>
<td>5.4.4.3</td>
<td>5(4).4.4.3</td>
<td>5.5.5.4</td>
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<td>No. of segment on P4 (♀:♂)</td>
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<td>1 : 1</td>
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<td>Armature on distal Endp P4 (spine + setae)</td>
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There are also differentiating features between the new species and *B. campaneri*, another member of Group II. The setation on allobasis and Endp-2 and 3 of antenna in the new species is one, five and seven setae, whereas *B. campaneri* has one, five and six setae, in the original paper, or two, six and six setae in Reid (1999). Coxa of P1 of the new species is without inner seta which is present in *B. campaneri*. The new species has a very short innermost seta (VI) whereas *B. bogoriensis* has one that is much longer (Table 2). The new species has the shortest anal operculum and innermost seta on the caudal rami compared to species within Group II, whereas *B. campaneri* has the longest anal operculum which extends beyond the tip of the caudal rami and it also has a relatively long innermost seta (Table 2).

Additionally, the new species differs from *B. caroli* in following characteristics: the number of setae on the allobasis, Endp-2 and Endp-3 of antenna (one, five and seven setae, respectively in the new species but there are one, four and six setae in *B. caroli* Bjornberg, 1985); the new species has no inner coxal seta on P1 but it is present in *B. caroli*. Setal and spine formula on P1–P4 Endp-2 in the new species is 2.3.1.2 and 1.1.1.0, respectively, whereas in *B. caroli* it is 3.3.5.4 and 1.1.1.1 in *B. caroli*. The new species has a smaller anal operculum and innermost seta on the caudal rami than those of *B. caroli* (Table 2).

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Table 2. Differential characteristics between members of the Group II sensu Lindberg (1954) and new species; the number in parentheses indicates the data in original species description.

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<td>Exp : Endp of P4</td>
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<td>1 : 2 (1)</td>
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<td>No. of setae/spine:</td>
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<td>alllobasis, Endp-2 and 3</td>
<td>1 : 5 : 7</td>
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<td>1 : 5 : 7</td>
<td>2 (1) : 6(5) : 6</td>
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<td>1 : 4 : 6</td>
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<td>on antenna</td>
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<td>Inner seta on P1 coxa</td>
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<td>Inner seta on P2 and P3 coxa</td>
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<td>Length</td>
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<td>anal operculum vs. caudal rami (CU) length</td>
<td>Shorter than ½ CU length</td>
<td>½ CU length</td>
<td>Exceeding ½ CU length</td>
<td>Exceeding ½ CU length / over its tip</td>
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<td>Innermost seta on CU Shape</td>
<td>Very short</td>
<td>Long</td>
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<td>anal operculum</td>
<td>Semi-circle, serrate</td>
<td>Ovate, serrate</td>
<td>Ovate, serrate</td>
<td>Ovate, serrate</td>
<td>Triangular, smooth</td>
<td>Semi-circle, serrate</td>
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<td>P3</td>
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<tr>
<td>Modified spine on Endp-2</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>??</td>
<td>Present</td>
</tr>
<tr>
<td>Male No. of setae on Endp-2 P4</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Male No. of spine and setae on Endp-2</td>
<td>0 + 2</td>
<td>1 + 3</td>
<td>1 + 3</td>
<td>1 + 3</td>
<td>1 + 3</td>
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