

A new species of *Clausidium* Kossmann, 1874 (Crustacea, Copepoda, Cyclopoida, Clausidiidae) associated with ghost shrimps from Iran

Vahid Sepahvand¹, Nasrullah Rastegar-Pouyani¹,
Terue C. Kihara² and Farzaneh Momtazi³

1 Department of Biology, Faculty of Science, Razi University, 6714967346 Kermanshah, Iran.

VS E-mail: sepahvandv@gmail.com

NRP E-mail: nasrullah.r@gmail.com

2 Senckenberg am Meer, DZMB - German Centre for Marine Biodiversity Research, Südstrand 44, 26382 Wilhelmshaven, Germany.

TCK E-mail: tckihara@gmail.com

3 Department of Marine Biological Sciences, Iranian National Institute for Oceanography and Atmospheric Science (INIOAS), Tehran, Iran.

FM E-mail: momtazi.f@gmail.com

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ABSTRACT

A new species of the *Clausidium* Kossmann, 1874 copepods is described, illustrated and provided by Confocal Laser Scanning Microscope photo on the basis of specimens from the gill chamber of *Callianidea typa* H. Milne Edwards, 1837 from the Iranian coast of the Persian Gulf. *Clausidium persiaensis* sp. nov is unique in the possession of a fine spine on endopod-1 of the antennae, large blade-like setae with an acute tip on P1, and the shape of P5 and the anal somite. The new species extends the group distribution into the northwest Indian Ocean and represents the first records of the genus in Iran.

KEY WORDS

CLSM, Persian Gulf, Indian Ocean, taxonomy, *Callianidea typa*.

INTRODUCTION

Ghost shrimps or mud shrimps, representatives of the infraorders Axiidea and Gebiidea, are among the most common benthic macro-invertebrates in littoral zones of the Persian Gulf and Gulf of Oman (Sepahvand *et al.*, 2013). These shrimps are adapted to a burrowing life-style and their burrows can also be occupied by a variety of organisms, including copepods (Dworschak *et al.*, 2012). According to Sepahvand *et al.* (2013), two out of 11 species of

CORRESPONDING AUTHOR

Vahid Sepahvand
sepahvandv@gmail.com

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burrowing shrimps recorded from the littoral zone of Iran have associated copepods from the genus *Clausidium* Kossmann, 1874 (Clausidiidae, Cyclopoida). Although these clausidiid copepods are relatively rarely recorded because of the cryptic lifestyle of their hosts, a total of 11 species of *Clausidium* have been described so far (Kihara and Rocha, 2013). This genus has seven species recorded from the Atlantic Ocean and two species from the Pacific Ocean. The remaining two species, *Clausidium travancorensis* Pillai, 1959 and *Clausidium chelatum* Pillai, 1959 have been found in the Indian Ocean (Pillai, 1959). In the present study, we investigate *Clausidium* copepods of Iranian coastal waters of the Persian Gulf, and describe a new species associated with the ghost shrimp *Callianidea typha* H. Milne Edwards, 1837. This new species extends the group distribution to the northwest Indian Ocean and represents the first record of the genus in Iran.

MATERIAL AND METHODS

Sampling was carried out at three localities along the Iranian coast of the Persian Gulf and Qeshm Island (Fig. 1). *Clausidium persiaensis* sp. nov. was collected from the gill chamber of *Callianidea typha* H. Milne Edwards, 1837 in three different sampling sites in the Persian Gulf and Qeshm Island. At the sampling site, the copepods were relaxed with drops of Menthol 1.5% added to the sea water and separated from the host by filtration through a 63 μ m mesh size net. The collected specimens were transferred to 75% ethanol. Whole specimens were temporarily mounted on slides with glycerin, and adhesive plastic discs were used to support the coverslip (Kihara and Rocha 2013). Specimens were dissected under a Leica MZ12 stereomicroscope (Leica, Wetzlar, Germany). Dissected parts were mounted on slides using glycerin as mounting medium, and preparations were sealed with transparent nail varnish. The material was studied with a Leica DMR differential interference contrast microscope (Leica, Wetzlar, Germany) equipped with a drawing tube. The descriptive terminology follows Huys *et al.* (1996). Abbreviations used in the text are: ae, aesthetasc; P1–P5, legs 1–5; exp and enp, exopod and endopod, respectively; exp (enp)-1 (-2, -3), proximal (middle, distal) segments of a ramus.

The body length was measured from the anterior apex of the cephalothorax to the posterior margin

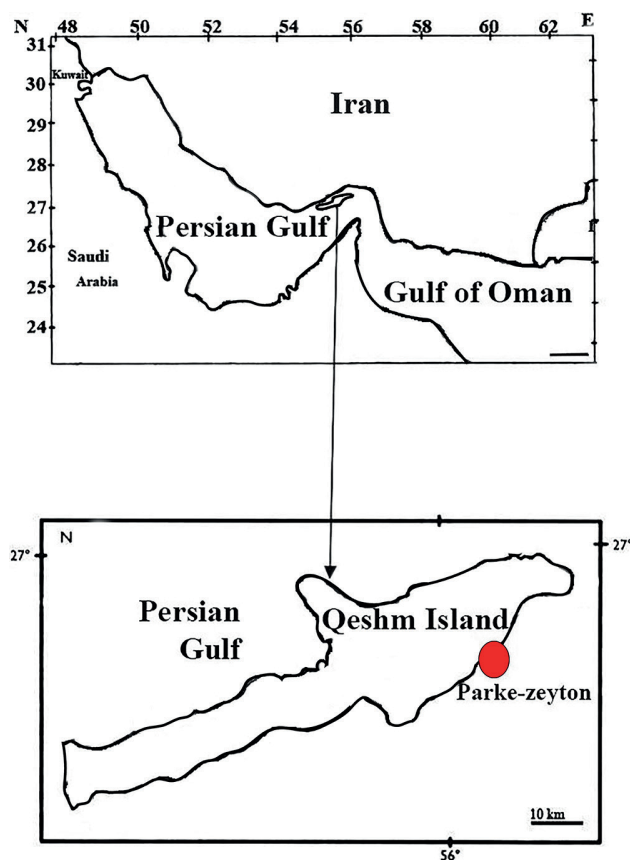


Figure 1. The type locality of *Clausidium persiaensis* sp. nov. (Red circle).

of the caudal rami, excluding caudal setae. The type material is deposited in the collection of the Zoological Museum, University of Tehran (ZUTC).

Confocal laser scanning microscopy (CLSM)

For confocal laser scanning microscopy (CLSM), selected material was stained with 1:1 solution of Congo Red and Acid Fuchsin overnight. Whole specimens and dissected parts were mounted on slides with glycerin following the procedure described by Michels and Büntzow (2010). The material was scanned using a Leica TCS SP5 (Leica, Wetzlar, Germany) equipped with a Leica DM5000 B upright microscope (Leica, Wetzlar, Germany) and 3 visible-light lasers (DPSS 10 mW 561 nm; HeNe 10 mW 633 nm; Ar 100 mW 458 nm, 476 nm, 488 nm and 514 nm), combined with the software Leica Application Suite Advanced Fluorescence (LAS AF 2.2.1) (Leica, Wetzlar, Germany). To obtain a three-dimensional representation from selected body parts, the data produced during the CLSM scanning was processed with the free software Drishti (<http://anusf.anu.edu.au/Vizlab/drishti/>).

Final plates were composed and adjusted for contrast and brightness using the software Adobe Photoshop CS4 (Adobe Systems, San José, U.S.A.).

SYSTEMATICS

Order Cyclopoida Burmeister, 1834

Family Clausidiidae Embletonn, 1901

Genus *Clausidium* Kossman, 1874

Clausidium persiaensis Sepahvand and Kihara sp.
nov.
(Figs. 2–12)

Type material. Holotype: dissected female on 25 slides (ZUTC 5913), 14.V.2015. Allotype: dissected male on 25 slides (ZUTC, 5914); undissected paratypes 21 couples (ZUTC 5915) deposited in ethanol at the Zoological Museum, University of Tehran Crustacean collection, Tehran, Iran. All material collected from the type locality by Vahid Sepahvand.

Type locality. Persian Gulf, Iran (Qeshm Island, Parke-zeyton 27°81'06"N 56°24'11"E, Fig. 1). Specimens collected from the gill chambers of the ghost shrimp *Callianidea typha*.

Description. Female: total length 1.31–1.36 mm (N = 10). Greenish in color when alive. Body broadly rounded (Figs. 8A, B, 11A, B), dorsoventrally compressed. Prosome (Fig. 8A, B) 2.5 times longer than urosome. Maximum width measured at posterior margin of second pedigerous somite. First pedigerous somite fused with cephalosome, forming cephalothorax. Body prosomites with minute integumental pits, sensilla and numerous pores distributed as illustrated in figures. Epimera of second and third pedigerous somites expanded posteriorly. Fourth pedigerous somite almost as long as the two anterior somites combined. Posterior margin of fourth pedigerous somite rounded.

Urosome (Figs. 5A, B, 8B) 3-segmented, distinctly narrower than prosome. Urosome comprising fifth pedigerous somite, genital double-somite and anal somite. Somite bearing P5 (Fig. 8B) 2.3 times wider than long in ventral view and with P5 arising

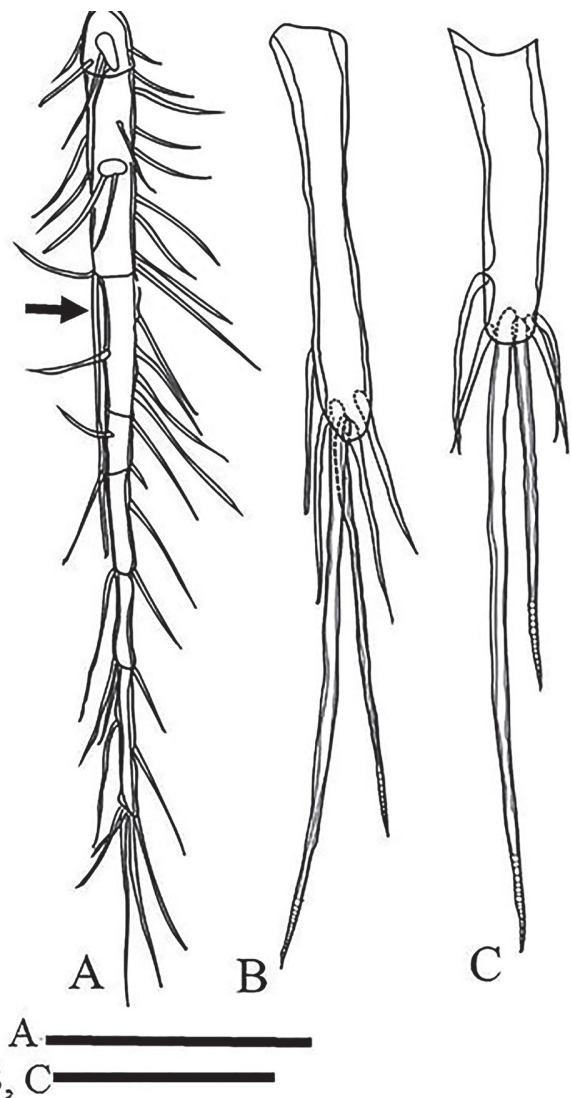


Figure 2. *Clausidium persiaensis* sp. nov. (female). A, antennule; B, caudal ramus; C, caudal rami (male). Scale bars: A, 50 μ m; B, C, 25 μ m.

ventrolaterally. Genital double-somite (Figs. 5, 9) wider than long. Genital apertures located dorsolaterally on each side (showed with white arrow in Fig. 12A), near fifth pedigerous somite posterior margin. Medial pore presence (showed with blue arrow in Fig. 12A) in dorsal surface. Anal somite (Figs. 9, 12) well developed, formed by second to fourth abdominal somites fused into one single segment; almost quadrate, incised medially, with intricate folders dorsally and along lateral margins, outer corners swollen and with special convoluted pattern as illustrated in figures. Caudal rami (Figs. 2B, 9) about 5 times longer than wide, and armed with 6 setae. Rostrum (Figs. 6A, 8B) incorporated into cephalothorax, with broad posteroventral margin.

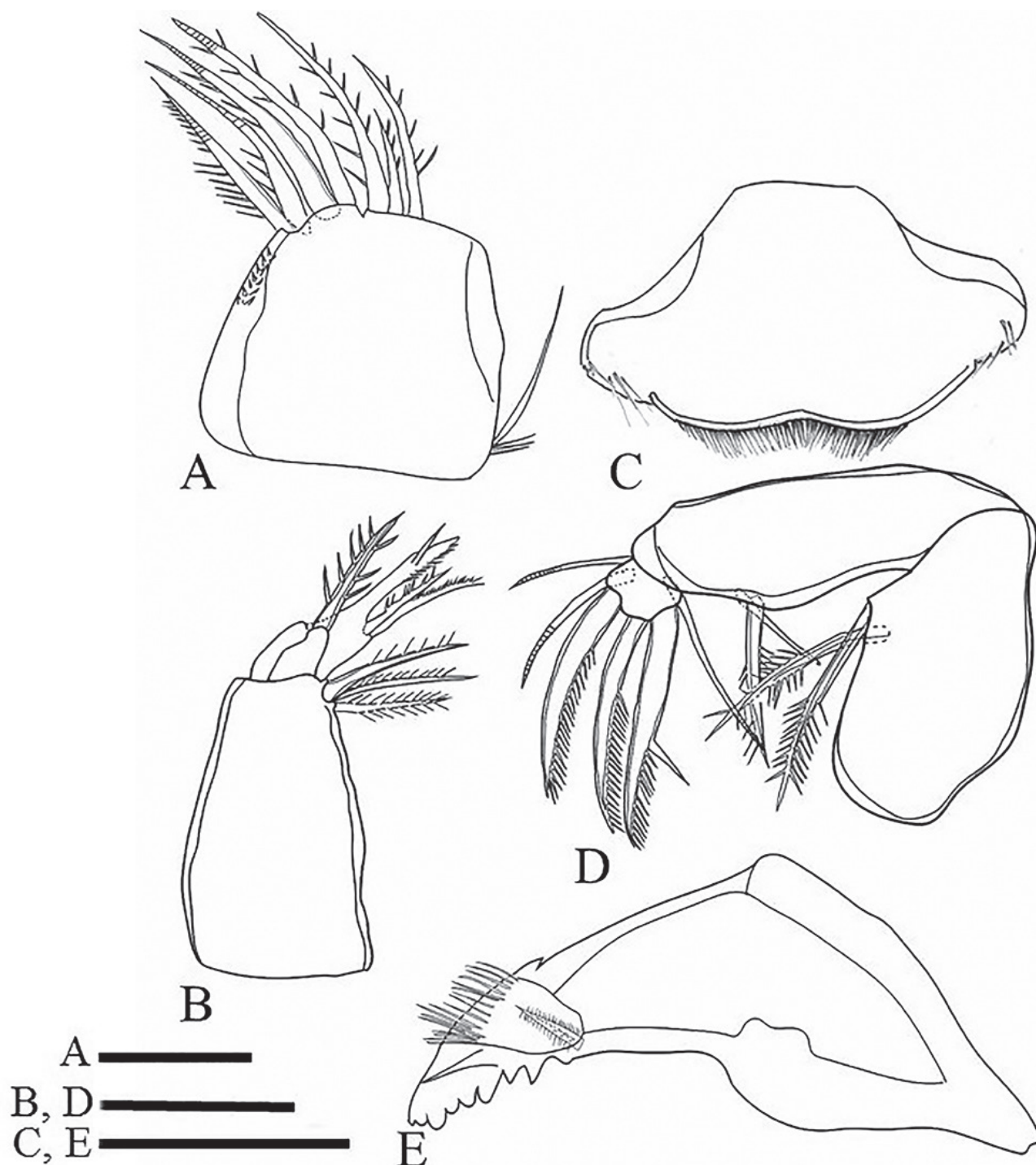


Figure 3. *Clausidium persiaensis* sp. nov. (female). A, maxillule; B, maxilla; C, labrum; D, maxilliped; E, mandible. Scale bars: A, 50 μm ; C, D, 25 μm .

Antennule (Figs. 2B, 8B) 7-segmented. Segment 2 longest, with long seta inserted on inner distal corner and almost reaching the tip of segment 5. Aesthetascs inconspicuous. Segment 7 with apical acrothek consisting of aesthetasc and 2 setae. Armature formula: I-[5], II-[15] III-[6], IV-[3], V-[5], VI-[3], VII-[8]. Antenna (Figs. 7C, 8C) 4-segmented. Coxobasis

elongated, with row of spinules along inner margin, with single seta on inner distal corner. Endopod 3-segmented; segment 1 with small seta along the inner margin; segment 2 with 2 pectinate spines and 2 setae (1 pinnate and 1 naked); segment 3 with 6 apical setae (2 pectinate, 2 pinnate and 2 naked).

Labrum (Figs. 3C, 8C) 2 times wider than long;

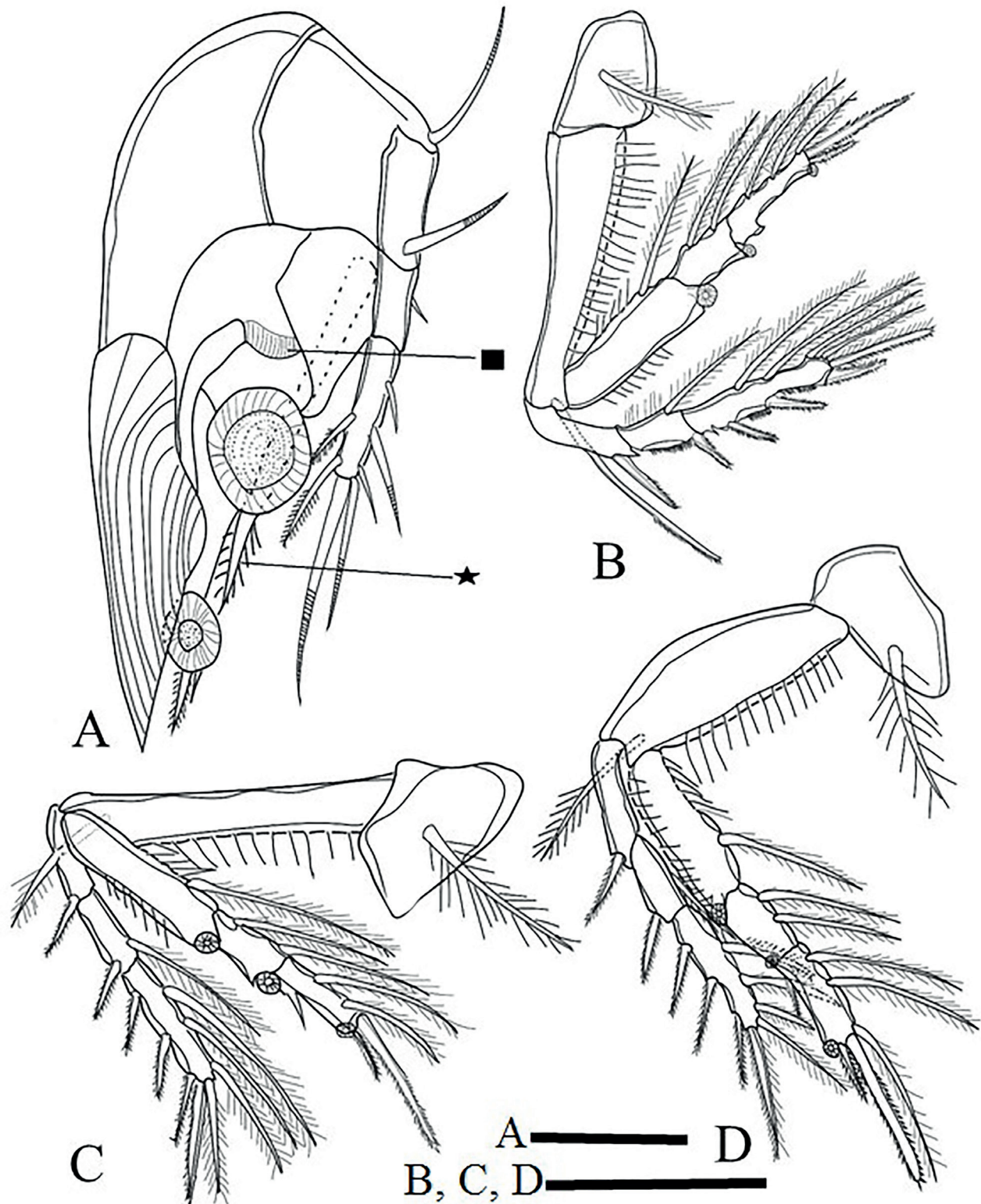


Figure 4. *Clausidium persiaensis* sp. nov. (female). A, P1; B, P2; C, P3; D, P4. Square = adhesive fringe; asterisk = long seta of endopod 2. Scale bars: A, 100 μm ; B, 50 μm .

distal area and lateral projections with row of long setules. Metastomal area ornamented as in Fig. 3C. Mandible (Fig. 3E) armed distally with 2 strong,

denticulate or spinulose elements and 1 small, spiniform seta, pulp represented by spinulose lobe. Maxillule (Figs. 3A, 10C) subrectangular, faintly bilobed, with

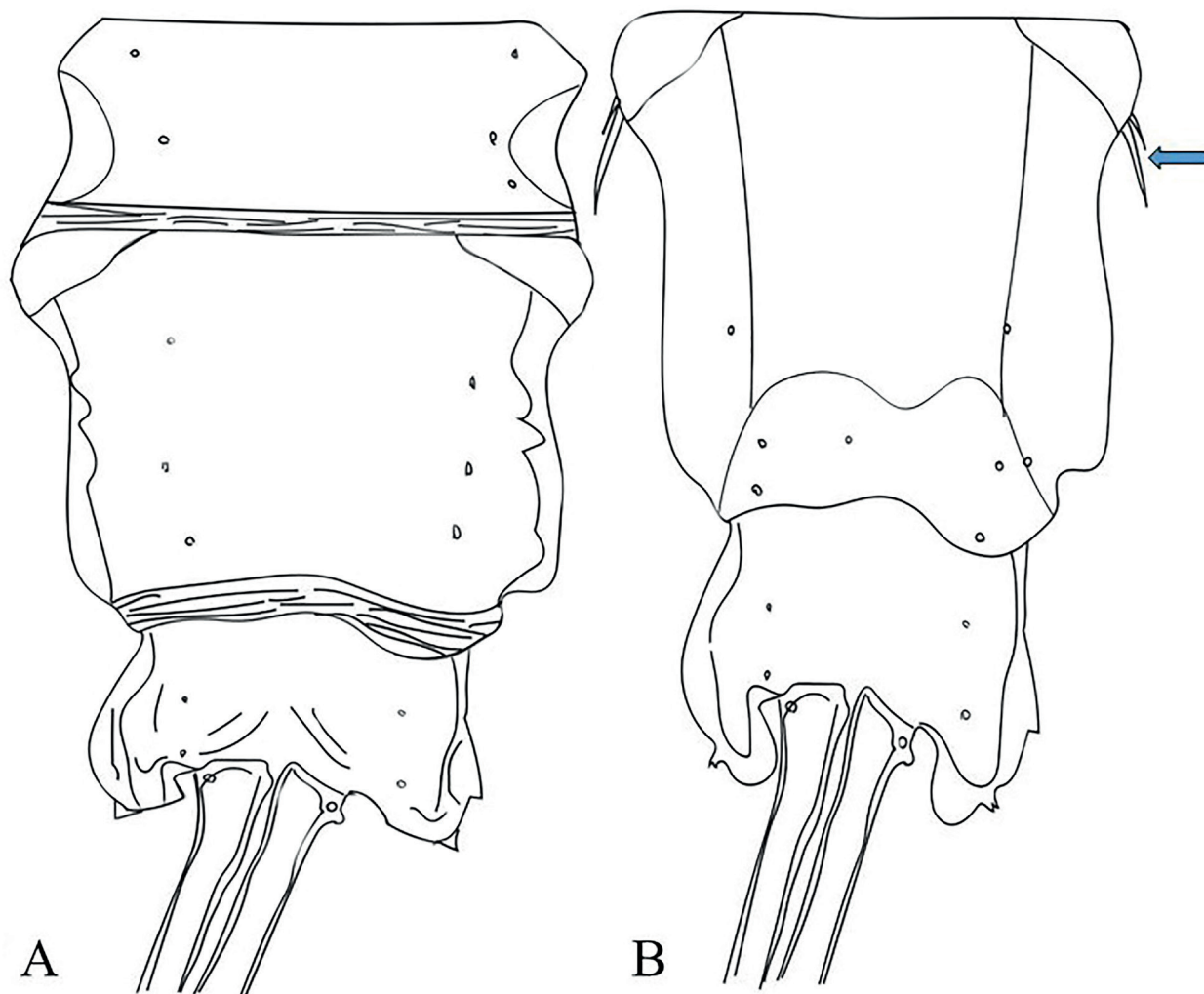


Figure 5. *Clausidium persiaensis* sp. nov. A, urosome, dorsal; B, urosome, ventral. Arrow indicates the P6 (female). Scale bar: 50 μ m.

row of spinules and 1 lateral seta at proximal margin. Outer lobe with row of spinules along outer margin and 4 pinnate setae apically. Inner lobe with 3 pinnate setae. Maxilla (Figs. 3B, 8C) 2-segmented. Syncoxa almost 2 times longer than wide, with 2 pinnate setae and 1 pinnate spine. Basis with large serrate process, bearing 3 setae (1 pinnate, 1 serrate and 1 naked) and 1 pinnate spine. Maxilliped (Figs. 3D, 5C) 4-segmented. Syncoxa with 2 pinnate setae along inner margin. Basis with 1 naked and 1 pinnate seta. Endopod 2-segmented; first segment armed with 1 naked seta; second segment bearing 1 naked lateral seta, 3 stout distal spines and 1 naked inner seta.

P1 (Figs. 4A, 12C) biramous, both rami 3-segmented, and modified for prehension. Coxa and basis fused forming protopod with naked seta on outer corner near exopod insertion; large blade-like element on inner corner, with rounded projection along the outer margin, concentric lines and acute apex. Exp-1 with outer seta. Exp-2 with 1 small seta. Exp-3 with 3 outer setae (proximal and distal ones reduced), 2 apical naked setae and 2 inner pinnate setae.

Enp-1 with 1 stout curved process with adhesive areas along the distal margin (marked with square in Fig. 4A). Enp-2 with a long seta (marked with asterisk in Fig. 4A). Enp-3 elongated, irregular segment

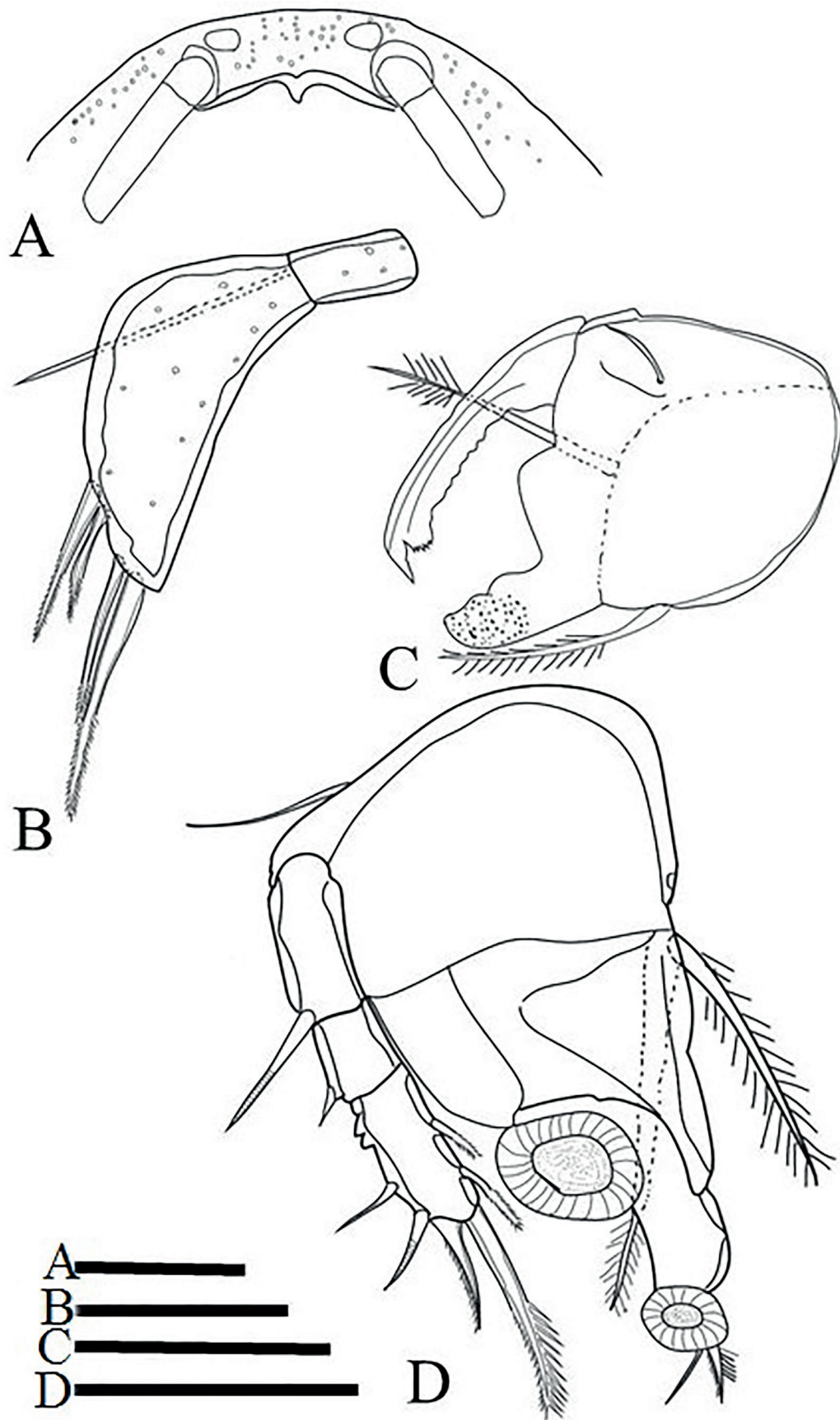


Figure 6. *Clausidium persiaensis* sp. nov. A, rostrum (female); B, P5 (female); C, maxilliped (male); D, P1 (male). Scale bars: A–C, 25 μm; D, 100 μm.

ending in a lobe armed with 1 seta and 2 sucking discs; proximal sucking disc 1.5 larger than distal one. P2–P4 (Fig. 4B–D) biramous, with both rami 3-segmented. Coxae with inner pinnate seta. Basis with pinnate seta on outer distal corner and row of setules along inner margin. Exopod outer spines serrate. Endopod outer apical spines serrate, sucking discs on distal outer edge of enp-1 and proximal and subterminal outer edges of enp-3. Armature formula of P2–P4 as follows (Roman numerals representing spines, Arabic numerals representing setae):

	Coxa	Basis	Exopod	Endopod
P2	0-1	1-0	I-0; I-1; III,I,4	0-1; 0-2; I,II,3
P3	0-1	1-0	I-0; I-1; III,I,4	0-1; 0-2; I,II,3
P4	0-1	1-0	I-0; I-1; III,I,4	0-1; 0-2; I,II,2

P5 (Fig. 6B) uniramous, 2-segmented and located laterally on somite. Protopod with 1 outer seta; free exopodal segment with characteristic shape as shown in Fig. 6B, with 3 serrate spines along outer margin and 1 serrate spine apically. P6 (Fig. 7A marked with blue square) consisting of 2 setae.

Male: total length 0.49–0.65 mm (N = 15). Body cyclopiform (Fig. 10A, B).

Prosome (Fig. 10A, B) longer than urosome (1.6:1). Maximum width measured at midlength of cephalic shield. First pedigerous somite fused with cephalothorax. Body prosomites with minute integumental pits, sensilla and pores distributed as illustrated in figures. Cephalothorax and 3 free prosomites with posterior borders smooth; somites bearing P2–P3 subequal; somite bearing P4 with distal margin concave medially.

Urosome (Figs. 7A, 10A, B) 5-segmented, narrower than prosome. Somite bearing P5 (Figs. 7A, 10B) 2 times wider than long in ventral view and with P5 arising ventrolaterally. Anal somite (Figs. 7A, 10B) extremely reduced and deeply incised medially. Caudal rami (Figs. 2C, 7A), shorter than to the female and seta V, 3 times longer than seta IV.

Antenna (Figs. 7B, 10C) 4-segmented. Coxobasis elongated, with row of spinules along inner margin, with seta on inner distal corner. Endopod 3-segmented; segment 1 with pinnate spine inserted along inner margin; segment 2 with row of denticles, 2 naked setae, 1 pinnate spine and 1 serrate spine; segment

3 with 4 pinnate setae and 2 spines (1 pinnate and 1 serrate). Mandible, maxilla and maxillule as in female. Maxilliped (Figs. 6C, 10C) strongly modified for prehension. Syncoxa with 2 pinnate setae. Basis with 1 naked seta, unequal denticulate projections and distal half of border curved and with irregular margin. Endopod represented by a strong serrate claw.

P1 (Figs. 6D, 12D) similar to female. Coxa and basis fused with 1 naked seta on outer corner and 1 pinnate seta on inner distal edge. Exp-1 with outer seta. Exp-2 with 1 small outer seta. Exp-3 with 2 outer setae, 1 serrate spine and 1 pinnate spine apically and 2 pinnate inner spines. Enp-1 with adhesive fringe along distal margin, stout curved process and long pinnate seta on inner distal corner. Enp-2 with a long seta. Enp-3 elongated, irregular segment ending in a lobe armed with 1 seta and 2 sucking discs.

P2–P4 (Fig. 10B) with larger coxae and not so elongated basis when compared with female. Armature formula of P2–P4 as follows (Roman numerals representing spines, Arabic numerals representing setae):

	Coxa	Basis	Exopod	Endopod
P2	0-1	1-0	I-0; I-1; III,I,4	0-1; 0-1; I,II,3
P3	0-1	1-0	I-0; I-1; III,I,4	0-1; 0-1; I,II,3
P4	0-1	1-0	I-0; I-1; III,I,4	0-1; 0-1; I,II,2

P5 (Fig. 7A) elongated, 7 times longer than wide and almost stright. Exopodal segment elongated with 2 serrate spines and naked seta along outer margin and 1 pinnate spine apically, spine I inserted near spine II, on the distal third of the segment. P6 (Fig. 7A) with 1 pinnate seta.

Etymology. The species name *persiaensis* is derived from the Latin meaning of or belonging to Persia referring to the provenance of the material.

DISCUSSION

There are a large number of different classification schemes proposed for the copepods in molecular, morphological or integrated approaches (Ho, 1990; 1994; Huys and Boxshall, 1991). The phylogenetic relationships of the copepods are very controversial as Huys and Boxshall (1991) argued. They proposed ten orders in their phylogenetics scheme and considered

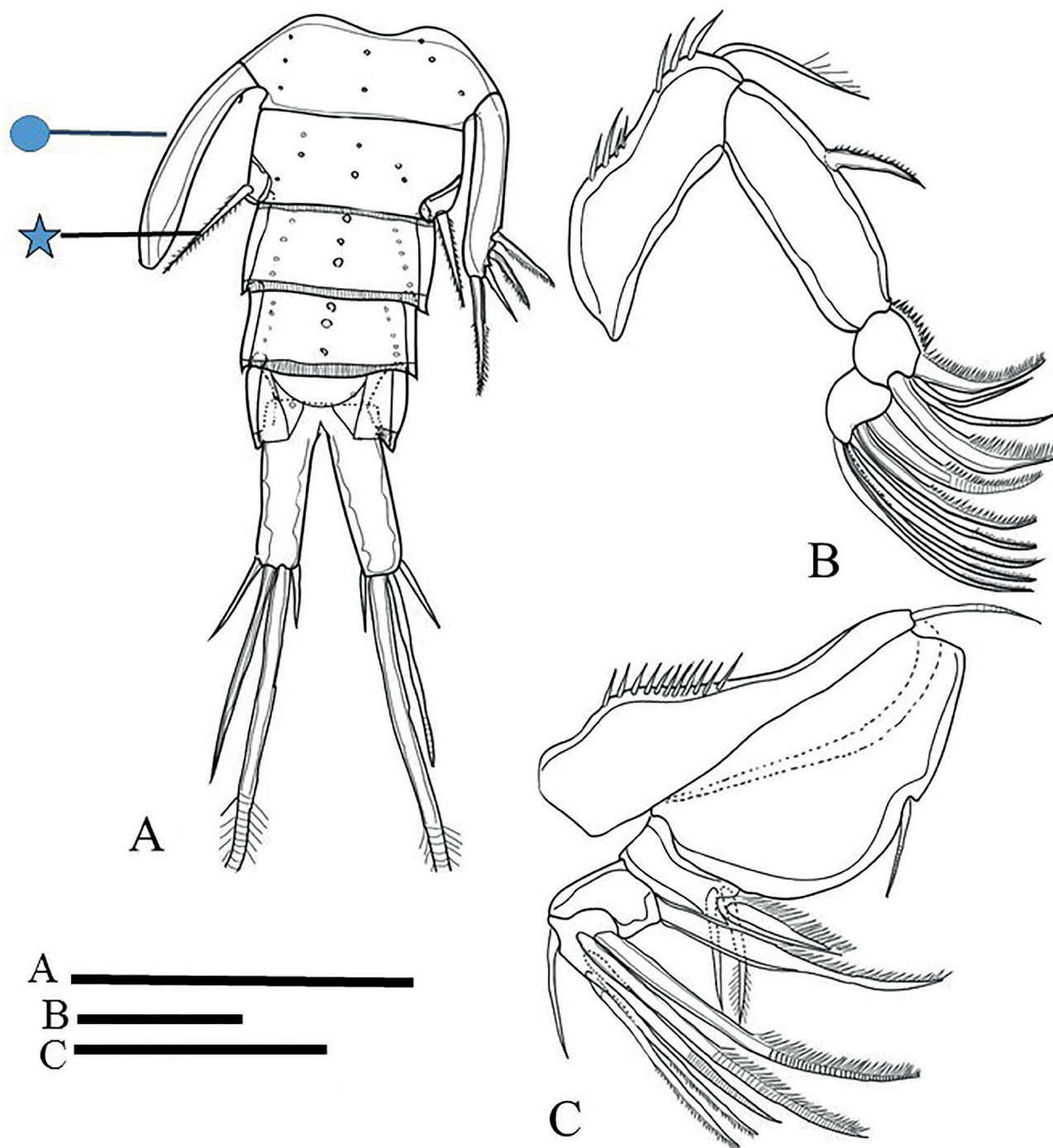


Figure 7. *Clausidium persiaensis* sp. nov. A, urosome (male); B, antenna (male); C, antenna (female). Circle = P5 (male); star = P6 (male). Scale bars: A, 100 μ m; B, 50 μ m.

Poecilostomatoida as a valid order. Boxshall and Hasley (2004) proposed 9 orders for copepods and Cyclopoida treated as an order that comprises all those families previously attributed to both the Cyclopoida and the Poecilostomatoida. It should be noted that Huys *et al.* (2012), by the use of molecular evidence, confirmed that the Poecilostomatoida order was not a monophyletic group, but rather it splits from the

Cyclopoida. Hence this supports the decision to include all these in one order.

In the present study, we accept the concept discussed by Boxshall and Hasley (2004) and assign our new species to Cyclopoida. The generic characters of *Clausidium* are not completely clear as many species descriptions lack male specimens, and so depend heavily on female morphology. On the

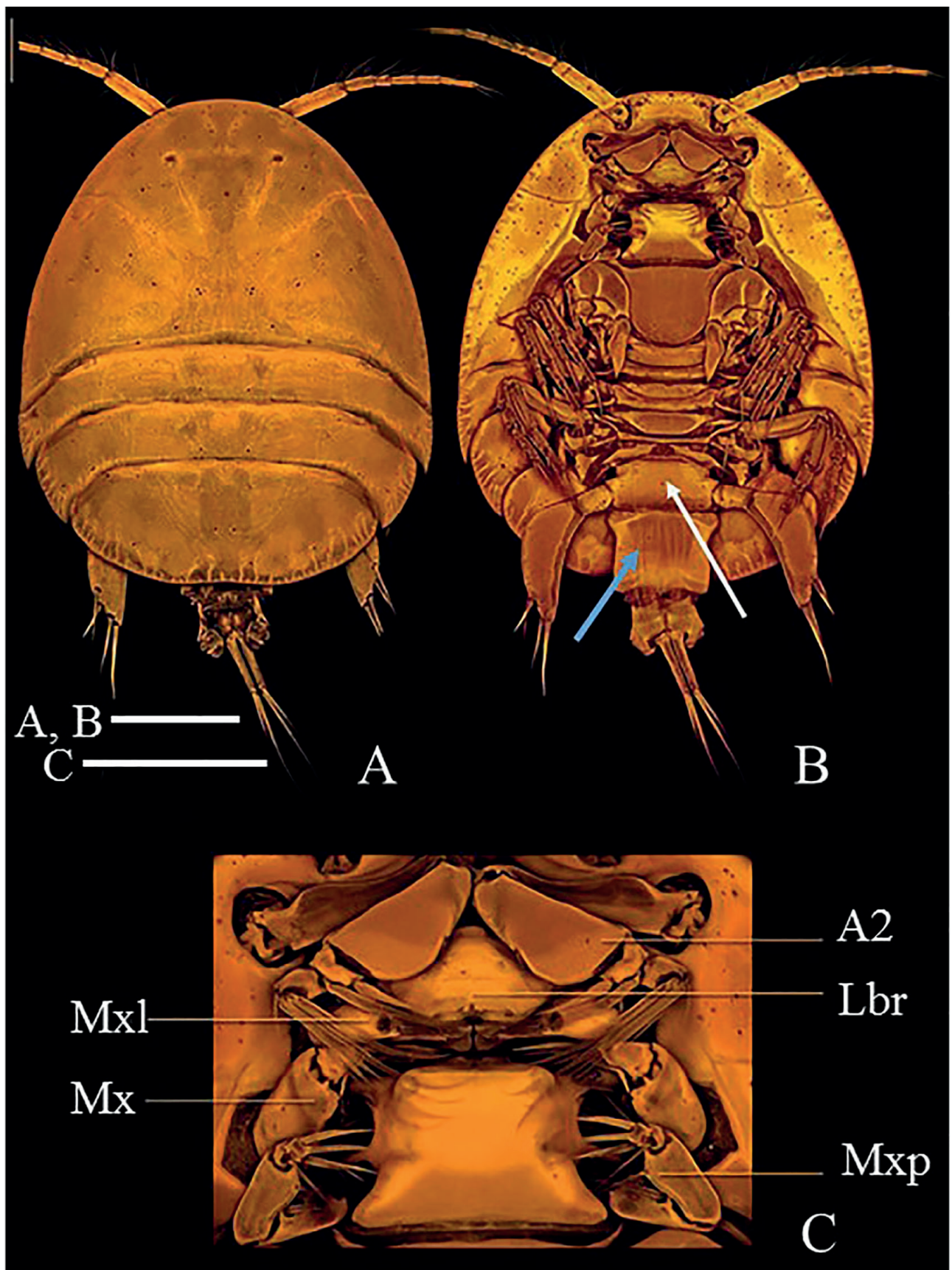


Figure 8. *Clausidium persiaensis* sp. nov. (female). Confocal laser scanning microscopy images. A, habitus, dorsal; B, habitus, ventral; C, oral region. White arrow = somite that bears PS; blue arrow = genital double somite. Scale bars: A, B, 200 μm; C, 100 μm.

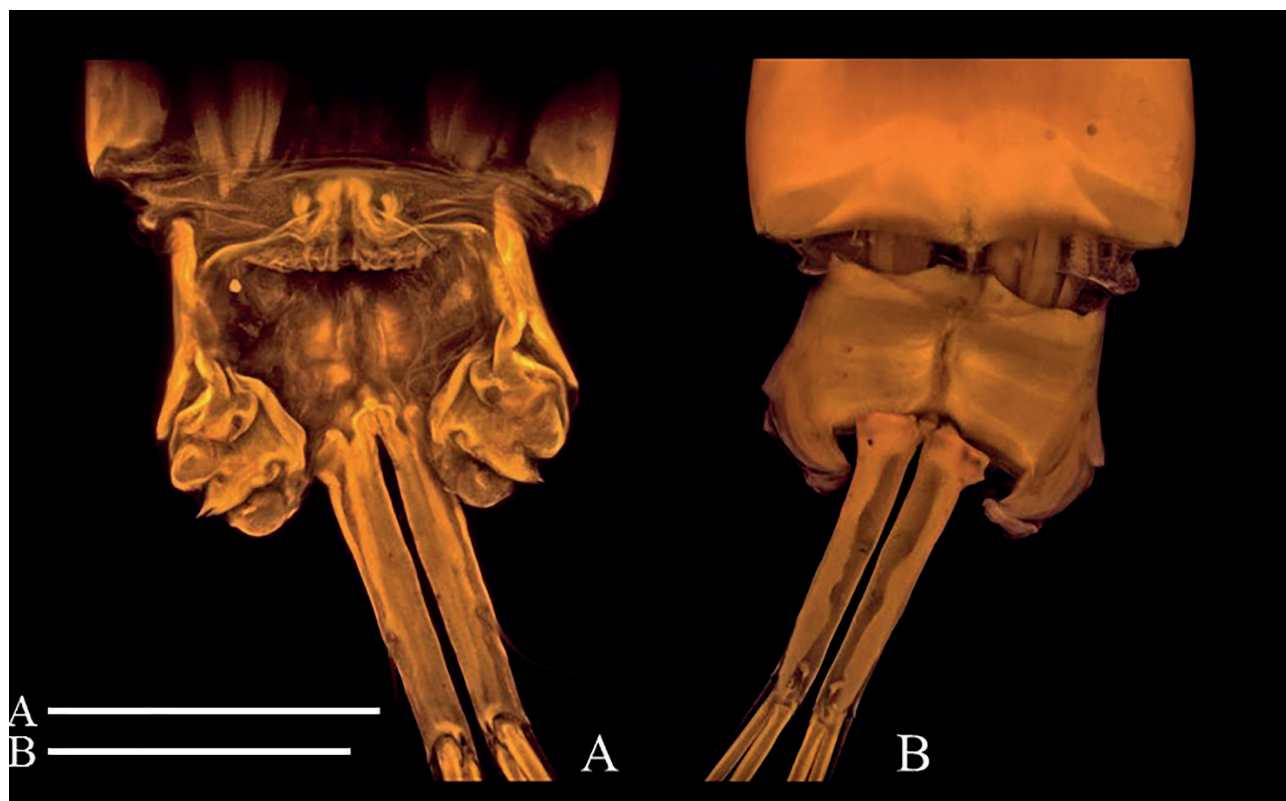


Figure 9. *Clausidium persiaensis* sp. nov. (female). Confocal laser scanning microscopy images. A, caudal rami and anal somite, dorsal; B, caudal rami and anal somite, ventral. Scale bars: 100 μ m.

other hand, many species of *Clausidium* described many years ago are not well illustrated and nor well described (see Pearse, 1947; Wilson, 1932; Kensley, 1974). Following Boxshall and Halsey's (2004) key, the present new species is assigned to *Clausidium* by the small sucker on endopods of legs 1 to 4. It seems that the mentioned character is the most reliable homology for recognition and to use in a phylogenetic study. Furthermore, the following characters can be used as supplementary ones for defining *Clausidium*: body oval and flattened, prosome comprising cephalothorax and 3 free pedigerous somites, urosome comprising 5 or 6 segments, antennule 7-segmented, antenna 4-segmented, legs 1 to 4 biramous, first pair highly modified, fifth leg uniramous and 2-segmented.

Clausidium persiaensis sp. nov. shares with *C. travancorensis* the armature formula of legs 2 to 4, but can be easily distinguished from its congener by the unique characteristics observed in the female's antenna with 2 strong pectinate spines on endopod 2, as well as in the armature and swimming legs with elongated basis. Another differential feature observed in males is the maxilliped with distinct projections. The most obvious differentiation between the two species is in

the shape of the body, P1 and P5. In a dorsal view of *C. persiaensis* sp. nov., the shape of the females body is suboval and the anterior margin is narrower than the posterior one, and the third pedigerous somites are 2.6 as wide as long. In contrast, *C. travancorensis* is more flattened, slightly narrowing posteriorly, the posterior margin tapers to a rounded apex, and the third pedigerous somite is 1.2 as wide as long. The P1 in *C. persiaensis* sp. nov. with a blade like process acute tip (*versus* blunt tip) and Exp2 of P1 with 1 setae (*versus* 2 setae) is also diagnostic. The shape and armature of the P5 are different between the two species. The free exopodal segment of *C. persiaensis* sp. nov. is wide, with a curved proximal part in the general outline, rather than being elongate and slender in *C. travancorensis*. The P5 in *C. persiaensis* sp. nov. also has 3 serrate spines along the outer margin and 1 serrate spine apically (*versus* 3 setae along outer margin and 1 seta apically).

Clausidium persiaensis sp. nov. further shares with *Clausidium rodriguesi* Kihara and Rocha, 2013 the armature of P2–P5, and maxilla and mandible, but is distinguished from the later by the free exopodal segment of P5, which is elongated about 2 times longer than wide and with 4 serrate spines (*versus*

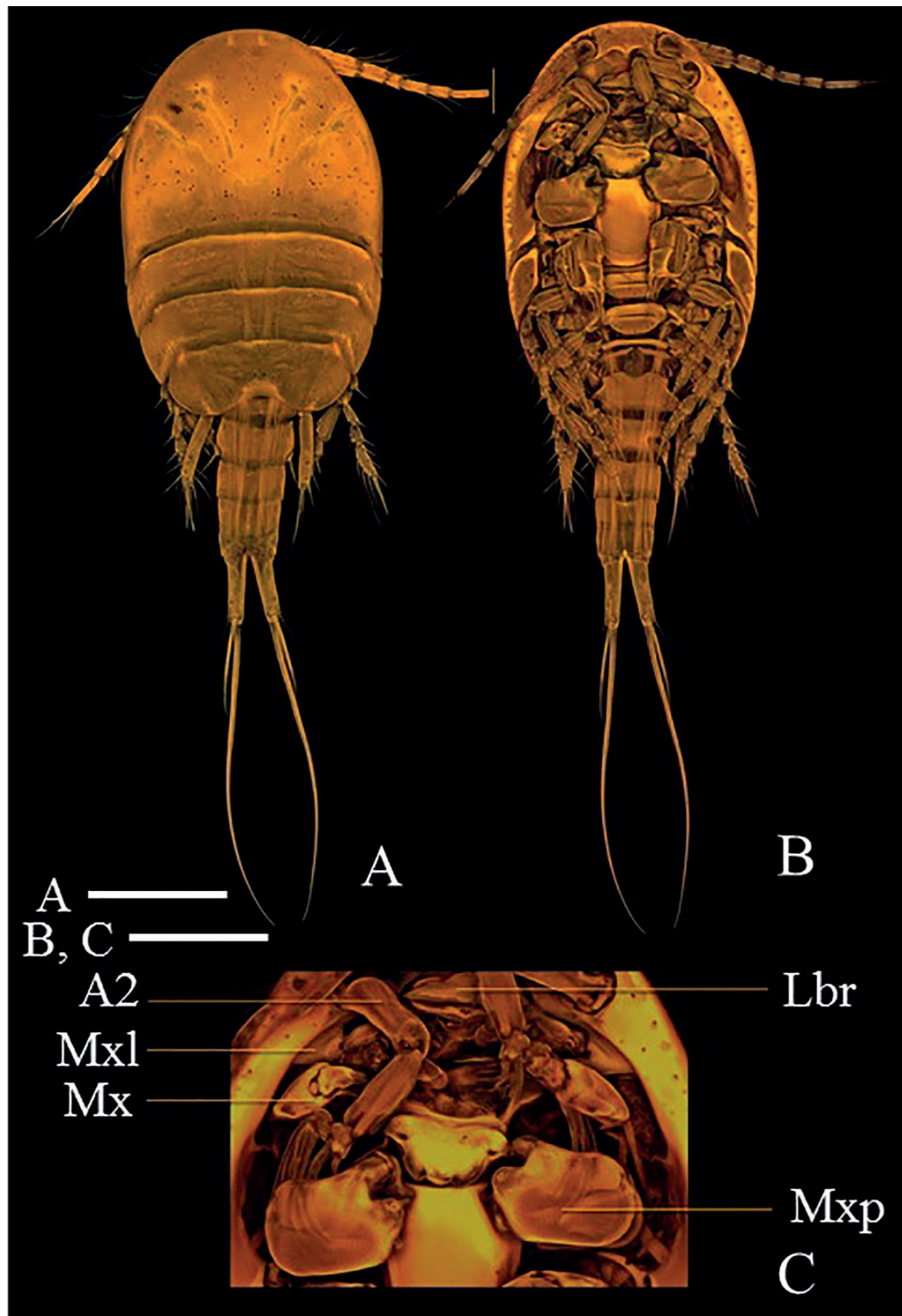


Figure 10. *Clausidium persiaensis* sp. nov. (male) Confocal laser scanning microscopy images. A, habitus, dorsal; B, habitus, ventral; C, oral region. Scale bars: A, B, 100 μ m; C, 50 μ m.

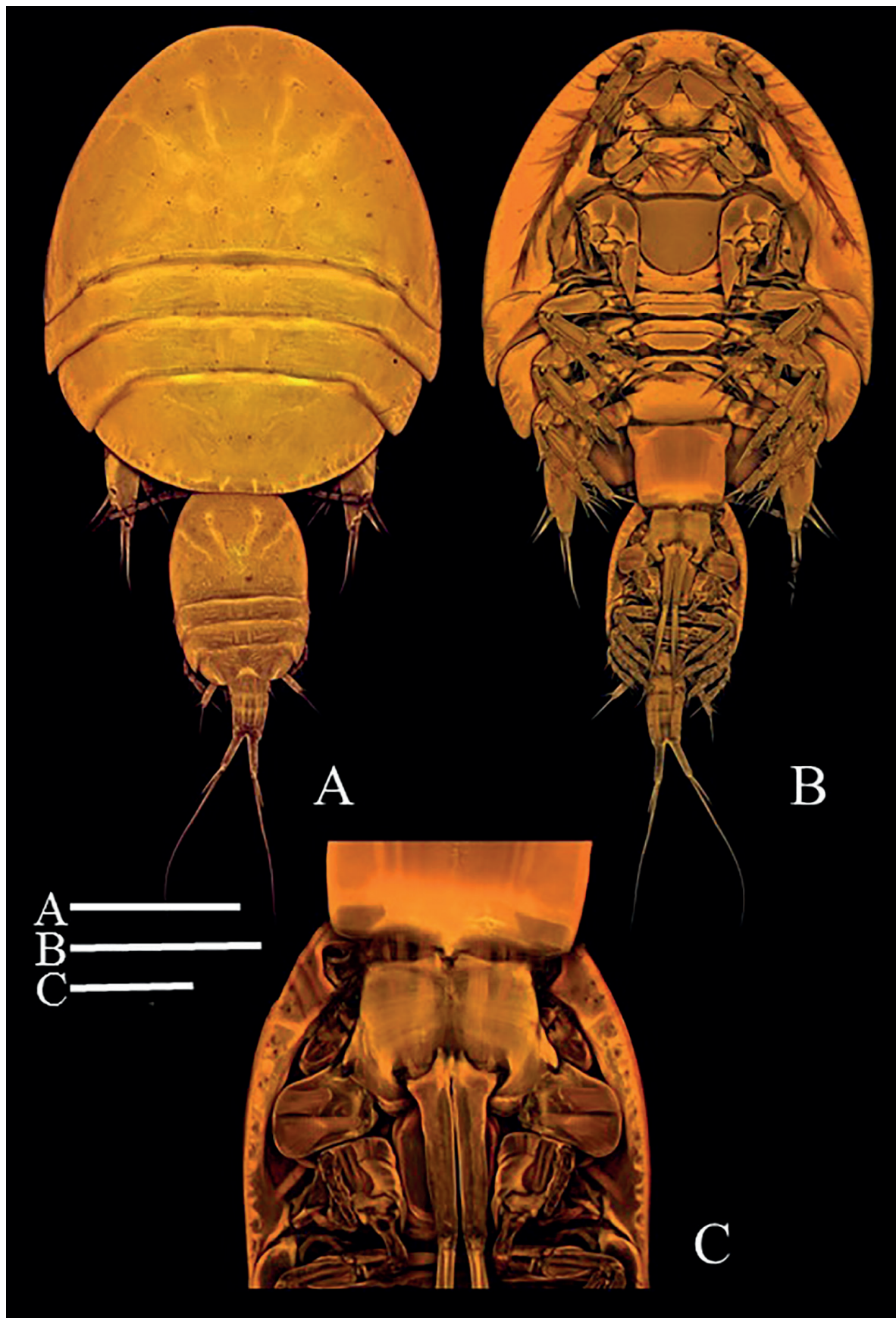


Figure 11. *Clausidium persiaensis* sp. nov. (couple). Confocal laser scanning microscopy images. A, habitus, dorsal; B, habitus, ventral; C, male grasping female with maxilliped. Scale bars: A, B, 200 μm ; C, 50 μm .

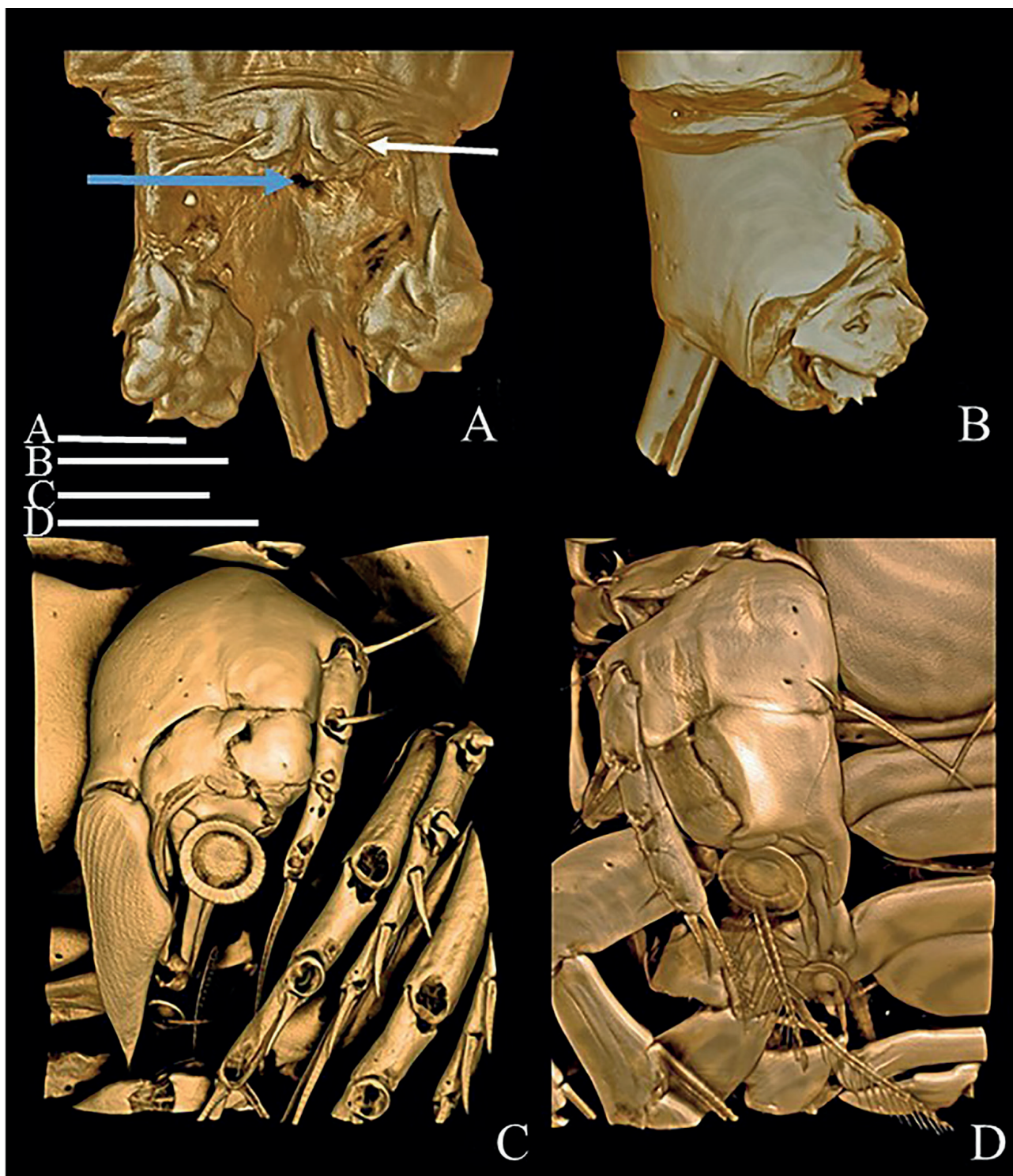


Figure 12. *Clausidium persiaensis* sp. nov. Three-dimensional representation (Drishti software) based on confocal laser scanning microscopy images. A, anal somite, dorsal (female); B, anal somite, lateral (female); C, P1 (female); D, P1 (male). Blue arrow = medial pore; white arrow = genital apertures. Scale bars: 50 μ m.

3 times longer than wide with 3 serrate spines and 1 naked seta). The basis of the syncoxa has a large serrate process, bearing 3 setae (1 pinnate, 1 serrate and 1 naked) and 1 pinnate spine (*versus* 2 pinnate, 1 naked

setae and 1 pinnate spine). The basis of the maxilliped has 1 naked and 1 pinnate seta (*versus* 1 pinnate seta and 1 spine), and the endopod is 2-segmented; first segment armed with 1 naked seta; second segment

bearing 1 naked lateral seta, 3 stout distal spines and 1 naked inner seta (*versus* endopod being 2-segmented; first segment unarmed; second segment bearing 2 naked lateral setae, 3 pinnate distal setae and stout distal spine).

The present species agrees with the female description of *Clausidium maximus* Hwang, Lee & Kim, 2016 in some aspects, which are: the armature formula of legs 1 to 4, maxilla, mandible and caudal ram. *Clausidium persiaensis* sp. nov. is easily defined from that species by the following characters: P5 in the later species having 4 serrate spine (*versus* 4 naked setae), segment 2 of the antennal endopod with 2 pectinate spines and 2 setae (*versus* 4 setae); segment 3 with 6 apical setae (*versus* 7 setae). Also, there are some differences between the male specimens of the above species that includes: free exopodal segment of P5 in *Clausidium persiaensis* elongated with armed with 3 serrate spines with 1 naked seta (*versus* 5 setae).

Early researchers studying *Clausidium* assumed some species were symbiotic and associated of ghost shrimps (Corsetti and Strasser 2003; Kihara and Rocha 2013), although some species were thought to be parasitic (Kossmann, 1874; Wilson, 1937; Pearse, 1947; Humes, 1949; Pillai, 1959). This complex interaction is a potentially important aspect of ghost shrimp biology. Because of the influential role that ghost shrimps play in aquatic systems, *Clausidium* copepods may have indirect effects on local communities and ecosystem processes via their direct effects on ghost shrimps.

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