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A new species of *Cymodoce* Leach, 1814 (Crustacea: Isopoda: Sphaeromatidae) based on morphological and molecular data, with a key to the Northern Indian Ocean species

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Abstract

Cymodoce waegelei **sp. nov.** is described from the subtidal zone of the Iranian coasts of the Persian Gulf using both morphological and molecular data. *C. waegelei* **sp. nov.** is most similar to *C. tribullis* Harrison & Holdich, 1984 from Australia, Vietnam and Singapore. Analysis of DNA barcodes and nuclear 28S rDNA: D8 expansion segments clearly support the existence of two distinct species. *Cymodoce waegelei* sp. nov morphologically differs from *C. tribullis* by lacking two continuous rows of tubercles on the pereonites 3 and 4. Moreover, the pleotelson has numerous scattered tubercles between two large prominent bosses, and small lateral tubercles rather than two prominent tubercles in *C. tribullis.* Based on our results we redescribe *Cymodoce tribullis* using specimens sampled from the type locality, Magnetic Island, Queensland. *Cymodoce lirella* Schotte & Kensley, 2005 from the Seychelles is placed in synonymy with *C. tribullis* Harrison & Holdich 1984. Furthermore we provide a key to the northern Indian Ocean species of this genus.

Key words: Indian Ocean, Persian Gulf, Sphaeromatidae, Isopoda, *Cymodoce*, new species, DNA barcoding, 28S rDNA: D8 expansion segment

Introduction

With about 75 described species (Schotte *et al*. 1995), the genus *Cymodoce* represents a species-rich genus of the family Sphaeromatidae Latreille, 1825 as part of the order Isopoda Latreille, 1817 (Schotte 2013). Isopods of this genus occur worldwide with the exception of polar waters, and are predominantly found on the continental shelf. They are usually collected from the intertidal and shallow subtidal zones (1–3 m) and can be found living in algal (e.g. *Sargassum*) and sea-grass beds, in dead intertidal and sub-tidal corals, dead barnacles, and amongst dead oyster shells (Harrison & Holdich 1984, Khalaji-Pirbalouty *et al*. 2013). To date, only two *Cymodoce* species are known from the Persian Gulf: *Cymodoce fuscina* Schotte & Kensley, 2005 has been reported from the Saudi Arabian coast and *Cymodoce delvarii* Khalaji-Pirbalouty, Bruce & Wägele, 2013 is known from the Iranian coast.

Here we redescribe *Cymodoce tribullis* Harrison & Holdich, 1984, placing *Cymodoce lirella* Schotte & Kensley, 2005 into synonymy, and describe a new species of *Cymodoce* from the Persian Gulf using morphological as well as DNA barcodes and the D8 expansion fragment of the nuclear 28S rDNA. During the last years, DNA barcoding has become a very efficient tool for the identification of species (Hebert *et al*. 2003). For animals, an approximately 660 base-pair fragment of the mitochondrial cytochrome *c* oxidase subunit I (CO1) gene was proposed as global standard for the identification of unknown animal specimens in terms of a given classification (Hebert *et al*. 2003). DNA barcoding has been successfully applied to numerous number of crustaceans (e.g. Costa *et al*. 2009, Radulovici *et al*. 2009, da Silva *et al*. 2011), and, in terms of an integrative taxonomic framework, an increasing number of taxonomic studies include barcode sequence data (e.g. Lörz *et al*. 2009, Yoshida *et al*. 2011, Chen *et al*. 2012, Riehl & Kaiser 2012, Carrison-Stone *et al*. 2013, Keikhosravi & Schubart 2013). In order to

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exclude effects of heteroplasmy, incomplete lineage sorting, introgressive hybridization and the presence numts which can affect the usefulness of mitochondrial DNA and DNA barcodes in particular (e.g. Funk & Omland 2003), hypervariable expansion or divergence segments of the nuclear 18S and/or 28S rDNA can be used as effective supplementary molecular markers for the identification of even closely related species (e.g. Markmann & Tautz 2005, Sonnenberg *et al*. 2007, Fisher & Smith 2008, Raupach *et al*. 2010). Therefore we also tested the usefulness of the D8 expansion segment of the 28S rRNA gene as molecular identification tool.

Material and methods

Morphological studies. Specimens for this study were collected primarily from subtidal habitats along the Iranian coastline of the Persian Gulf. *Cymodoce tribullis* and *C. lirella* were obtained from the Museum of Tropical Queensland (Townsville, Australia) and the National Museum of Natural History (Smithsonian Institution, Washington, United States of America), respectively. Appendages were dissected from the analysed specimens using a dissecting microscope (Olympus SZX12) and fixed in stained antibacterial glycerine-gelatine (Merck). Drawings were made using a compound microscope (Olympus BX 51). Following the inking, all images were processed using Adobe Photoshop (version CS5). SEM micrographs were taken using a Hitachi S-2460N SEM. All loaned specimens returned to the responsible national history collections with the end of our analysis.

Molecular Studies. DNA extraction, amplification and sequencing. Genomic DNA was extracted from one to three dissected legs of five specimens of *Cymodoce delvarii*, four specimens of *C. fuscina*, five specimens of *C. tribullis*, and four specimens of *C. waegelei* **sp. nov.** (see Table 1), using the QIAmp© Tissue Kit (Qiagen GmbH, Hilden, Germany), and following the extraction protocol. Polymerase chain reaction (PCR) was performed for the amplification CO1 barcode fragment using the newly designed degenerated isopod-specific primer pair ISO-LCO (5'-DTT CCA CHA ACC AYA ARG AYA TTG G-3') and ISO-HCO (5'-TAH ACY TCW GGG TGN CCA AAR AAY CA-3'). We added derived M13 forward and reverse tails to both primers to provide defined base sequence for sequencing (Ivanova *et al*. 2007). The complete primer sequences were therefore 5'-TGT AAA ACG ACG GCC AGT DTT CCA CHA ACC AYA ARG AYA TTG G-3' for ISO-LCO-M13F and 5'-CAG GAA ACA GCT ATG ACT AHA CYT CWG GGT GNC CAA ARA AYC A-3' for ISO-HCO-M13R (M13 tail sequences were underlined). All PCR products were amplified using illustra™ puReTaq Ready-To-Go PCR Beads (GE Healthcare, Buckinghamshire, UK) in a total volume of 20 µl, containing 17.5 µl sterile molecular grade H_2O, 2 µl DNA template with an DNA amount between 2 to 150 ng/µl and 0.25 µl of each primer (20 pmol/µl). The PCR thermal conditions included an initial denaturation at 94 °C (5 min), followed by 38 cycles at 94 °C (denaturation, 45 s), 48 °C (annealing, 45 s), 72 °C (extension, 80 s), and a final extension step at 72 °C (7 min). In addition to the CO1 barcode region, an app. 850 base pair (bp) region of the ribosomal 28S rDNA: D8 expansion segment was amplified for selected specimens of *Cymodoce delvarii* (*n* = 2), *C. tribullis* (*n* = 3) and *C. waegelei* (*n* = 3) with the newly designed primer pair AD8F (5'-CGG GAG AAG GAT TGG CTC T-3') and AD8R (5'-GGG CAG AAA TCA CAT CGC G-3') in 20 µl volume reaction tubes, containing 4 µl Q-Solution©, 2 µl 10x Qiagen PCR buffer, 2 µl dNTPs (2 mmol/µl), 0.2 µl of each primer (both 25 pmol/µl), 2 µl of DNA template with an DNA amount between 2 to 150 ng/µl and 0.2 µl Qiagen Taq polymerase (5 U/µl). The reaction tube was filled up to 20 µl with sterile molecular grade H_2O. The temperature profile used consisted of an initial denaturation of 94 °C (5 min), followed by 38 cycles of 94 °C for denaturation (45 s), 58 °C for annealing (45 s), and 72 °C for extension (80 s). The amplification reactions were completed by a final extension (72 °C) for 7 min. All PCR amplification reactions were conducted using an Eppendorf Mastercycler© Pro system (Eppendorf, Hamburg, Germany). Negative and positive controls were included with each round of reactions. Two µl of the amplified products were verified for size conformity by electrophoresis in a 1% agarose gel with GelRed™ using commercial DNA size standards, whereas the remaining PCR product was purified with the QIAquick© PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Purified PCR products were cycle sequenced and sequenced in both directions at a contract sequencing facility (Macrogen, Seoul, Korea or GATC, Konstanz, Germany) using the M13 sequence tails for the DNA barcodes or the same primers as used in PCR in the case of 28S rDNA fragment. Double stranded sequences were assembled with the Geneious version 7.0.4 program package (Biomatters, Auckland, New Zealand). BLAST searches were performed to confirm the identity of all new sequences (Zhang *et al*. 2000; Morgulis *et al*. 2008). All aligned CO1 sequences were translated to amino acid sequences to check for nuclear mitochondrial pseudogenes

TABLE 1. Table of all *Cymodoce* specimens that were analysed using molecular methods, including individual codes, gender, collection sites with coordinates and dates, and GenBank accession numbers of both analysed markers.

Code	Genus and species	Gender	Sample locality and sample date	Longitude	Latitude	Depth [m]	Collector	Accession number CO1	Accession number 28S rDNA: D8
ZMH-K-42597-T1	*Cymodoce delvarii*	male	Iran, Golestan-e-Saheli; 28.04.2010	N 28.2388	E 51.2793	1.5	V. Khalaji, A. Samimi	KJ410460	
ZMH-K-42597-T2	*Cymodoce delvarii*	male	Iran, Golestan-e-Saheli; 28.04.2010	N 28.2388	E 51.2793	1.5	V. Khalaji, A. Samimi	KJ410461	
ZMH-K-42597-T3	*Cymodoce delvarii*	male	Iran, Golestan-e-Saheli; 28.04.2010	N 28.2388	E 51.2793	1.5	V. Khalaji, A. Samimi	KJ410462	JN247574
ZMH-K-42597-T4	*Cymodoce delvarii*	male	Iran, Golestan-e-Saheli; 28.04.2010	N 28.2388	E 51.2793	1.5	V. Khalaji, A. Samimi	KJ410463	JN247575
ZMH-K-42597-T5	*Cymodoce delvarii*	male	Iran, Golestan-e-Saheli; 28.04.2010	N 28.2388	E 51.2793	1.5	V. Khalaji, A. Samimi	KJ410464	
SMF 39654-N1	*Cymodoce fuscina*	male	Saudi Arabia, N. Jubail; 22.02.1993	N 27.3191	E 49.4222	1-3	M. Apel	KJ410465	
SMF 39654-N2	*Cymodoce fuscina*	male	Saudi Arabia, N. Jubail; 22.02.1993	N 27.3191	E 49.4222	1-3	M. Apel	KJ410466	
SMF 39654-N3	*Cymodoce fuscina*	male	Saudi Arabia, N. Jubail; 22.02.1993	N 27.3191	E 49.4222	1-3	M. Apel	KJ410467	
SMF 39654-N4	*Cymodoce fuscina*	male	Saudi Arabia, N. Jubail; 22.02.1993	N 27.3191	E 49.4222	1-3	M. Apel	KJ410468	
MTQ W31864-A1	*Cymodoce tribullis*	male	Australia, Nelly Bay, Magnetic Island; 09.03.2010	S 19.1701	E 146.8480	1	N.L. Bruce, C. Buxton	KJ410455	
MTQ W31864-A2	*Cymodoce tribullis*	male	Australia, Nelly Bay, Magnetic Island; 09.03.2010	S 19.1701	E 146.8480	1	N.L. Bruce, C. Buxton	KJ410456	JN247568
MTQ W31864-A3	*Cymodoce tribullis*	male	Australia, Nelly Bay, Magnetic Island; 09.03.2010	S 19.1701	E 146.8480	1	N.L. Bruce, C. Buxton	KJ410457	JN247569
MTQ W31864-A4	*Cymodoce tribullis*	male	Australia, Nelly Bay, Magnetic Island; 09.03.2010	S 19.1701	E 146.8480	1	N.L. Bruce, C. Buxton	KJ410458	JN247570
MTQ W31864-A5	*Cymodoce tribullis*	male	Australia, Nelly Bay, Magnetic Island; 09.03.2010	S 19.1701	E 146.8480	1	N.L. Bruce, C. Buxton	KJ410459	
ZUTC Iso. 1106-C2	*Cymodoce waegelei*	male	Iran, Bousher coast, Jofreh Mahini; 09.08.2009	N 28.9670	S 50.8183	1-3	V. Khalaji	KJ410469	
ZUTC Iso. 1106-C3	*Cymodoce waegelei*	male	Iran, Bousher coast, Jofreh Mahini; 09.08.2009	N 28.9670	S 50.8183	1-3	V. Khalaji	KJ410470	JN247571
ZUTC Iso. 1106-C4	*Cymodoce waegelei*	male	Iran, Bousher coast, Jofreh Mahini; 09.08.2009	N 28.9670	S 50.8183	1-3	V. Khalaji	KJ410471	JN247572
ZUTC Iso. 1106-C5	*Cymodoce waegelei*	male	Iran, Bousher coast, Jofreh Mahini; 09.08.2009	N 28.9670	S 50.8183	1-3	V. Khalaji	KJ410472	JN247573

(numts) using Geneious. All analyzed sequences are available in GenBank (28S rDNA: D8: *Cymodoce delvarii*: JN247574–JN247575, *C. tribullis*: JN247568–JN247570, and *C. waegelei*: JN247571-JN247573; CO1: *C. delvarii*: KJ410460–KJ410464, *C. fuscina*: KJ410465–KJ410468, *C. tribullis*: KJ410455–KJ410459, and *C. waegelei*: KJ410469–KJ410472).

Sequence analysis. All sequences were aligned using MUSCLE version 3.6 (Edgar 2004) with default settings. Intra- and interspecific genetic distances based on *p*-distances and, in the case of the CO1 data, Kimura-2-parameter distances (K2P; Kimura 1980) as well as base frequencies of all *Cymodoce* sequences were calculated using MEGA 6.0.5 (Tamura *et al*. 2013). Furthermore, both alignments were tested for a nucleotide bias using a chi-square test of base composition homogeneity across the analyzed taxa implemented in PAUP*4.0b10 (Swofford 2002). The program MEGA 6.0.5 was also used to perform a neighbour-joining cluster analysis (Saitou & Nei 1987) based on *p*-distances for a graphical representation of nucleotide divergence for both molecular markers. Bootstrap supporting values were calculated by resampling and analyzing 1,000 replicates (Felsenstein 1985)

Abbreviations

BMNH—Natural History Museum, London; MTQ—Museum of Tropical Queensland, Queensland Museum, Townsville; SMF—Senckenberg Museum, Frankfurt am Main, Germany; USNM—Smithsonian Institution Natural Museum of Natural History, USA; ZFMK—Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany; ZMH—Zoologisches Museum Hamburg, Hamburg, Germany; ZMUC—Zoological Museum, University of Copenhagen, Denmark; ZUTC—Zoological Museum, University of Tehran, Iran. RS—Robust seta/e; SPS—Sensory palmate seta/e; PMS—Plumose marginal setae.

Systematics

Family Sphaeromatidae Latreille

Genus *Cymodoce* Leach, 1814

Cymodoce Leach, 1814: 433.—Dumay, 1972: 642.—Kussakin, 1979: 417.—Harrison & Holdich, 1984: 301.—Schotte & Kensley, 2005: 1243.—Khalaji-Pirbalouty *et al.*, 2013: 501.

Type species: *Cymodoce truncata* Leach, 1814; by monotypy.

Diagnosis. The most recent diagnosis to the genus can be found in Khalaji-Pirbalouty *et al*. (2013).

Cymodoce tribullis Harrison & Holdich, 1984
(Figs. 1–5)

Cymodoce tribullis Harrison & Holdich, 1984: 304.—Kussakin & Malyutina, 1993: 1171
Cymodoce lirella Schotte & Kensley, 2005: 1248.
Cymodoce longistylis.—Pillai, 1965: 76 (not *Cymodoce longistylis* Miers, 1884).
Cymodoce mammifera.—Pillai, 1965: 78 (not *Cymodoce mammifera* Haswell, 1881).

Material examined. ♂ (8.8 mm) paratype, from type locality, Magnetic Island, Queensland, 19 °10’S, 146 °50’E, 24 April 1976, coll. D. M. Holdich (QM W9643). 5 adults ♂, numerous ♀ and subadult ♂, Magnetic Island, Nelly Bay, Queensland, 19 °10’12.25”S, 146 °50’52.64”E, 9 March 2010, 1 m. depth, coll. N. L. Bruce & C. Buxton (MTQ W31864).

Diagnosis. Head and pereonites 1–2 smooth, lacking tubercles, pereonites 3–7 with two transverse rows of small tubercles. Pleon posterior margin with two extensions bearing two tufts of long setae on either side. Pleotelson with 2 large, apically bifid bosses, lacking small tubercles between 2 bosses; dorsolateral sides possess 2 prominent tubercles, one posterior to other; posterior half of the pleotelson with medial, large smooth hemispheric

dome; posterior margin trilobed, medial lobe extending well beyond lateral lobes. Appendix masculina extending beyond endopod by one-third of length, tapering evenly to a narrowly rounded apex, distally bearing cuticular spines on most of surface. Uropod rami extending beyond pleotelsonic medial lobe apex.

Description of male (from type locality). Body about 2.2 times as long as greatest width (pereonite 6). *Head* and pereonites 1–2 dorsal surfaces smooth; pereonites 3–7 with two transverse rows of small, and increasingly more prominent tubercles. Pereonites 2–7 coxal plates with evident sutures, and bearing some long setae on distal surface (Fig. 1A).

Pleon with a row of tubercles over two, long, straight, separate and parallel sutures at each side, dorsally bearing scattered uneven tubercles of various sizes; posterior margin with 2 extensions and 2 pronounced tufts of simple long setae on each side; posterolateral margins with a fringe of very long sub-marginal setae.

Pleotelson (Fig. 1A, B; 5A–C) bearing scattered tubercles of various sizes over most of surface, with 2 large prominent apically bifid bosses; dorsally with tufts of long setae especially in the cleft and below of the bifid process, large bosses medial regions lacking small tubercles, lateral regions with 2 prominent tubercles, one posterior to the other, each bearing tuft of long simple setae; posterior region of pleotelson in midline with a hemispheric dome. Apex of pleotelson clearly trilobed; lateral lobes blunt; medial lobe extending well beyond the level of lateral lobes, bearing 2 apical conical tubercles and tuft of long setae, dorsally tuberculated.

Antennula (Fig. 1C) peduncle article 1 bearing scattered weak tubercles, with 4 small sensory palmate submarginal setae on dorsal margin; articles 2 short with some small SPS on ventral and dorsal margins, article 3 with 3–4 long setae on distal margin; flagellum with 17 articles, articles 5–16 each bearing single aesthetasc.

Antenna (Fig. 1D) peduncle articles 1–3 short, subequal in length, article 5 about 1.2 times as long as article 4; articles 2–5 superodistal margins with long simple setae; flagellum with 21 articles, extending to posterior margin of pereonite 2.

Epistome (Fig. 1E) granulose, with triangular acute apex, lateral margins concave.

Left mandible (Fig. 2C) incisor with 3 cusps, lacinia mobilis bluntly dentae, with 3 cusps, spine row of 6 serrate spines.

Maxillula (Fig. 2A) lateral endite with long fine setae on mesial margin, outer margin bearing small stout setae, apical margin with 10 simple or serrate RS, 2 short RS and 1 small serrate submarginal seta; mesial endite with 4 long, robust, comb and 1–2 short simple setae.

Maxilla (Fig. 2B) lateral and middle endites each with 10 curved pectinate RS; mesial endite with 2 rarely plumose, 2 long robust comb, 7–8 robust proximally plumose and distally biserrate, and some slender simple setae.

Maxilliped (Fig. 2D) endite lateral margin sinuate, mesial margin with single coupling hook, distal margin with 4 blunt rarely plumose RS and 7 longer circumplumose RS, inner surface with 3 long circumplumose robust and transverse row of stout finely serrate setae; palp article 2 with single long seta on superodistal angle.

Pereopod 1 (Fig. 2E) basis about 2.6 times as long as greatest width, ischium superior margin with 1 curve, acute RS on proximal corner and 1 long and 1 small RS on medial angle; merus superodistal angle with 4 biserrate RS, inferior margin with 5 biserrate RS and single long apically palmate seta; carpus inferior margin with 3 biserrate RS; propodus inferior margin with 5 biserrate RS set in amongst some acute scales; dactylus inferior margin with serrate cuticular scales.

Pereopod 2 (Fig. 2F) basis about 2.6 times as long as greatest width, with 3 small SPS; ischium superior margin with 1 long RS on medial angle; merus superodistal angle with 3 RS, inferior margin with 4 biserrate RS and single long apically palmate seta; merus, carpus and propodus inferior margin fringed with short setae; carpus inferior margin with 6 biserrate RS, and long apically palmate seta, superodistal angle with 1 RS; propodus inferior margin with 4 biserrate RS, superodistal angle with 3 long simple seta and a single SPS; dactylus inferior.

Pereopod 3 (Fig. 3A) is similar to pereopod 2 as illustrated.

Pereopods 4 and 5 (Fig. 3B, Fig. 3C) similar as illustrated.

Pereopod 6 (Fig. 3D) basis about 3 times as long as greatest width, inferodistal angle with 1 simple setae, superior margin with several simple and 2 SPS; ischium superior margin with 1 long and some small RS; merus superior distal margin with 4 long distally biserrate setae, inferior margin with 6 roust biserrate setae and a single long apically palmate seta; carpus subequal in length to merus, inferior margin with 7 biserrate RS and single long apically palmate seta, distal margin with 4 biserrate RS; propodus superodistal corner with 4 slender and single SPS, inferior margin with 4 biserrate RS; dactylus inferior margin with cuticular scales.

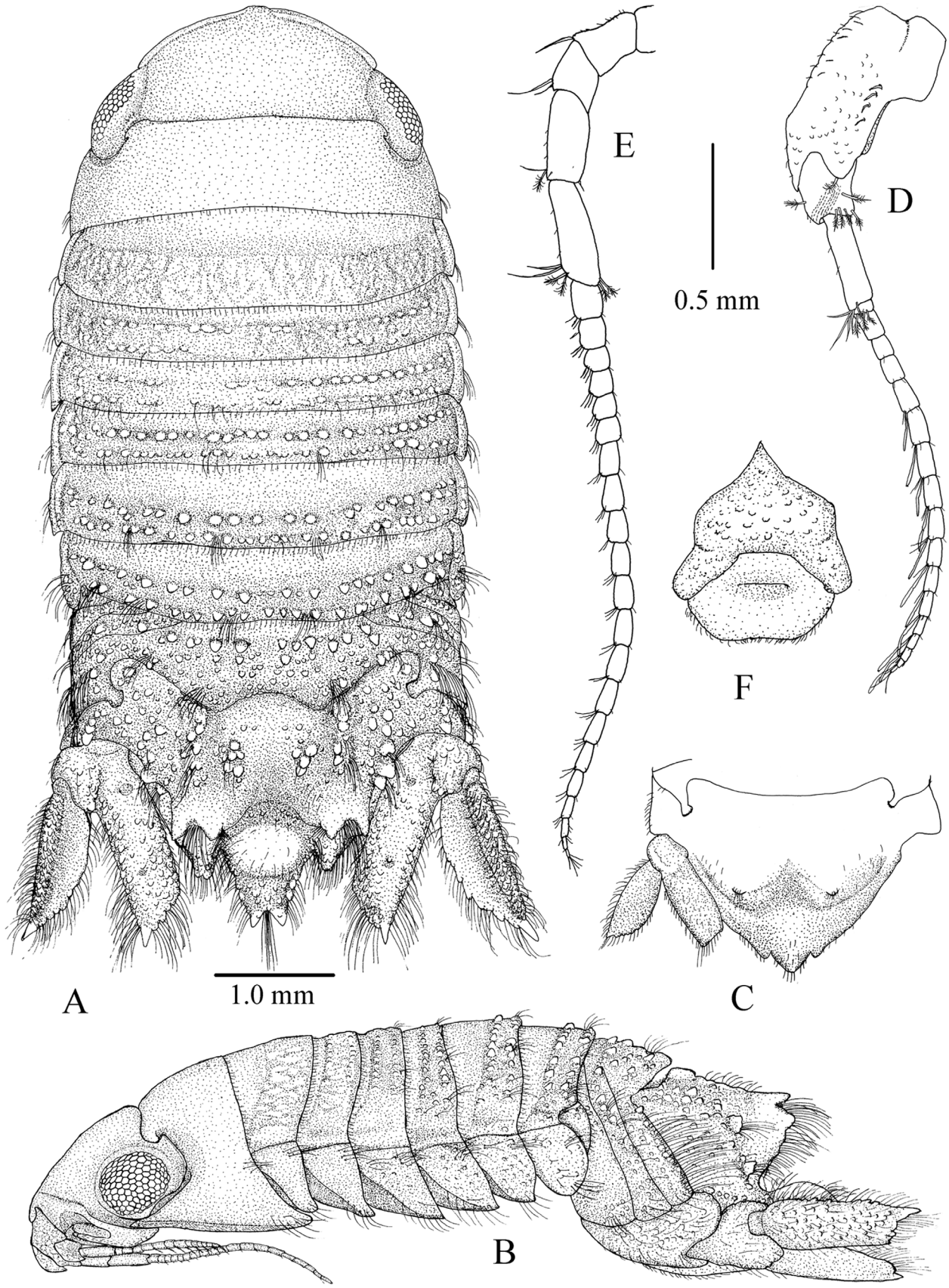

FIGURE 1. *Cymodoce tribullis* Harrison & Holdich 1984, paratype (QM W9643); A, dorsal view; B, lateral view; C, antennula; D, antenna; E, epistome; F, female (QM W9643).

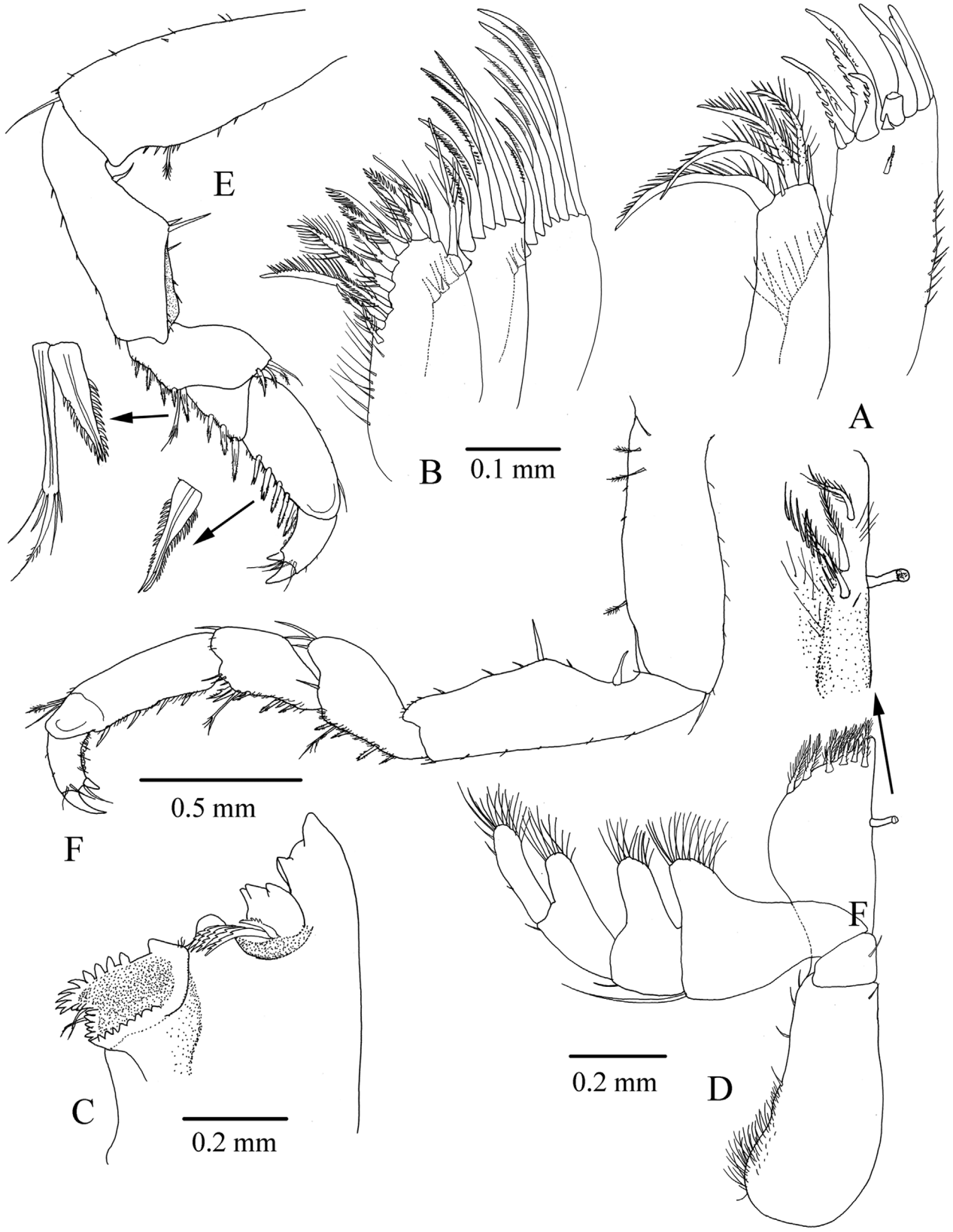

FIGURE 2. *Cymodoce tribullis* Harrison & Holdich 1984, paratype (QM W9643); A, maxillula; B, maxilla; C, left mandible; D, maxilliped; E, pereopod 1; F, pereopod 2.

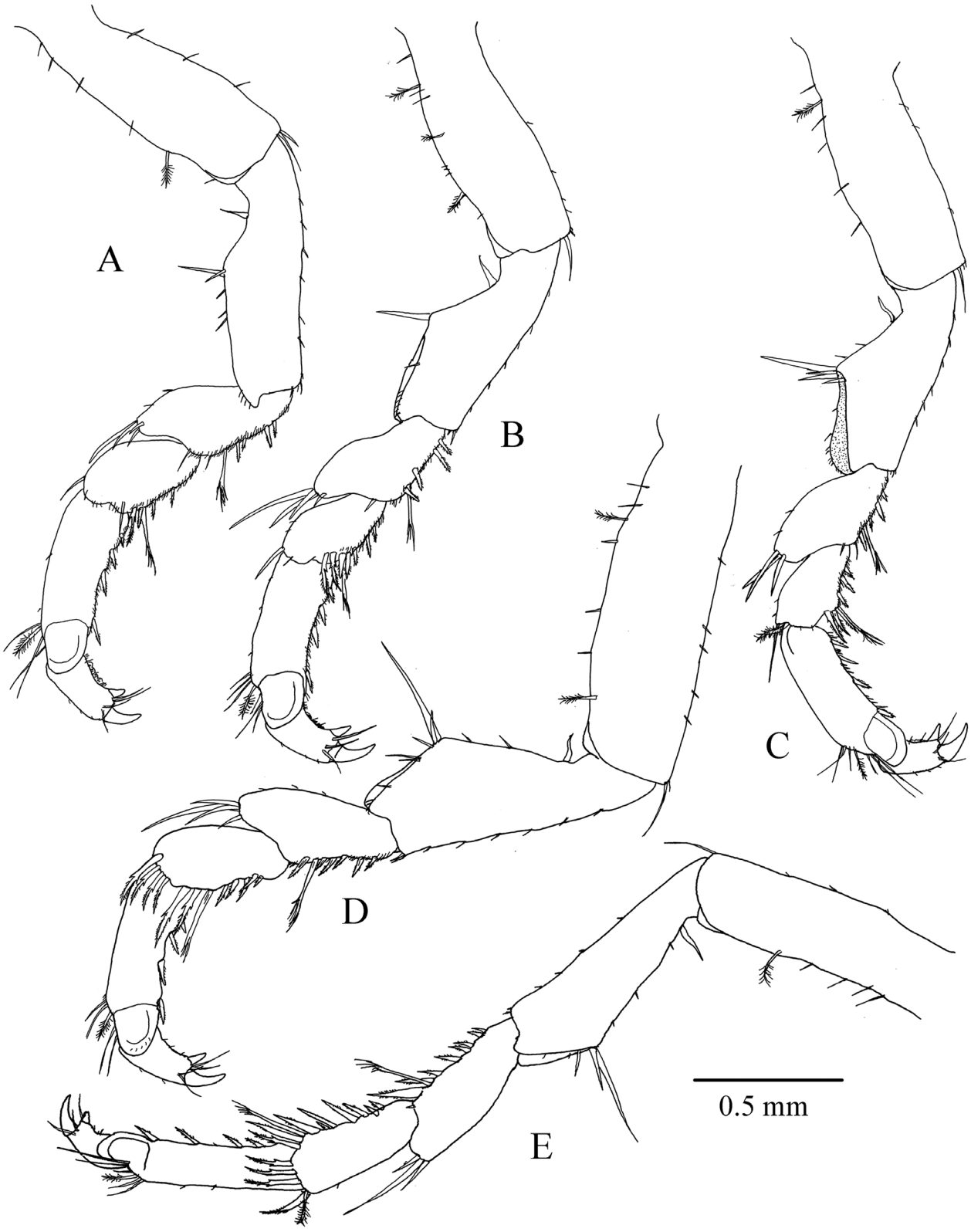

FIGURE 3. *Cymodoce tribullis* Harrison & Holdich 1984, paratype (QM W9643); A–E, pereopods 3–7.

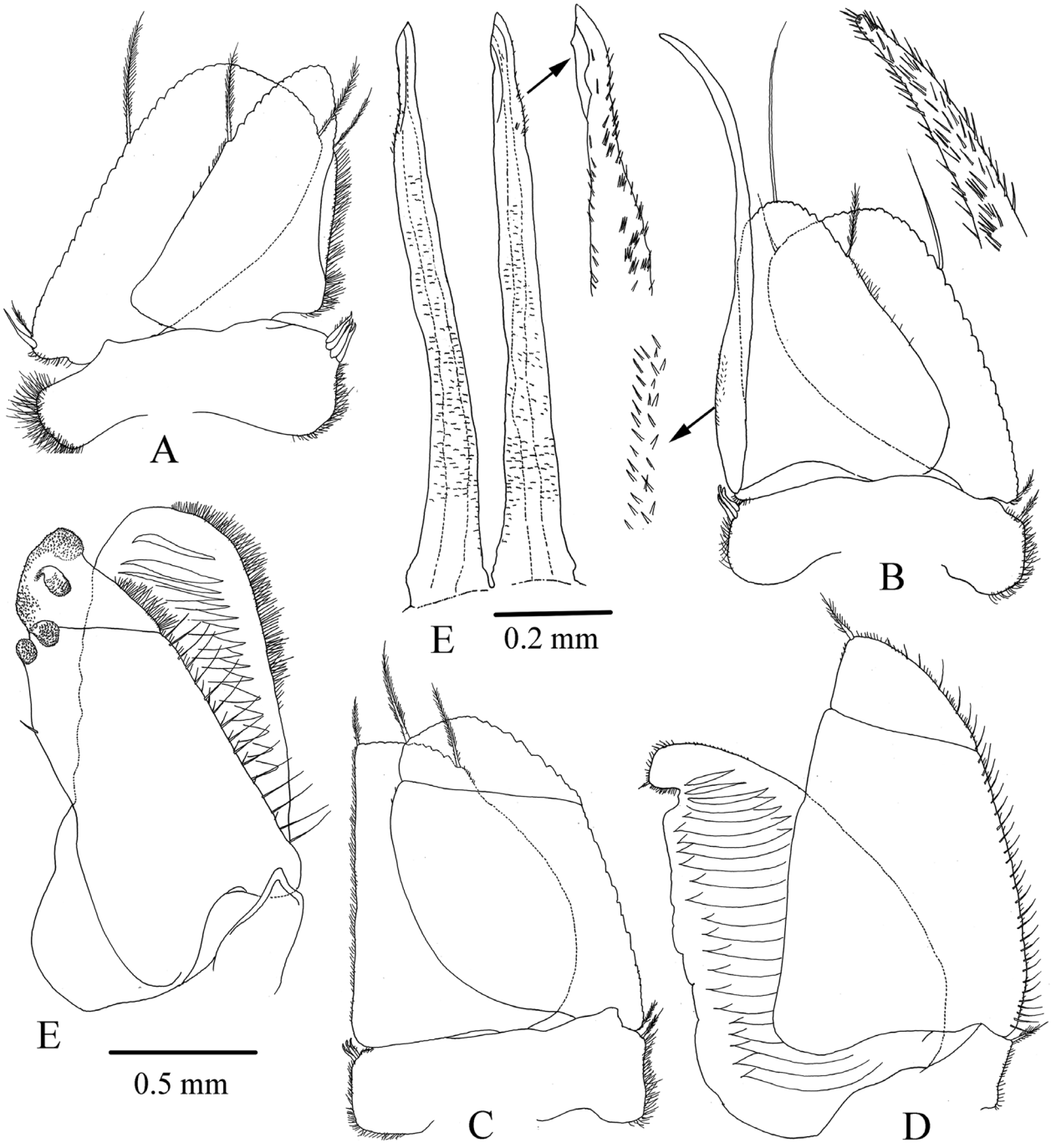

FIGURE 4. *Cymodoce tribullis* Harrison & Holdich 1984, paratype (QM W9643); A–E, pleopods 1–5; F, penes.

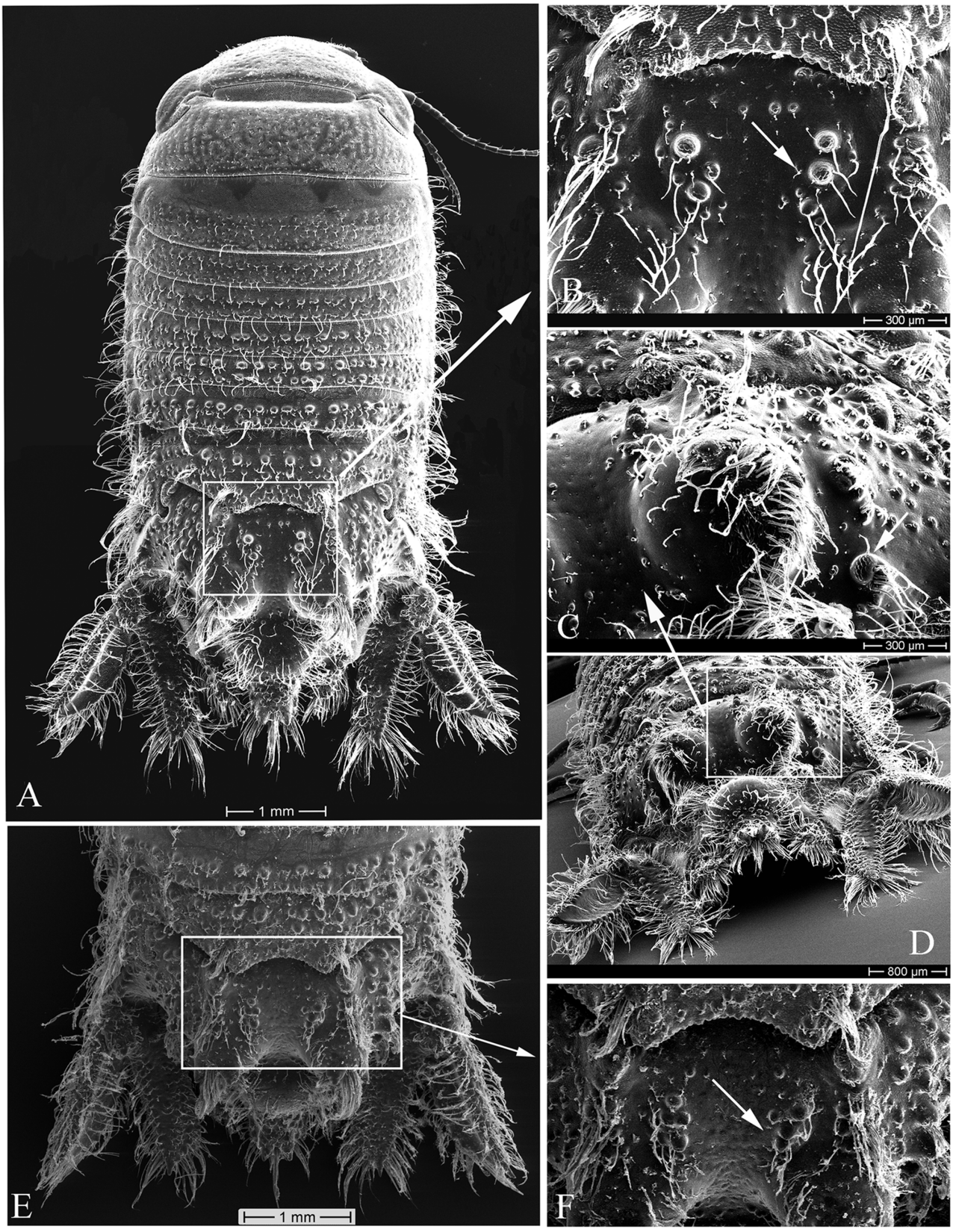

FIGURE 5. *Cymodoce tribullis* Harrison & Holdich 1984, (QM W31864); A, SEM, dorsal view; B, pleotelson anterodorsal part; C, pleotelson dorsal boss with lateral tubercles; D, pleotelson dorsal view; E, paratype (QM W9643); E, pleotelson dorsal view; F, pleotelson anterodorsal part.

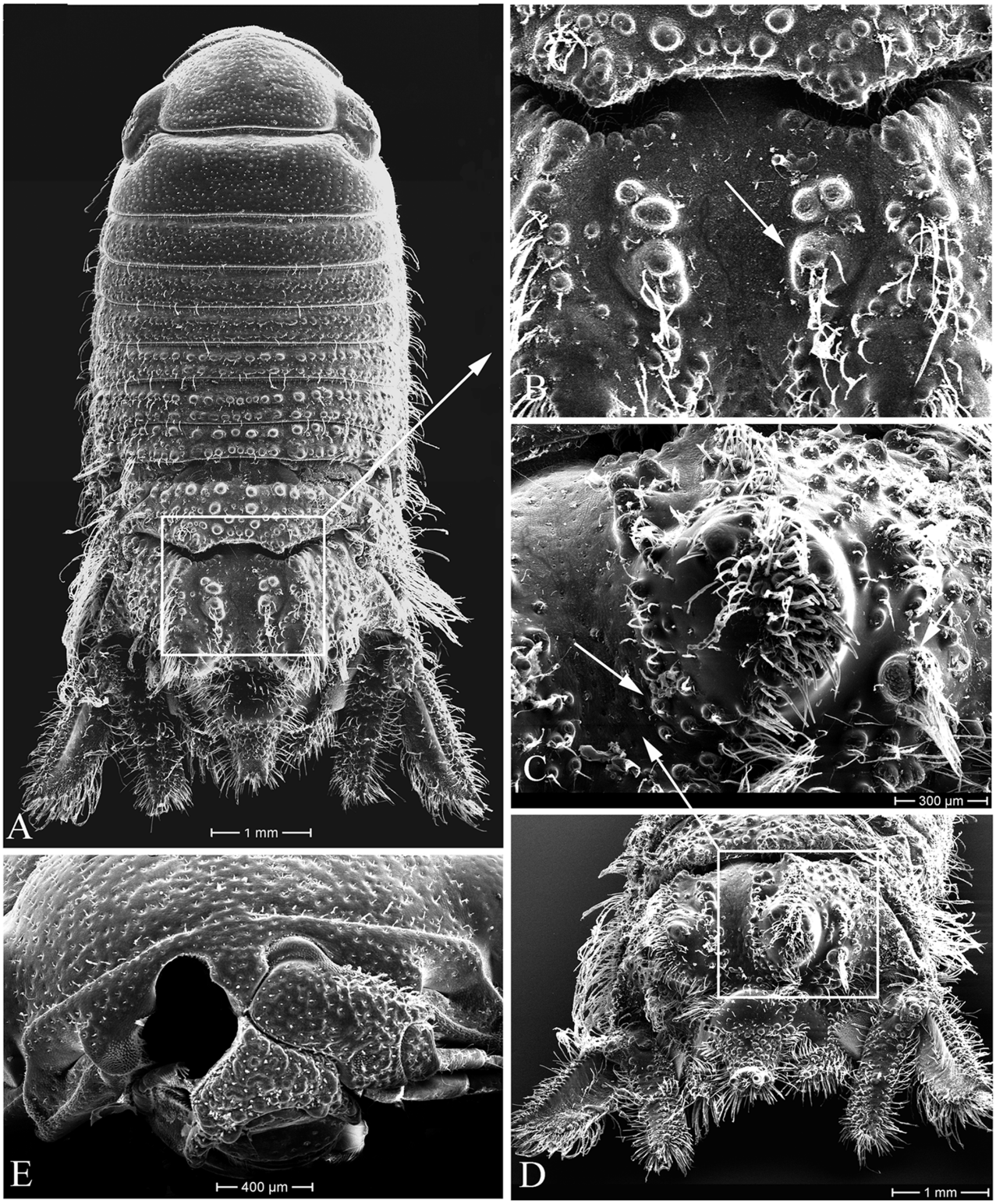

FIGURE 6. *Cymodoce tribullis* Harrison & Holdich 1984 (paratype of *Cymodoce lirella* Schotte & Kensley, 2005, SEM, (USNM 280295); A, dorsal view; B, pleotelson anterodorsal part; C, pleotelson dorsal boss with lateral tubercles; D, pleotelson dorsal view; E, frons and anterior of head.

Pereopods 7 (Fig. 3E) similar to pereopod 6, except in some details such as number of biserrate RS on distal margin of carpus.

Penial processes (Fig. 4E) about 9 times as long as basal width, tapering to narrowly rounded apex; distal fourth covered with cuticular tiny spines, rest part covered with cuticular branched scales.

Pleopod 1 (Fig. 4A) exopod and endopod with approximately 37 and 30 PMS; exopod proximally with single RS on lateral angle; endopod nearly triangular, medial margin bearing longitudinal fold fringed with fine setae; sympod mesial margin with 4 coupling hooks.

Pleopod 2 (Fig. 4B) exopod and endopod shape as in pleopod 1, with 38 and 19–21 PMS; *appendix masculina* slender, arising basally, extending beyond endopod by about one-third of length, tapering evenly to a narrowly rounded apex, distally bearing cuticular tiny spines on most of surface, proximally with single cuticular spines on medial marginal; sympod with 3 distomesial coupling hooks, lateral margin with long plumose RS.

Pleopod 3 (Fig. 4C) exopod and endopod with approximately 38 and 15 PMS; endopod distally truncate; exopod sub-elliptical, narrowing proximally, with transverse suture; sympod with 3 distomesial coupling hooks, lateral margin with fringe of thin setae and single long plumose setae on distolateral corner.

Pleopod 4 (Fig. 4D) endopod narrower than exopod, with pronounced and curved apical lob, bearing single stout apical seta; exopod bearing 1 single apical plumose seta, lateral margin with 30 slender simple and numerous short setae (6 distally of and 24 under transverse suture); sympod with a plumose setae on distolateral corner.

Pleopod 5 (Fig. 4E) exopod with 5 scale patches (3 distally of and 2 under transverse suture), lateral margin with 16 slender simple marginal setae (all under transverse suture); endopod distally broadly rounded or truncate, distolateral margin fringed with fine setae on 0.45 its length.

Uropodal rami subequal, extending just beyond pleotelsonic medial lobe apex; endopod distally oblique with distolateral conical acute tip, bearing scattered small tubercles over surface, distal and lateral margins fringed with long setae; exopod wider than endopod, lateral margin stout, straight and tuberculated, medial margin convex, with conical acute tip.

Subadult male. Cephalon, pereon and pleon lacking tubercles, pereon and pleon dorsal surface covered with scattered short setae. Pleon posterior margin with two tufts of setae on either side; penes with separate, short and broad rami; appendix masculina joined with endopod of pleopod 2.

Female. Apart from sexual character similar to sub-adult male (Fig. 1C).

Remarks. *Cymodoce tribullis* Harrison & Holdich, 1984 being recognized by the presence of two large, apically bifid bosses on the pleotelson, flanked on either side by two prominent tubercles, one posterior to other. The detailed examination of type specimens of *C. tribullis* from Queensland and *C. lirella* Schotte & Kensley, 2005 from the Seychelles failed to reveal any clear differences. Interestingly, the published drawings revealed some differences between *C. tribullis* and *C. lirella* in the shape of the penial process, appendix masculina ornamentation, and setation of pleopods 4 and 5. However, the examination of both type specimens confirmed the similarity between *C. tribullis* and *C. lirella*. Here, the shape and ornamentation of the penial process, the appendix masculina, and the pleotelson ridges are identical in both species. However, SEM micrographs show some minor differences between *C. tribullis* and *C. lirella* in the pleotelson tuberculation. *Cymodoce lirella* has a pleotelson (see Fig. 6A, B) with two prominent and some small tubercles on the anterodorsal surface (rather than some small separated tubercles) and also some small scattered tubercles (see Fig. 6C, D) on medial surface of the prominent apically bifid bosses. However, these prominent tubercles arise from the joining of two small tubercles and also there is variation in the size of these tubercles in the different populations of *C. tribullis* (Fig. 5B, F). Therefore, *Cymodoce lirella* Schotte & Kensley, 2005 is here regarded as a junior subjective synonym of *C. tribullis*.

The same similarities also exist between *C. tribullis* and *C. madrasensis* (Srinivasan, 1959) from India. The later species was redescribed by Loyola e Silva (1998) with material from Madras, India (USNM 102151, Fig. 28E). However, there is no available type material for this species, and the given original descriptions and drawings are of insufficient to confirm the identity of the species in relation to other similar congeneric *Cymodoce* species. This present work clearly reveald cryptic species within *Cymodoce*, and as the identity of *C. madrasensis* is unlikely to ever be resolved, we here regard this species as nomen dubium. Moreover, *Cymodoce longistylis* Miers, 1884 and *C. mammifera* Haswell, 1881 were reported from South India by Pillai (1965). According to Pillai's drawings and descriptions of these species, both appear to be identical with *C. tribullis*. Pillai's specimens differ from *C. longistylis* by having a less tuberculate pleotelson, medial lobe of pleotelsonic apex extending well beyond the level of lateral lobes, uropodal endopod with straight margins and extending only slightly beyond the pleotelsonic apex. *Cymodoce mammifera*, which Pillai (1965) recorded from the same locality is almost certainly a subadult male (i.e. with short penial process and unseparated appendix masculina to endopod).

Distribution. Eastern Australia (Harrison & Holdich, 1984), southeastern Vietnam (Kussakin & Malyutina, 1993), Singapore (Bruce and Wetzer collections, but not published yet; also Bruce in press), south India (Pillai,

1965), Seychelles (Schotte & Kensley, 2005). Distribution of all known species from the northern Indian Ocean and their present status provided in table 2.

TABLE 2. Distribution and present status of all known *Cymodoce* species of the northern Indian Ocean.

Species	Present status	Distribution
C. bicarinata Stebbing, 1904	*C. bicarinata*	Maldives, northern Indian Ocean (Kussakin & Malyutina 1993).
C. delvarii Khalaji-Pirbalouty, Bruce & Wägele, 2013	*C. delvarii*	Persian Gulf
C. erythraea erythraea Nobili, 1906.	Incertae sedis	Red Sea
C. fuscina Schotte & Kensley, 2005	*C. fuscina*	Persian Gulf
C. longistylis Miers, 1884	*C. longistylis*	Southern China, southeastern Vietnam, Nicobar Island, Queensland, Torres Strait, Philippine Islands, Indonesia, Singapore, Nicobar Islands, Low Isles (Harrison & Holdich 1984; Kussakin & Malyutina 1993).
C. madrasensis (Srinivasan, 1959)	Nomen dubium	India, (Loyola e Silva1998)
C. richardsoniae Nobili, 1906	*C. richardsoniae*	Red Sea (Khalaji-Pirbalouty *et al*, 2013)
C. spinula Yousuf & Javed, 2001	*C. spinula*	Pakistan
C. tribullis Harrison & Holdich, 1984	*C. tribullis*	Southern Vietnam, northeastern Australia, Singapore (Harrison & Holdich 1984; Kussakin & Malyutina 1993).
C. waegelei **sp. nov.**	*C. waegelei*	Persian Gulf

***Cymodoce waegelei* sp. nov.**
(Figs 7–12)

Material examined. All material from Iran, northern Persian Gulf.

Holotype. ♂ (5.8 mm), Bushehr, Bahmani, 1.5 m depth, on algae, 9 July 2009, 28 °51'461"N, 56 °50'584"E, coll.V. Khalaji (ZMH–K–42594). Paratypes: 7 ♂ (5.5–5.7 mm), 4 sub-adult ♂ (4–4.5 mm), 30 ♀ (3.8–5 mm), same data as holotype (ZMH–K–42595); 3 ♂ (4.5, 5, 5.8 mm), 2 ♀ (5.5 mm), Gloestan-e-Saheli, sub tidal, 1.5 m depth, on green algae, 28 April 2010, 28 °14'198"N, 51 °16'455"E, coll. A. Samimi and V. Khalaji (ZUTC Iso. 1102); 5 sub-adult ♂ (3.5–5.0 mm), 2 ♀ (3.0, 5.0, 5.0 mm) Brikan, 22 November 2007, 28 °17'294"N, 51 °13'494"E, coll. V. Khalaji (ZUTC Iso. 1103); 2 ♀ (4.0, 5.5 mm), Zyarat, 4 December 2008, 27 °05'444"N, 53 °05'044"E, coll. V. Khalaji (ZUTC Iso. 1104); 5 sub-adult ♂, (3.5–4.2 mm), 9 ♀ (3–4.1 mm), Chahak, Genaveh, rocky shore, sandy flat with algae, 10 July 2009, 29 °40'113"N, 50 °23'355"E, coll.V. Khalaji (ZUTC Iso. 1105); 5 adult♂ (5.9–6.2 mm), 3 sub-adult ♂ (4.5, 4.9, 5.0 mm), 3 ♀ (4.8, 4.9, 5.0 mm), Bushehr, Jofreh Mahini, 9 July 2009, 28 °58'022" N, 50 °49'100" E, coll.V. Khalaji (ZUTC Iso. 1106).

Diagnosis. Head and pereonites 1–3 smooth, lacking tubercles, pereonites 4–7 with two transverse rows of small tubercles. Pleotelson with two large prominent, apically bifid bosses; anterodorsal surface two irregular, longitudinal rows of various sizes of tubercles medially; dorsolateral sides with numerous of small tubercles; posterior half of the pleotelson with peripherally tuberculated hemispheric dome; posterior margin trilobed, medial lobe extending well beyond lateral lobes.

Description of male. Body 2.17 times as long as greatest width (pereonite 6). *Head* dorsal surfaces smooth, rostral process not visible in dorsal view. Pereonites 1–3 smooth, 4–5 with 2 transverse rows of non-continuous weak tubercles; pereonites 6–7 with 2 rows of continuous tubercles. Coxal plate 2–7 bearing some long setae below the suture line and distal part (Fig. 7A, 11A).

Pleon: with a row of tubercles over two, long, straight, separate and parallel sutures at each side, dorsally bearing row of prominent tubercle and numerous scattered uneven tubercles of various sizes, with two pronounced tufts of simple long setae on each side; posterolateral margins with fringe of very long sub-marginal setae.

Pleotelson bearing scattered tubercles of various sizes over most of surface, with two large prominent apically bifid bosses, dorsally with tufts of long setae especially in cleft and below of bifid process, laterally with 2 tufts of

long simple setae, posterior region of pleotelson in midline with hemispheric dome, medial margins of bifid bosses and peripheral margin of hemispheric dome bearing numerous small tubercles. Apex of pleotelson clearly trilobed; lateral lobes blunt; medial lobe extending well beyond the level of lateral lobes, bearing two apical conical tubercles and tuft of long setae, dorsally tuberculated (Figs. 7A, 11A–D).

Antennula (Fig. 7C) first peduncle article bearing scattered small setae and some SPS, articles 2 short with 4 small SPS on ventral and a single SPS on dorsal margin, article 3 slender, about 1.6 times as long as article 2; flagellum with 16 articles, article1 bearing 3–4 small SPS, articles 5–15 each bearing single aesthetascs and 2 simple setae.

Antenna (Fig. 7D) peduncle articles 1 fringed with small setae dorsally, article 5 about 1.3 times as article 4; articles 2–5 superodistal margins with long simple setae; flagellum with 18 articles, extending to posterior margin of pereonite 4.

Epistome (Fig. 7E) granulose, with triangular acute apex, anterolateral margin straight, lateral margins concave.

Left mandible (Fig. 8C) incisor with 3 cusps, lacinia mobilis with 3 cusps, spine row of 6 serrate spines.

Maxillula (Fig. 8A) lateral endite with long fine setae on mesial and lateral margins, outer, apical margin with 10 simple or serrate RS; mesial endite with 4 long, robust, comb and 1short simple setae.

Maxilla (Fig. 8B) lateral and middle endites each with 10 curved pectinate RS; mesial endite with 2 rarely plumose, 2 long robust comb, 7–8 robust proximally plumose and distally biserrate, and some slender simple setae.

Maxilliped (Fig. 8D) endite lateral margin sinuate, mesial margin with single coupling hook, distal margin with 4 blunt rarely plumose RS and 7 longer circumplumose RS; palp article1 with single simple seta on distomedial corner, article 2 with single long seta on superodistal angle.

Pereopod 1(Fig. 8E) basis about 2.4 times as long as greatest width, ischium superior margin with 1 curve, acute RS on proximal corner and 1 long and 1 small RS on medial angle; merus superodistal angle with 4 robust biserrate or simple setae, inferior margin with 5 biserrate RS and single long apically palmate seta; carpus inferior margin with 3 biserrate RS; propodus inferior margin with 5 biserrate RS set in amongst some acute scales and 1 submarginal biserrate seta; dactylus inferior margin with serrate cuticular scales, secondary unguis simple.

Pereopod 2 (Fig. 8F) basis about 2.6 times as long as greatest width, with 3 small SPS; ischium superior margin with 2 RS on medial corner; merus superodistal angle with 3 RS, inferior margin with 4 biserrate RS and single long apically palmate seta; merus, carpus and propodus inferior margin fringed with short setae; carpus inferior margin with 6 biserrate RS, and long apically palmate seta, superodistal angle with 1 RS; propodus inferior margin with 4 biserrate RS, superodistal angle with 3 long simple seta and a single SPS.

Pereopod 3 (Fig. 9A) is similar to pereopod 2. *Pereopods* 4 (Fig. 9B) and 5 (Fig. 9C) are similar as illustrated.

Pereopod 6 (Fig. 9D) basis about 3 times as long as greatest width, inferodistal angle with 1 long simple setae, superior margin with several simple and 3 SPS; ischium superior margin with 1 long and some small RS; merus superodistal margin with 3 long setae, inferior margin with 4 roust biserrate setae and single long apically palmate seta; carpus subequal in length to merus, inferior margin with 6 biserrate RS and single long apically palmate seta, distal margin with 2 biserrate RS; propodus superodistal corner with 5 slender and single SPS, inferior margin with 4 biserrate RS; dactylus inferior margin with cuticular scales. Articles carpus, propodus and dactylus of *pereopods* 7 (Fig. 9E) and *6* are similar except in some details such as number of biserrate RS on distal margin of carpus.

Penial processes about 7 times as long as basal width, tapering to narrowly rounded apex; medial margin and apical parts covered with small cuticular tiny spines, medial surface covered with cuticular scales (Fig. 10F).

Pleopod 1(Fig. 10A) exopod and endopod with 39 and 29 PMS; exopod proximally with single biserrate RS on lateral angle; endopod nearly triangular, medial margin bearing longitudinal fold fringed with fine setae; sympod mesial margin with 4 coupling hooks.

Pleopod 2 (Fig. 10B) exopod and endopod shape as for pleopod 1, with approximately 39 and 18 PMS; *appendix masculina* slender, arising basally, extending beyond endopod (by about 0.33 its length), tapering evenly to a narrowly rounded apex, distally bearing cuticular branched scales on most of surface, proximally with single cuticular scales on medial margin; sympod with 3 distomesial coupling hooks, lateral margin with long plumose RS.

Pleopod 3 (Fig. 10C) exopod and endopod with approximately 41 and 15 PMS; endopod distally truncate; exopod sub-elliptical, narrowing proximally, with transverse suture; sympod with 3 distomesial coupling hooks, lateral margin with fringe of thin setae and single long plumose setae on distolateral corner.

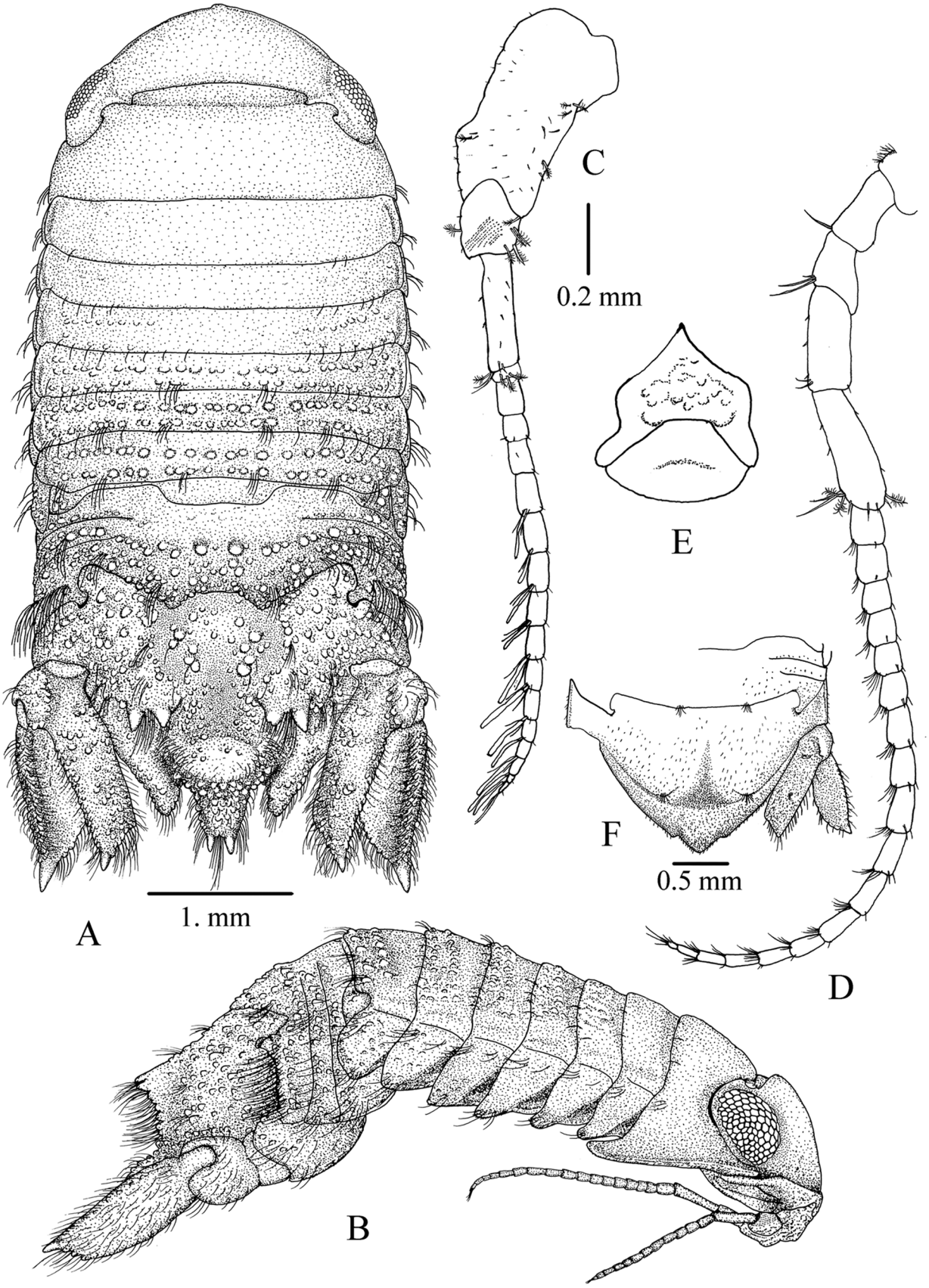

FIGURE 7. *Cymodoce waegelei* **sp. nov.**, holotype (ZMH-K-42594); A, dorsal view; B, lateral view; C, antennula; D, antenna; E, epistome; F, female.

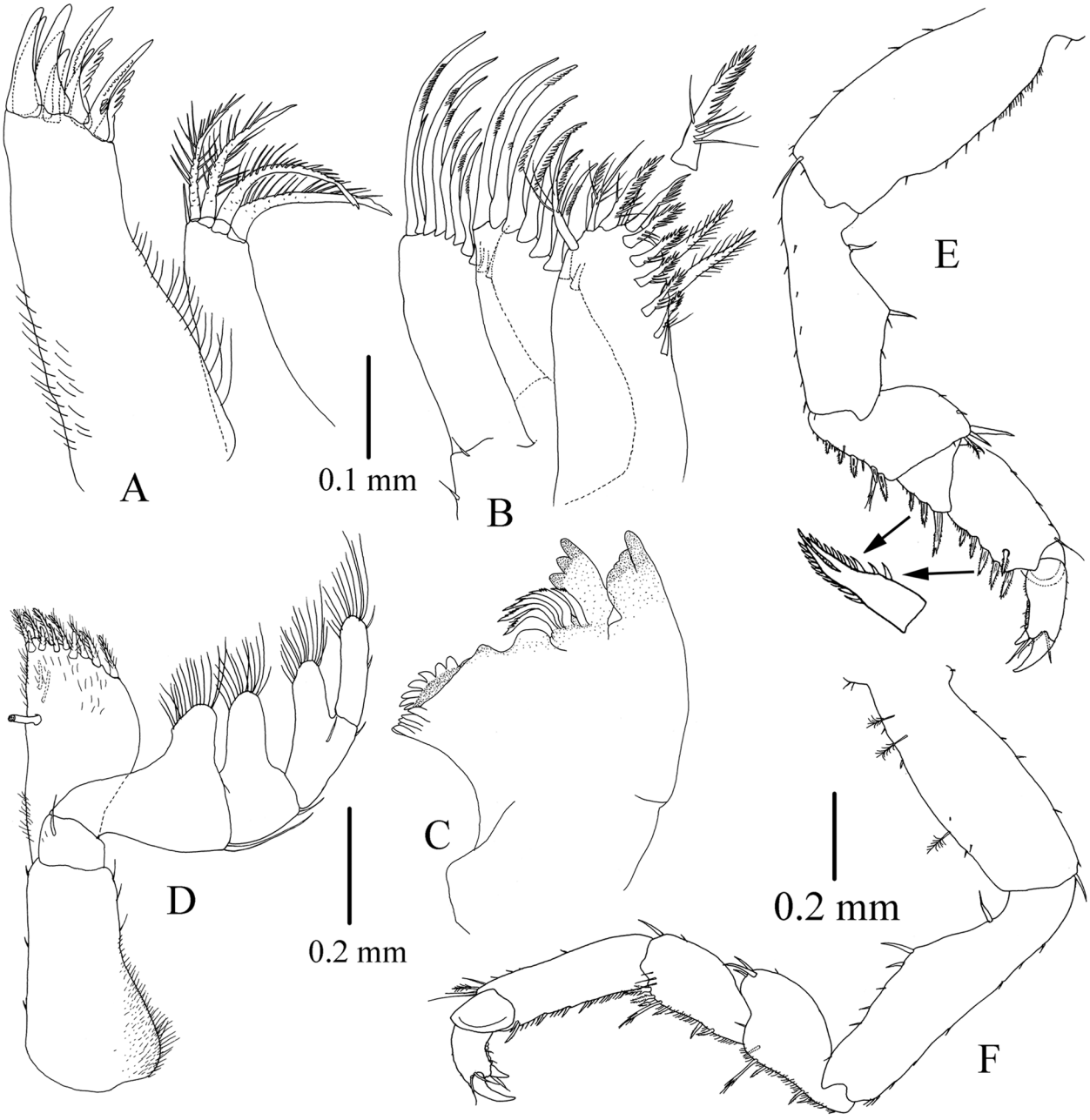

FIGURE 8. *Cymodoce waegelei* **sp. nov.**, holotype (ZMH-K-42594); A, maxillula; B, maxilla; C, left mandible; D, maxilliped; E, pereopod 1; F, pereopod 2.

Pleopod 4 (Fig. 10D) endopod with pronounced and curved apical lob, bearing 1 stout apical seta; exopod bearing 1 single apical plumose seta, lateral margin with approximately 34 slender simple setae and numerous short setae (6–8 distally of and 26–28 under transverse suture); sympod with plumose setae on distolateral corner.

Pleopod 5 (Fig. 10E) exopod with 5 scale patches (3 distally of and 2 under transverse suture), lateral margin with 18 slender simple marginal setae (1 distally of and 17 under transverse suture); endopod distolateral margin fringed with fine setae on 0.52 its length.

Uropodal rami (Figs 7A, 11A) subequal, extending just beyond pleotelsonic medial lobe apex; endopod distally oblique with distolateral conical acute tip, bearing scattered small tubercles on dorsal surface, with prominent proximal tubercle, distal and lateral margins fringed with long setae; exopod wider than endopod, lateral margin stout, straight and tuberculated, medial margin convex, tapering distally to conical acute tip, distal and lateral margins and ventral surface bearing long setae.

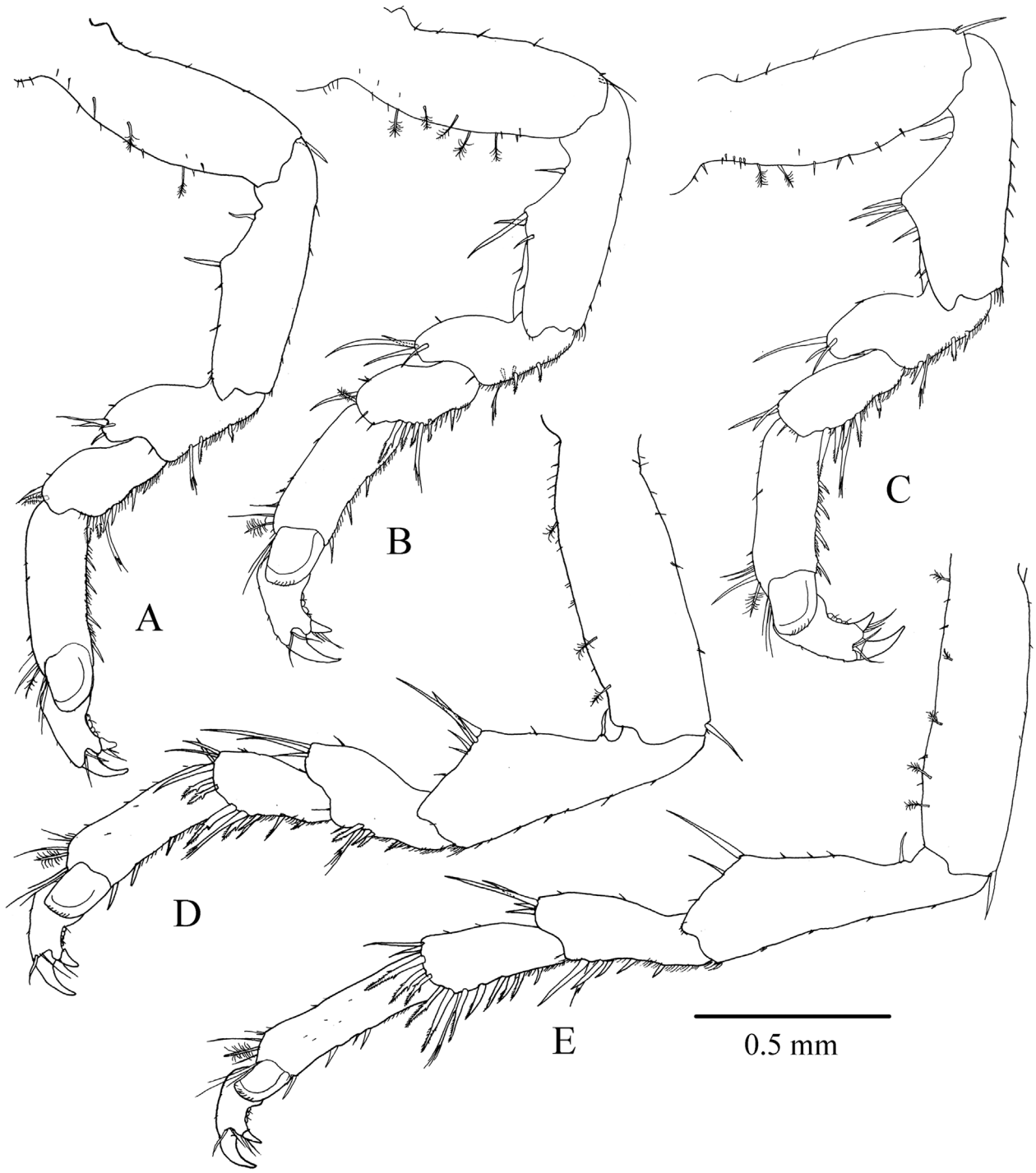

FIGURE 9. *Cymodoce waegelei* **sp. nov.**, holotype (ZMH-K-42594); A–E, pereopods 3–7.

Subadult male. Cephalon, pereon and pleon lacking tubercles, dorsal surface covered with scattered short setae. Pleon posterior margin with two tufts of setae on either side, posterolateral margins with transverse row of long sub-marginal setae. Pleotelson bearing weak, sparsely setose boss mid-dorsally on either side, with trifid apex, medial lobe broad and extending well beyond level of small lateral lobes. Uropodal rami smooth with sparsely setose; endopod distally oblique; exopod lanceolate, with acute tip and serrated medial margin; penes with separate, short and broad rami; Appendix masculina joined with endopod of pleopod 2, bearing single plumose seta on rounded apex (Fig. 12A, B).

Female. Apart from sexual character similar to sub-adult male, medial lobe of pleotelsonic apex not extended as sub-adult male, whereas in ventral surface apical depression is longer than sub-adults male (Fig. 7F; 12C, D).

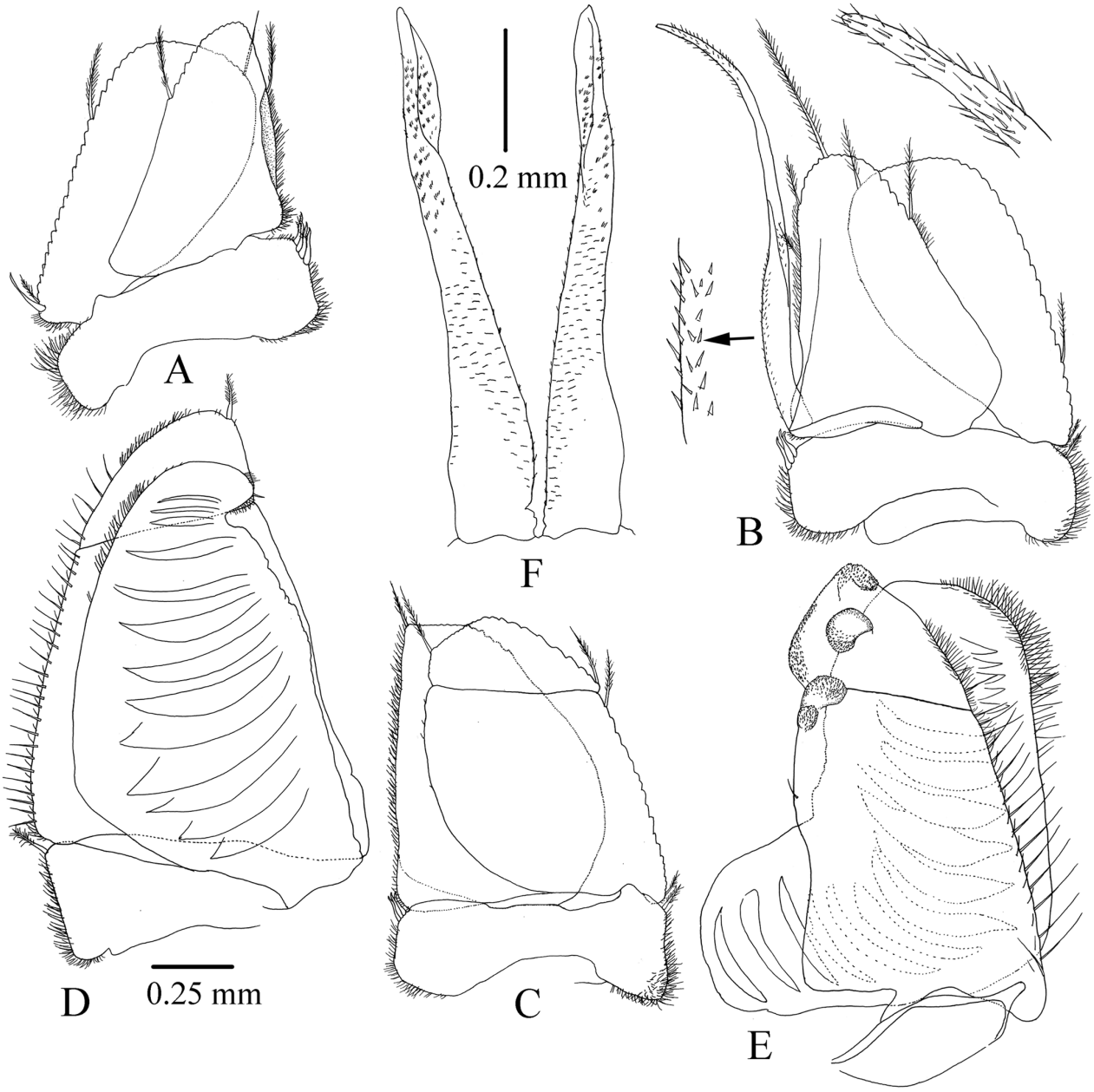

FIGURE 10. *Cymodoce waegelei* **sp. nov.**, holotype (ZMH-K-42594); A–E, pleopods 1–5; F, penes.

Remarks. *Cymodoce waegelei* **sp. nov.** can be recognized by a pleotelson with two widely separated bifid bosses, dorsolateral sides with small tubercles, and posterior margin with well extending narrow medial lobe beyond lateral lobes. *Cymodoce waegelei* is most similar to *C. tribullis* Harrison & Holdich, 1984, and differs from it by lacking two continuous rows of tubercles on the pereonites 3 and 4. Furthermore, the pleotelson has numerous scattered tubercles between two large prominent apically bifid bosses, and small lateral tubercles rather than two prominent tubercles in *C. tribullis*. Both bifid bosses are more widely separated, and the medial lobe of the pleotelson is more extended and narrower than in *C. tribullis.* In the new species the antenna extends to posterior margin of the pereonite 4, whereas in *C. tribullis* it extends to posterior margin of pereonite 2. Moreover, the shape of the penial process differs in cuticular scales pattern, and the length ratio to basal width (in the new species is about 7 times as long as basal width but in *C. tribullis* is about 9). Beside this, there are differences between *C. tribullis* and *C. waegelei* **sp. nov.** in the shape and marginal setation of pleopod 5 (in *C. waegelei* **sp. nov.** exopod has 1 slender simple marginal setae upper the transverse suture whereas in *C. tribullis* all setae are under the transverse suture and endopod distolateral margin fringed with fine setae on 0.52 of length rather than 0.45 mm of length). Finally, the maximum size of *C. waegelei* sp. nov (5.8 mm) is less than *C. tribullis* (7.9 mm).

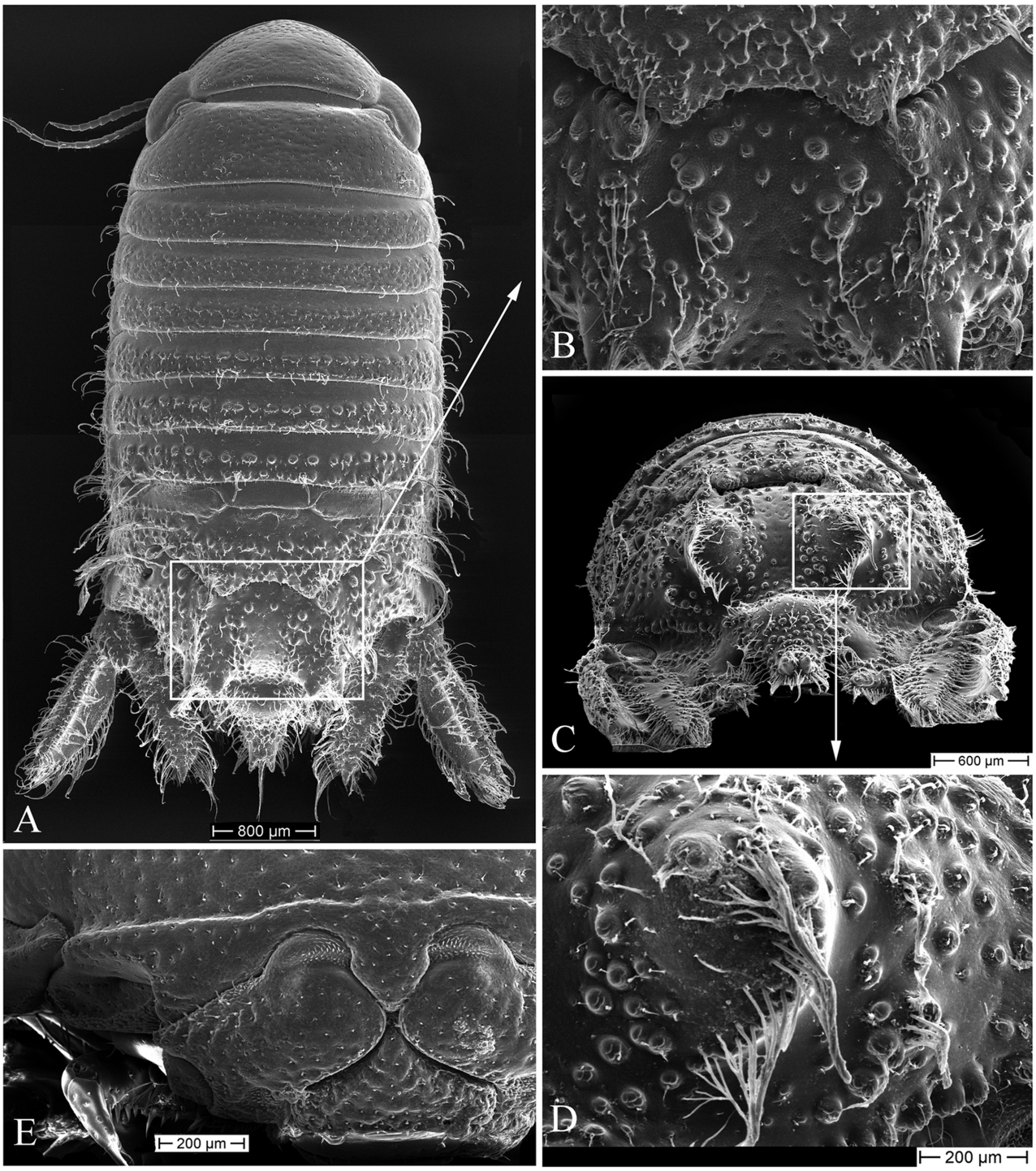

FIGURE 11. *Cymodoce waegelei* **sp. nov.**, SEM, paratype (ZMH-K-42595); A, dorsal view; B, pleotelson anterodorsal part; C, pleotelson dorsal view; D, details of pleotelson dorsal boss; E, frons and anterior of head.

Etymology. This species is named for Prof. Dr. J. Wolfgang Wägele (Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany) to acknowledge many years of contributions to the taxonomy and phylogeny of Isopoda.

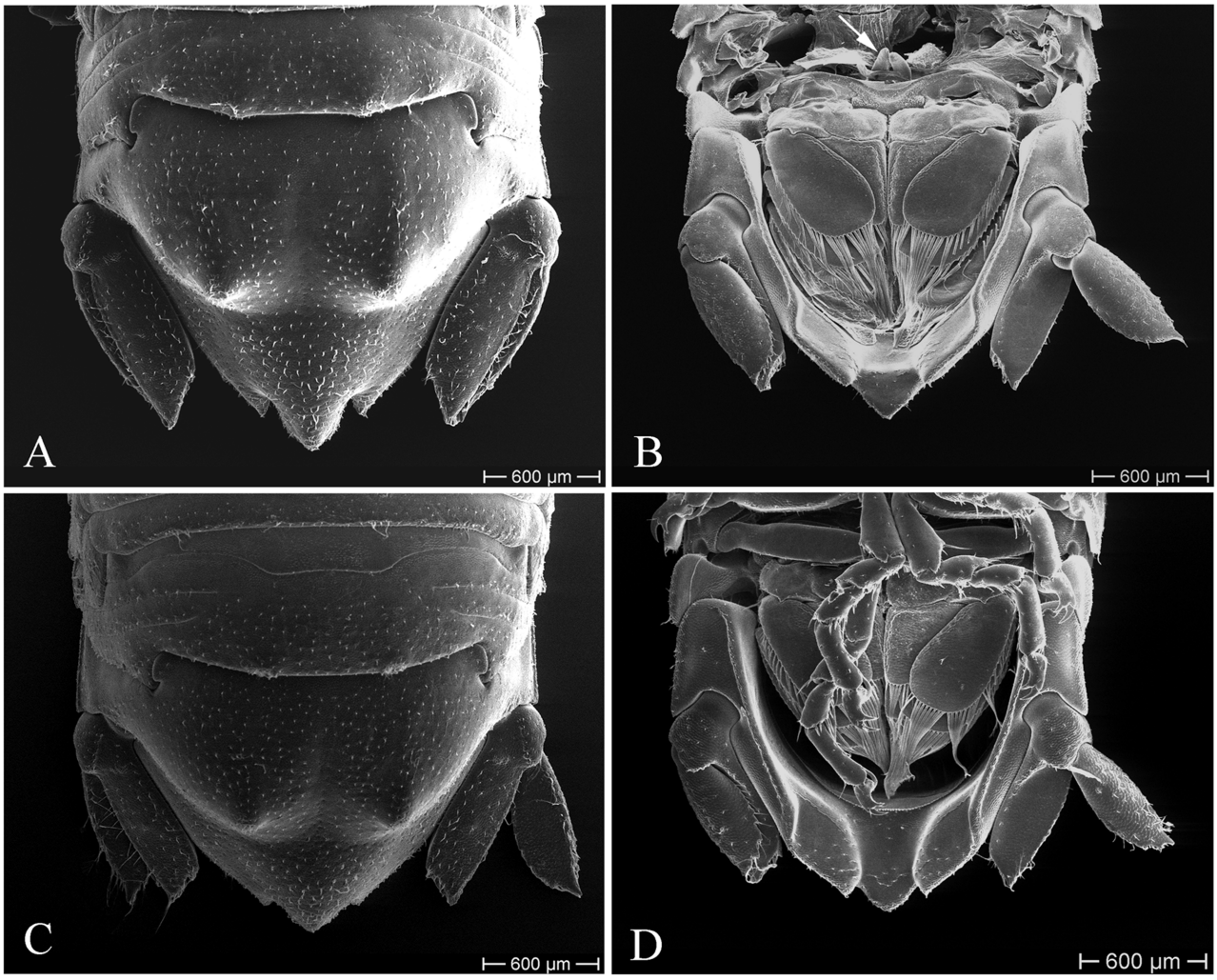

FIGURE 12. *Cymodoce waegelei* **sp. nov.**, SEM, paratype (ZMH-K-42595), sub-adult male; A, dorsal view; B, ventral view; C, female, dorsal view; D, female ventral view.

Key to the northern Indian Ocean species of *Cymodoce* (adult males)

1.	Appendix masculina straight, extending slightly beyond endopod apex (by about < one-fifth of length	2
-	Appendix masculina curving distally, extending well beyond endopod apex (by about one-third of length)	5
2.	Pleotelson anterior region with 2 prominent longitudinal ridges, posterior region with a domed boss	3
-	Pleotelson anterior region lacking 2 prominent longitudinal ridges, posterior region lacking domed boss	*C. fuscina* Schotte & Kensley, 2005
3.	Pleotelson with a large domed boss, and deep apical notch	4
-	Pleotelson with a very large domed boss, longitudinal row of tubercles on either side, shallow apical notch.	*C. spinula* Yousuf & Javed, 2001
4.	Each pleotelsonic longitudinal ridges flanked on lateral side by 3 prominent tubercles, uropod rami extending beyond pleotelsonic medial lobe apex	*C. richardsoniae* Nobili, 1906
-	Each pleotelsonic longitudinal ridges flanked on lateral side by 2 prominent tubercles, uropod rami not extending to level of pleotelsonic medial lobe apex	*C. delvarii* Khalaji-Pirbalouty, Bruce & Wägele, 2013
5	Uropodal endopod subequal to exopod, medial margin straight, distally oblique	6
-	Uropodal endopod longer than exopod, medial margin convex, distal margin acute	*C. longistylis* Miers, 1884
6	Pleotelson anterodorsal part without 2 slightly divergent ridges	7
-	Pleotelson anterodorsal part with 2 slightly divergent ridges	*C. bicarinata* Stebbing, 1904
7	Pleotelson lacking scattered tubercles between two large prominent bifid bosses, flanked on lateral side by 2 prominent tubercles. Pereonites 3 and 4 with 2 continuous rows of tubercles	*Cymodoce tribullis* Harrison & Holdich, 1984
-	Pleotelson with numerous scattered tubercles between two large prominent bifid bosses, lacking 2 prominent lateral side tubercles	*C. waegelei* **sp. nov.**

Molecular results

The 18 new barcode fragment showed no significant differences in base composition (χ-square test: 26.67, *df* = 51, *p* = 0.99). Nevertheless, the barcode fragments were AT rich (A = 26%, C = 17%, G = 20% and T = 37%), as it is typically known from this gene fragment for arthropods (e.g. Simon *et al*. 2006; Raupach *et al.* 2010; Wesener *et al.* 2010; Rajaei *et al*. 2013). All specimens of the same species grouped together (Fig. 13). Our analysis of pairwise COI nucleotide divergences based on patristic as well as K2P distances for all four *Cymodoce* species revealed higher interspecific versus intraspecific divergences (Table 3). Maximum intraspecific divergence was observed in *Cymodoce fuscina* (*p*-distances: 0.61%, K2P distances: 0.61%), followed by *C. tribullis* (0.45%/0.46%) and *C. waegelei* (0.45%/0.46%). Interspecific distances ranged between 3.93%/4.07% and 19.82%/23.37%. Lowest interspecific distances were found between *Cymodoce delvarii* and *C. fuscina* (3.93–4.39%/4.07–4.55%; see Fig. 13).

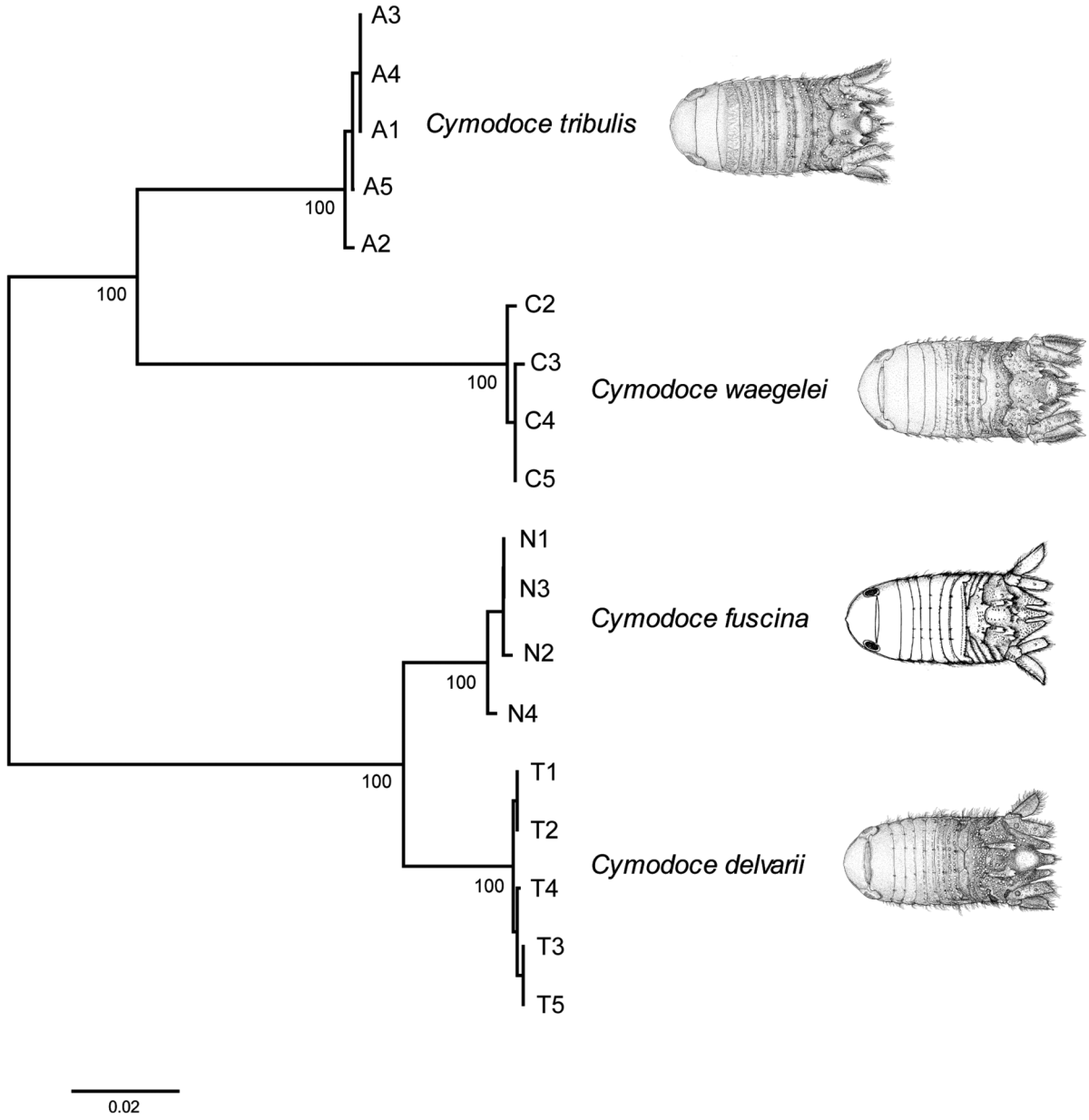

FIGURE 13. Unrooted Neighbour-joining phylogram of the analyzed CO1 sequences of the four *Cymodoce* species. Numbers next to internal branches are bootstrap values (in %).

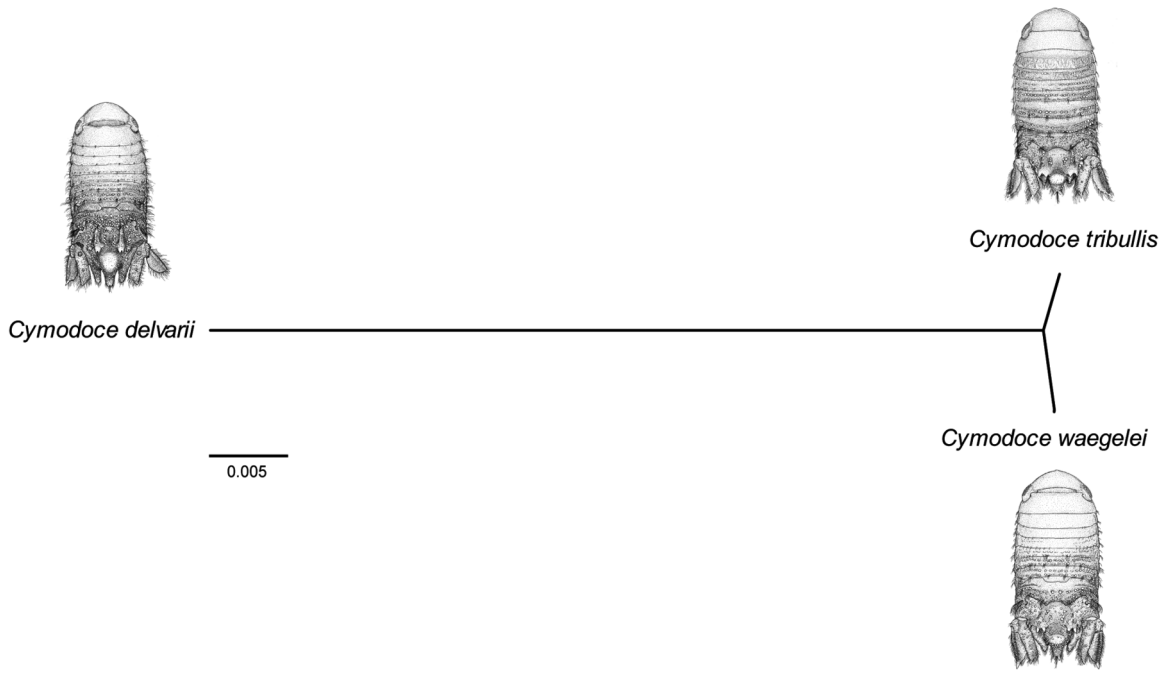

FIGURE 14. Unrooted Neighbour-joining phylogram the 28S rDNA: D8 expansion fragment of *Cymodoce delavrii*, *C. tribullis* and *C. waegelei* based on *p*-distances.

In terms of the 28S rDNA: D8 fragment, eight sequences were successfully amplified and sequenced: *Cymodoce tribullis*: three sequences with a length of 851 bp, *C. waegelei*: three sequences (850 bp), and *C. delvarii*: two sequences (881 bp). For the analyzed fragments, average base frequencies were A = 15%, C = 29%, G = 31% and T = 25%. The alignment showed no significant differences in base composition (χ-square test: 0.44, *df* = 21, *p* = 1). We also found no intragenomic or intraspecific variations within the studied marker fragments. In our study we observed seven base changes between *Cymodoce tribullis* and *C. waegelei*, 48 base changes and insertions between *Cymodoce tribullis* and *C. delvarii*, and 49 base changes and insertions between *Cymodoce waegelei* and *C. delvarii* (Fig. 14, Table 4).

TABLE 3. Uncorrected (*p*-distances, upper values) and K2P estimates (lower values) of the DNA barcodes of the four analysed *Cymodoce* species. With *n* = number of specimens.

	Cymodoce delvarii	*Cymodoce fuscina*	*Cymodoce tribullis*	*Cymodoce waegelei*
Cymodoce delvarii	0–0.003			
(*n* = 5)	0–0.003			
Cymodoce fuscina	0.0393–0.0439	0–0.0061		
(*n* = 4)	0.0407–0.0455	0–0.0061		
Cymodoce tribullis	0.1604–0.1634	0.1619–0.1649	0–0.0045	
(*n* = 5)	0.1816–0.1856	0.1836–0.1879	0–0.0046	
Cymodoce waegelei	0.1952–0.1982	0.1861–0.1906	0.1135–0.1150	0–0.0045
(*n* = 4)	0.2293–0.2337	0.2162–0.2244	0.1243–0.1261	0–0.0046

TABLE 4. Uncorrected *p*-distances of the 28S rDNA: D8 fragment of the three analysed *Cymodoce* species (lower triangle). Upper triangle: total number of observed base changes (insertions, deletions, and substitutions).

Cymodoce delvarii	*Cymodoce tribullis*	*Cymodoce waegelei*
-	48	49
0.0585	-	7
0.0597	0.0085	-

Discussion—Molecular Part

Our study strongly confirmed the benefits of an integrated taxonomy, combining molecular and morphological data. In this context, the analysis of both mitochondrial as well as nuclear markers represents a highly efficient method as part of a comprehensive morphological species description. Both analysed markers revealed distinct clusters of all four analysed *Cymodoce* species. Our molecular data also revealed *Cymodoce tribullis* as sister taxon of the new species *C. waegelei*, as it is also suggested by morphology, with patristic distances ranging from 11.35–11.5% (K2P: 12.43–12.61%) for CO1 and seven observed substitutions for the 28S rDNA: D8 marker. The given barcode data indicated an even closer relationship of *Cymodoce fuscina* and *C. delvarii* (3.93–4.39%/ 4.07–4.55%). However, it should be kept in mind that our use of both marker focus on species delineation and identification, and not on phylogenetic inference.

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