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A NEW SPECIES OF *ACARTIA* SUBGENUS *EUACARTIA* (COPEPODA: CALANOIDA: ACARTIIDAE) FROM KOREAN ESTUARIES BASED ON MORPHOLOGICAL AND MOLECULAR EVIDENCE

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ABSTRACT

A new species of the subgenus *Euacartia*, *Acartia forticrusa* n. sp., is described from Korean estuaries and compared with its congeners, *A. southwelli* Sewell, 1914 and *A. sarojus* Madhupratap and Haridas, 1994 based on morphological and molecular characteristics. This is the third species from the *Acartia* subgenus *Euacartia*. The new species differs from its congeners in the longer antennules, the higher ratio of length to width of caudal rami, and the shape of fifth legs of both sexes. The mitochondrial gene cytochrome oxidase subunit I (mtCOI) sequences differ from *A. southwelli* by 21 to 23%. The geographical range of *A. forticrusa* is predicted to meet the range of *A. southwelli*, in the Indo-Malayan region.

KEY WORDS: *Acartia*, copepod, estuary, *Euacartia*, mtCOI, new species, taxonomy

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INTRODUCTION

The copepod genus *Acartia* Dana, 1846, presently comprised of 56 species, lives in estuarine and coastal waters worldwide (Razouls et al., 2005-2012). This genus has been divided into six subgenera: *Acartiura* Steuer, 1915, *Euacartia* Steuer, 1915, *Hypoacartia* Steuer, 1915, *Acanthacartia* Steuer, 1915, *Odontacartia* Steuer, 1915, and *Acartia* Dana, 1846 (= *Planktacartia* Steuer, 1915). Of these, only two species, *A. southwelli* Sewell, 1914 and *A. sarojus* Madhupratap and Haridas, 1994, occupy the subgenus *Euacartia* (Steuer, 1915, 1923; Sewell, 1924, 1932; Madhupratap and Haridas, 1994; Razouls et al., 2005-2012). However, Madhupratap and Haridas (1994) questioned the validity of *Euacartia* and the other subgenera of the genus *Acartia* based on the examination of literatures and figures of the mouthparts. Additionally, Barthélémy (1999) found that the female genitalia did not support Steuer's subgeneric system. In this study, *Euacartia* is retained because the species are well defined in having rostral filaments, relatively undecorated or naked fifth pedigerous somite posteriodorsally, and a heavy terminal spine on the fifth female leg.

During our study on the diversity of fauna in Korean brackish waters, a species of *Acartia* closely related to species previously assigned to *Euacartia*, very closely related to *A.* (*Euacartia*) *southwelli* from China was collected. In this study, we compare its morphological and molecular differences with those of *A. southwelli* collected recently from India, the type locality. We also discuss the geographical distribution of species in *Euacartia*.

MATERIALS AND METHODS

Materials Examined

Zooplankton sampling was carried out monthly, for one year from January to December, 2000, from a range of salinities in the Seomjin River estuary, southern Korea, using a NORPAC net (mesh size 200 μm; diameter 45 cm). Additional collections were also made from seven Korean estuaries (the Mankyung and Youngsan Rivers of western Korea; the Beolgyo Stream, Seomjin and Nagdong Rivers of southern Korea; the Taehwa and Hyungsan Rivers of eastern Korea) between the years 2000 and 2008. All samples were preserved in 6% neutralized formalin/seawater immediately after the collection. Specimens for molecular analysis were separately collected and preserved in 99.9% ethyl alcohol. The speciemsn of *Acartia* were sorted out from the ethyl alcohol-fixed samples.

Acartia southwelli was also collected from the Pulicat Lake (13°24′57″N, 80°19′14″E), located on the southeast coast of India, Chennai ca. 500 km from the type locality to compare their DNA sequences with the Korean forms. After the specimens were placed in distilled water for 24 hours, appendages were dissected and mounted in CMC medium (Masters Chemical). The bodies and appendages were observed using a differential interference contrast microscope (Nikon Eclipse 80i) equipped with a drawing tube. The body length was measured from the anterior head to the tip of the right caudal ramus under a stereo microscope (Nikon SMZ-1000) using an ocular micrometer and image analysis software. The rostrum, female urosomites including the female genital double-somite, and the fifth legs of both sexes were examined with a scanning electron microscope (Hitachi S-4700) to show selected morphological features in detail. The morphological terminology follows that of Huys and Boxshall (1991) and Boxshall and Halsey (2004). Abbreviations used in the text and figures are: ae, aesthetasc; P1-P5, first to fifth legs. The interpretation of antennule segmental homologies, relative to the theoretical ancestral copepod (Huys and Boxshall, 1991), is made using the developmental sequences (Boxshall and Huys, 1998), especially using the segmental location of aesthetacs as points of reference. The interpretation of the maxillule is made according to Boxshall and Halsey (2004). The type specimens are deposited in the

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collection of National Institute of Biological Resources (NIBR), Incheon, Korea. Scale bars in figures are indicated in μm.

Genetic Analysis

DNA sequences were determined for portions of the mitochondrial gene cytochrome oxidase subunit I (mtCOI). Each individual for mtCOI analysis was placed in a capped 0.2 ml microcentrifuge tube, 20 μl dHOH was added, and microwaved for several minutes. The 18 μl template DNA and 1.0 μl of 10 μM forward and reverse primer solutions each were placed in 0.2 ml microcentrifuge tube including AccuPower™ HotStart PCR Premix (Bioneer), and was well mixed. PCR primers for mtCOI were LCO1490 and HCO2198 (Folmer et al., 1994). The reaction condition was 94°C (1 min); 47°C (2 min); 72°C (3 min); repeated for 40 cycles. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced directly using the BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing products were purified using the Qiagen DyeEx 20.0 spin Kit (Qiagen).

DNA sequencing was carried out in an Automated DNA Sequencer, Model 377 (Applied Biosystems). The machine-read sequences were compiled using Sequencing Analysis (Ver. 3.3, ABI prism) and manually checked for accuracy. Multiple-sequence alignments were made using CLUSTAL X version 2.0 (Larkin et al., 2007). Pairwise distance measures and phylogenetic analyses were conducted using MEGA 4 software (Tamura et al., 2007). Ambiguous sites were eliminated from the dataset. The Neighbor-Joining (Saito and Nei, 1987) tree with the p-distance model was used for likelihood optimization. Significance of the tree topology for NJ was estimated by the bootstrap test (Felsenstein, 1985) with 1000 replicates. The mtCOI DNA sequence for *Acartia* (*Acartiura*) *omorii* Bradford, 1976, collected from Jangmog bay, southern Korea (GenBank accession No. JX982268), was used as an outgroup for the phylogenetic analysis.

RESULTS

Systematics

Order Calanoida G. O. Sars, 1903
Acartiidae G. O. Sars, 1900
Acartia Dana, 1846
Subgenus *Euacartia* Steuer, 1915

***Acartia forticrusa* n. sp.**

(Figs. 1-6)

Acartia southwelli: Shen and Song, 1979, pp. 160-161, Fig. 84a-d.

Type materials.—Holotype: Adult female dissected and mounted on 2 glass slides, total body length: 0.86 mm, NIBRIV0000266727; allotype: adult male dissected and mounted on two glass slides, NIBRIV0000266728; Paratypes: 17 females undissected in vial and 6 male undissected in vial, NIBRIV0000266729; all collected from the Seomjin River estuary (35°01′06.01″N 127°47′06.77″E), southern Korea by E. O. Park on 15 June 2000. Additional paratypes: undissected 40 females and 2 males in total in vial (MABIK CR00178441) from Beolgyo Stream estuary (34°49′43.34″N 127°23′20.63″E), southern Korea collected by H. Y. Soh on 5 August 2008.

Female.—Body (Fig. 1A, B) length 0.79 to 0.88 mm (n = 20, holotype 0.86 mm). Anterior margin of cephalosome rounded in dorsal view (Fig. 1D). Rostral filaments paired, curved, thin and long (Figs. 1C, 5A). Prosome:Urosome ratio 4.05:1. Prosome 5-segmented; cephalosome and first pedigerous somite completely separate; fourth and fifth pedigerous somites fused (Fig. 1A). Posterior corners of fifth pedigerous somite rounded, naked (Fig. 1A). Urosome 3-segmented; genital double-somite most swollen anterolaterally, with paired copulatory and gonopores ventromedially (Figs. 1E, F, 5D-F), each gonopore covered with epicuticle; second urosomite sometimes with row of spinules on dorsoposterior margin. Proportional lengths of urosomites and caudal ramus 47:19:12:22 = 100.

Antennule 20-segmented, extending to midlength of genital double-somite (Fig. 2A): ancestral segments XVIII and XIX with 1 row of setules, XXI with 3 rows of setules, XXV with 1 row of setules wide distally, and XXVI with 2 rows of setules. Segmentation and setation patterns as follows: I-[3], II-V-[5 + ae], VI-VII-[2 + ae], VIII-X-[2 (1spini-form)], XI-[1 + ae], XII-[0], XIII-[0], XIV-XV-[2 + ae], XVI-[1 + ae], XVII-[1], XVIII-[1 + ae], XIX-[1], XX-[1], XXI-[1 + ae], XXII-[1], XXIII-[1], XXIV-[2 (1 + 1)], XXV-[2 (1 + 1) + ae], XXVI-[2(1 + 1)], XXVII-XXVIII-[4 + ae].

Antenna (Fig. 2B) coxa with 1 seta; basis and first endopodal segment fused to form elongated allobasis bearing 8 setae medially, one seta terminally along inner margin, and distal spinules in 2 rows; second endopodal segment elongated, with 7 setae; third endopodal segment short, with 7 setae. Exopod short, 4-segmented; setation formula 1, 2, 2, 3. Mandible (Fig. 2C): coxa well developed gnathobase bearing 9 large and 3 small sharp teeth; basis with 1 medial seta and row of short and long setules on lateral and posterior margins, respectively; endopod 2-segmented, proximal and distal endopods each with 2 and 9 setae; exopod 4-segmented, first segment with row of setules and setation formula as 1, 1, 1, 2.

Maxillule (Fig. 2D, E): praecoxa and coxa incompletely fused, praecoxal arthrite with 9 elements; coxal endite with 3 setae; 9 setae on coxal epipodite; basal endite 1 with 1 seta; basal exite with 1 seta; endopod (with 5 terminal setae) and exopod (with 2 outer setae) fused with hairs along inner margin.

Maxilla (Fig. 3A) precoxa and coxa incompletely fused, setation formula of endites 4, 2, 2, 3; basis with 1 long seta and row of setules on distal margin; endopod 3-segmented, last two segments incompletely fused, with setation formula 2, 2, 3.

Maxilliped (Fig. 3B) precoxa and coxa incorporated into syncoxa bearing 6 setae; basis with 1 spiniform seta; endopod 2-segmented, first and second endopodal segments with 3 and 2 spiniform setae, respectively.

Swimming legs (P1-P4) (Fig. 3C-G) biramous, with 3-segmented exopods and 2-segmented endopods; coxa without seta; only basis of P4 with outer seta; distal endopodal segments of P1-P4 with setules on anterior distal regions; each exopodal segment of P1 with spiniform seta. Spine and setal formula as follows:

	Coxa	Basis	Exopod	Endopod
P1	0-0	0-0	I-1; I-1; II, I, 4	0-1; 1, 2, 3
P2	0-0	0-0	0-1; 0-1; 0, I, 5	0-2; 1, 2, 4
P3	0-0	0-0	0-1; 0-1; 0, I, 5	0-2; 1, 2, 4
P4	0-0	1-0	0-1; 0-1; 0, I, 5	0-3; 1, 2, 3

P5 (Figs. 3H, 5G) symmetrical, 3-segmented: both coxae completely incorporated in intercoxal sclerite; basis oblong-ovate, with outer seta inserted at about midlength; exopod tapering, distally short, thick, bent at midlength, distal portion serrated, base slightly swollen.

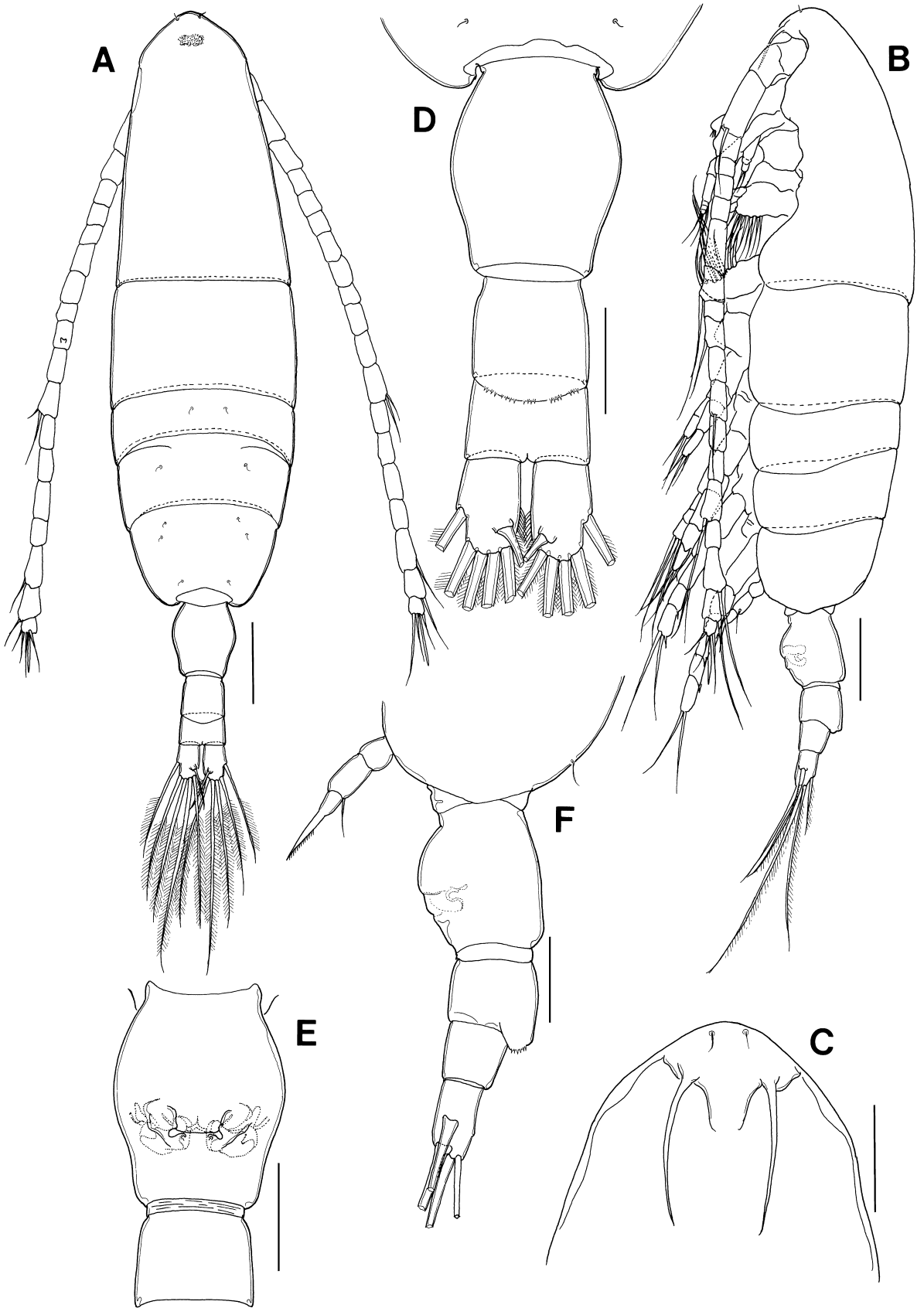

Fig. 1. *Acartia forticrusa* n. sp. female (holotype). A, habitus, dorsal view; B, habitus, lateral view; C, rostral filaments, ventral view; D, genital double-somite and urosome, dorsal view; E, genital double-somite, ventral view; F, genital double-somite and urosome, lateral view. Scale bars: A, B = 100 μm; C-F = 50 μm.

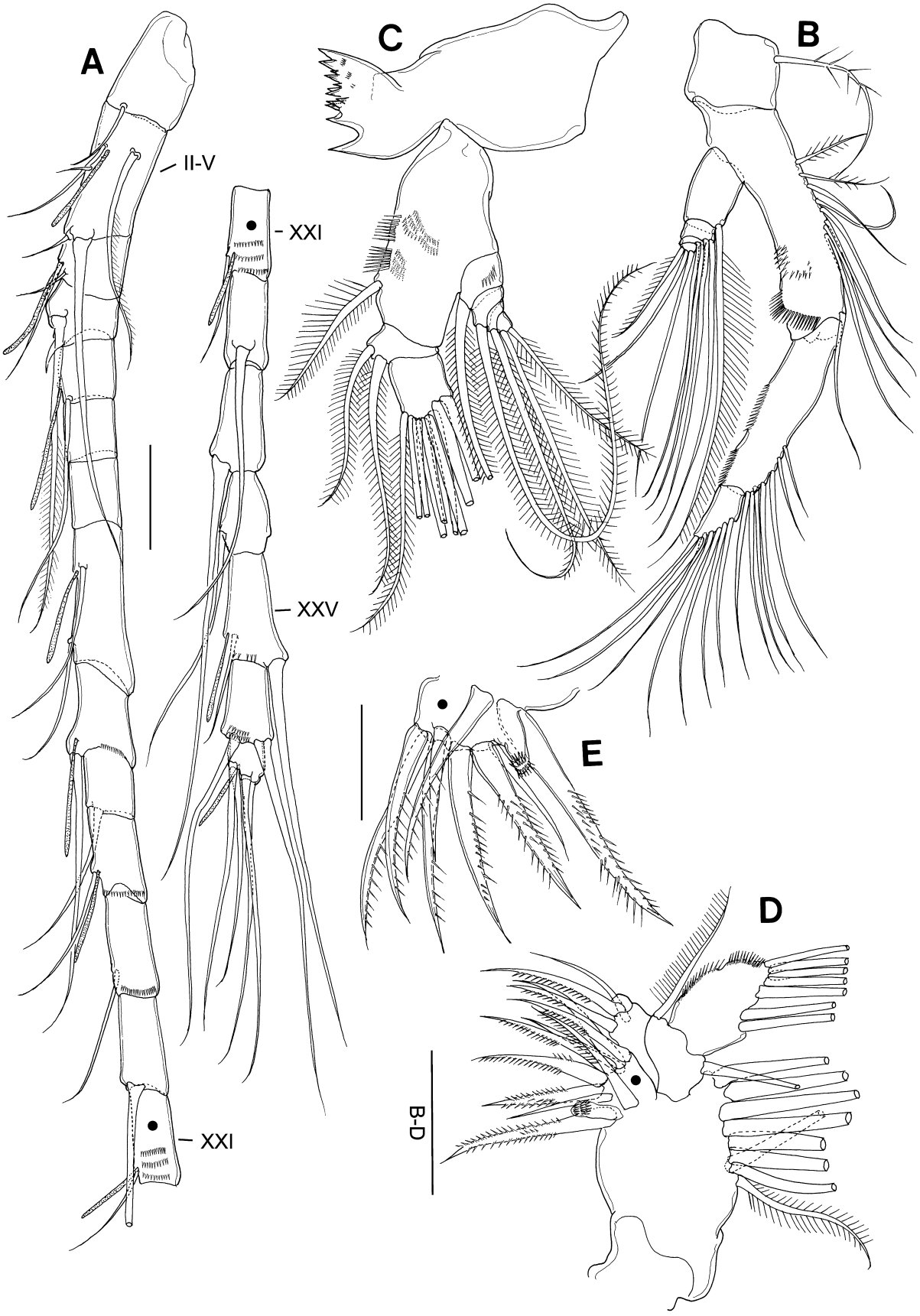

Fig. 2. *Acartia forticrusa* n. sp., female (holotype). A, antennule; B, antenna; C, mandible; D, maxillule; E, precoxa arthrite of maxillule. Scale bars: A-D = 50 μm; E = 25 μm.

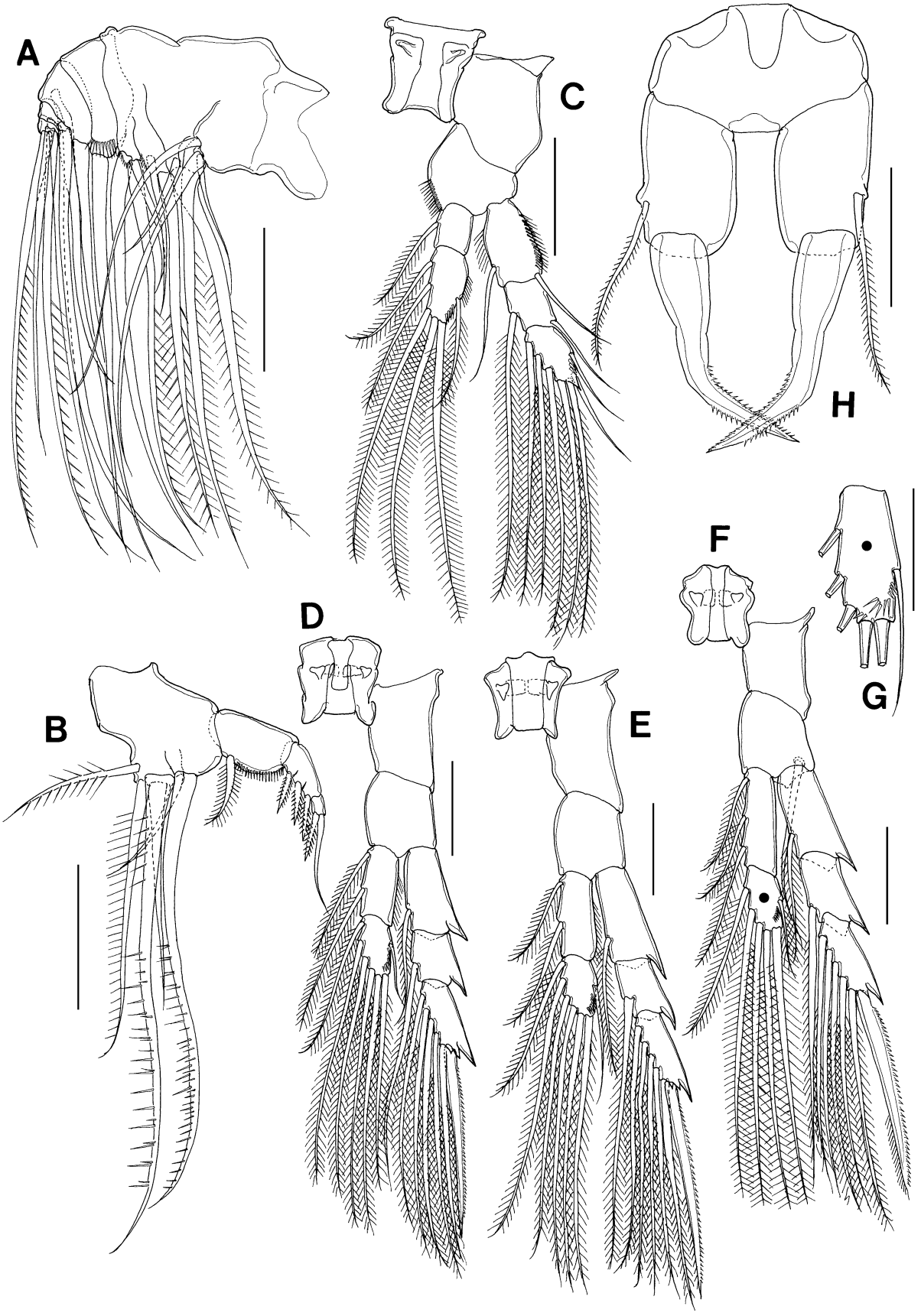

Fig. 3. *Acartia forticrusa* n. sp., female (holotype). A, maxilla; B, maxilliped; C, P1, anterior view; D, P2, anterior view; E, P3, anterior view; F, P4, anterior view; G, third exopodal segment of P4, anterior view; H, P5. Scale bars: A-F = 50 μm; G, H = 25 μm.

Male.—Body (Fig. 4A, B) length 0.67 to 0.80 mm (n = 20, paratype 0.76 mm). Prosome:Urosome ratio 3.47:1. Posterior corners of fifth pedigerous somite naked. Urosome 5 somites; each somite usually unarmed, but sometimes second somite furnished with rows of setules (Fig. 5E-H). Caudal rami nearly as broad as long. Proportions of urosomites and caudal rami 11:34:22:8:11:14 = 100.

Right antennule 18-segmented, geniculate between segments XX and XXI (Fig. 4C): ancestral segments IX-XI and XIX with row of transverse spinules, XX and XXI-XXIII with longitudinal row of denticles (Fig. 4D-F). Segmentation and setation patterns as follows: I-[1], II-IV-[3 + ae], V-[2], VI-VIII-[1 + ae], IX-XI-[3 + spiniform + ae], XII-[0], XIII-[0], XIV-[1 + ae], XV-[1], XVI-[1 + ae], XVII-[1], XVIII-[1 + ae], XIX-[1 + spiniform + ae], XX-[1], XXI-XXIII-[1 + II + ae], XXIV-XXV-[4 (2 + 2) + ae], XXVI-2 (1 + 1)], XXVII-XXVIII-[4 + ae]. Left antennule 22-segmented. Segmentation and setation patterns as follows: I-[1], II-VI-[4 + ae], VIII-[2], IX-[1 + ae], X-[2 (1spiniform)], XI-[2 + ae], XII-[0], XIII-[0], XIV-[2 (1spiniform) + ae], XV-[1], XVI-[1 + ae], XVII-[1], XVIII-[1 + ae], XIX-[1], XX-[1], XXI-[1 + ae], XXII-[1], XXIII-[1], XXIV-[2 (1 + 1)], XXV-[2 (1 + 1) + ae], XXVI-[2 (1 + 1)], XXVII-XXVIII-[4 + ae].

Other mouthparts and P1 to P4 as in female. Fifth leg (Fig. 4G, H) asymmetrical: intercoxal sclerite completely fused to both coxae. Left leg 3-segmented: basis armed with outer seta and round lobe on posterior surface, basis slightly longer than outer seta; exopod 2-segmented, first segment short, unarmed; distal segment furnished with proximal tuft of hair, one long medial spine with two rows of teeth along its inner margin and blunt projection at its base; distal segment terminates in one short process and one terminal spine (Fig. 5H-J). Right leg 4-segmented: basis short, with outer seta longer than its segment; exopod 3-segmented, first segment with long slender seta posteromedially, second segment with oblong inner lobe, grooved terminally, bearing one spine on distal border, third segment with very short spine on medial margin and curved thick terminal spine.

Remarks.—The new species, *A. forticrusa*, closely resembles *A. southwelli* and *A. sarojus* Madhupratap and Haridas, 1994, but it differs from the latter two species in the following characteristics (see Table 1) in the female: (1) the antennule extends to the middle of the genital-double somite (shorter in the other 2 species), (2) the caudal ramus length about 1.4 times longer than wide (shorter in the other 2 species), (3) the fifth leg ratio of the basis to exopod less than two (>2 in the other 2 species), (4) the basal seta of the fifth leg is longer than the basis (shorter than the basis in the other 2 species), (5) the exopod of the fifth legs is short, thick and bent at midlength (more elongate in the other 2 species); in the male: (1) the left fifth leg has a rounded process on the posterior surface (this process is absent in the other 2 species), (2) the distal exopodal segment of the left fifth leg with one long medial spine with two rows of teeth along its inner margin and with a blunt projection at its base, (3) inner process of the proximal segment of right fifth leg conspicuously grooved (rounded or only slightly grooved in the other species). Some individuals are variable in having setules on the dorsoposterior margin of the female genital-double-somite and the second urosomite (Fig. 6). Shen and Song (1979) described *A. southwelli* from Chinese waters, but according to their figures this species is identical with our new species *A. forticrusa* from Korean waters in having the above characteristics.

Etymology.—the specific name *forticrusa* (Latin *fortis*: strong; *crus*: leg) refers to the strong feature of the female fifth leg.

Molecular diversity.—A 592-bp region of the mtCOI was obtained for six individuals of *A. forticrusa* (GenBank accession Nos JX982260-JX982265) collected from the Seomjin River estuary, the southern Korea and five individuals of *A. southwelli* (GenBank accession Nos JX982266, JX982267) from the Pulicat Lake, the south east coast of India. Individuals of the same species differed in mtCOI sequences by 0-3%, while individuals of different species differed by 21-24% (Table 2). The mtCOI gene tree also showed that *A. forticrusa* is clearly an undescribed species separate from *A. southwelli* (Fig. 7).

Distribution.—*Acartia forticrusa* occurs in salinities ranging from 5 to 20 in the estuaries of Beolgyo Stream and Seomjin River, southern Korea, between June and November. However, its peak abundance was in a salinity of ca. 15 in June and ca. 20 in September (Fig. 8). When the water temperature is less than 20°C, it is replaced by *A. hudsonica* Pinhey, 1926. Also, *A. forticrusa* generally occurs together with the brackish calanoid copepods *A. ohtsukai* Ueda and Bucklin, 2006 and *Tortanus dextrilobatus* Chen and Zhang, 1965 in the Seomjin River estuary, southern Korea.

Discussion

Since *A. forticrusa* has the morphological features of *Euacartia* it is provisionally located in that subgenus until the re-evaluation of Steuer's subgeneric system through morphological descriptions and genetic analysis of more number of the species of *Acartia*.

The mitochondrial gene cytochrome oxidase subunit I (mtCOI) has been used as an appropriate marker for species-level studies because it contains sufficient diversity to address intra- and interspecific phylogenetic relationships in calanoid copepods (Bucklin and Wiebe, 1998; Ueda and Bucklin, 2006; Eyun et al., 2007; Bucklin and Frost, 2009; Sakaguchi and Ueda, 2010; Soh et al., 2012). The differences between *A. forticrusa* from Korea and *A. southwelli* collected from India were 21-24%. Ueda and Bucklin (2006) showed that mtCOI DNA sequences of species in *Acartia* differed by 23-27%. The differences among 11 species of *Acartia*, including more than one species among 6 nominated subgenera (*Acartia*, *Acartiura*, *Acanthacartia*, *Euacartia*, *Hypoacartia*, *Odontacartia*), also ranged between 14 and 27% (Soh et al., unpublished data), confirming that *A. forticrusa*, in addition to its morphological distinctness, is an independent species separated from *A. southwelli*.

Of the three species within *Euacartia*, *A. forticursa*, *A. sarojus* and *A. southwelli* (Fig. 9), *A. southwelli* is widely distributed in brackish waters of India (Sewell, 1914, 1924, 1932; Steuer, 1915, 1923; Tranter and Abraham, 1971; Madhupratap and Haridas, 1994), while *A. sarojus* is confined to the salt pans located along the Gulf of Kutch, northwestern

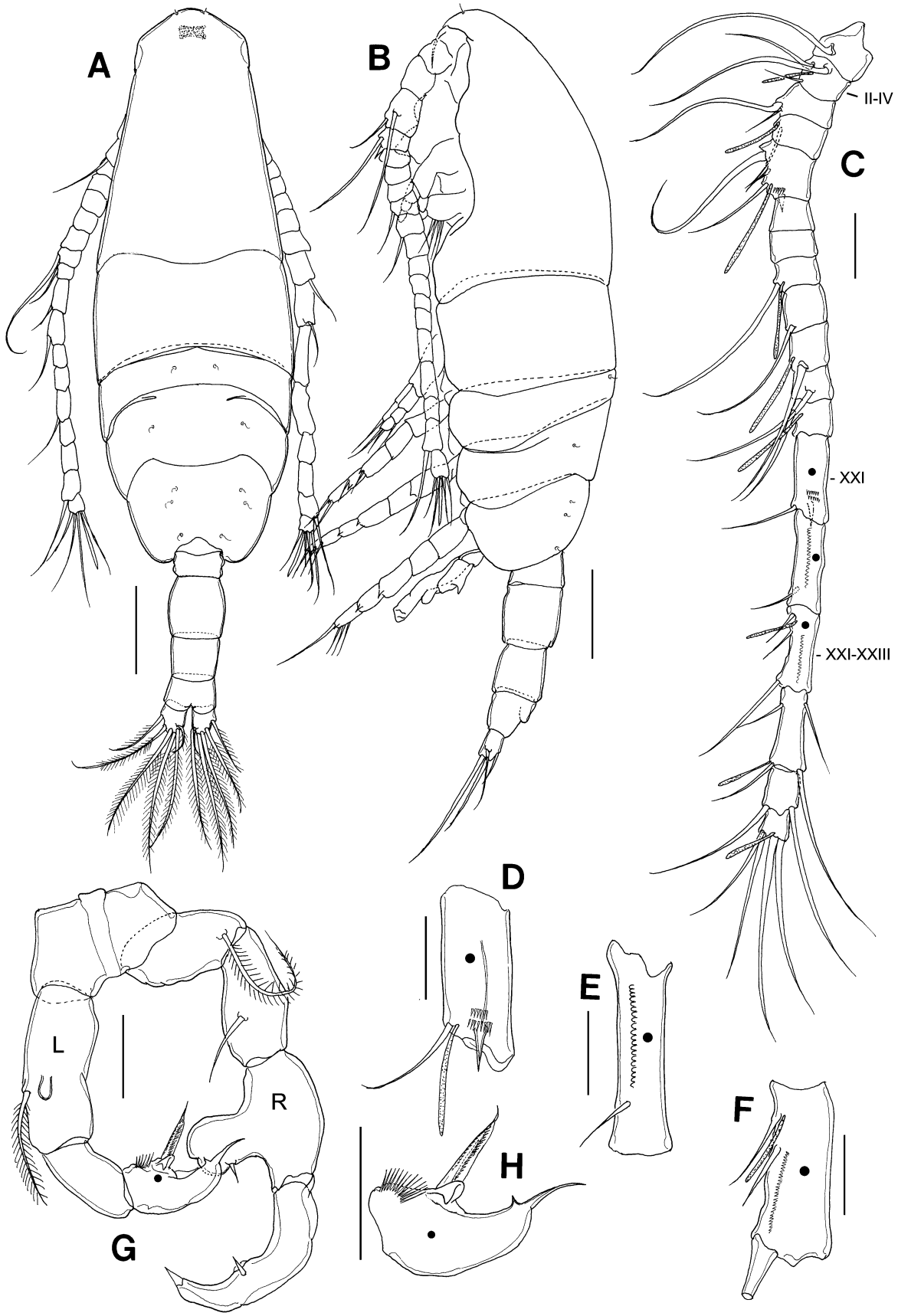

Fig. 4. *Acartia forticrusa* n. sp., male (paratype). A, habitus, dorsal view; B, habitus, lateral view; C, right antennule; D-F, 13-15 segments of right antennule; G, P5, anterior view; F, third segment of left P5. Scale bars: A, B = 100 μm; C = 50 μm; D-H = 25 μm.

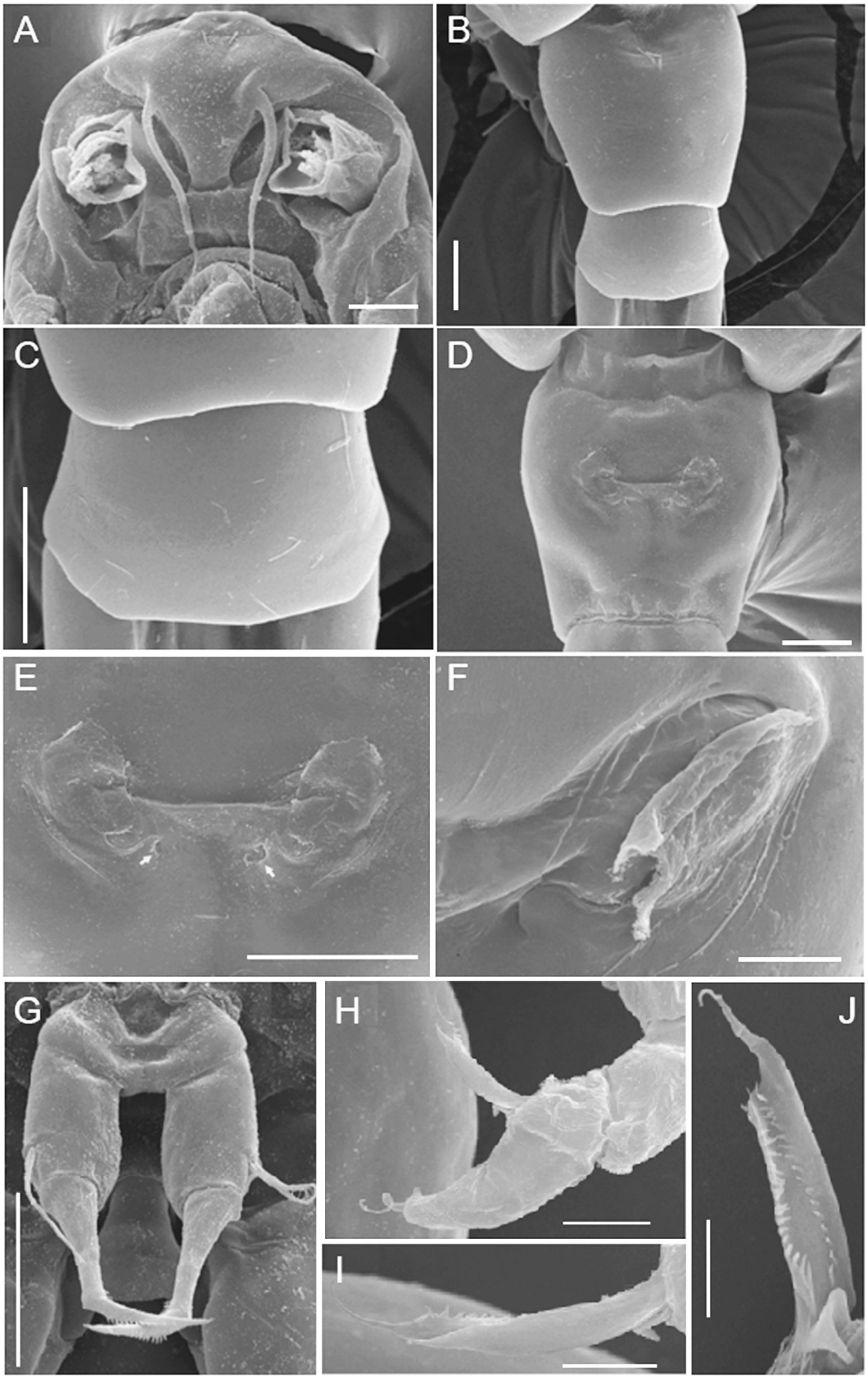

Fig. 5. Scanning electron micrographs of *Acartia forticrusa* n. sp., female. A, rostral area, ventral view; B, genital double-somite, dorsal view; C, first urosomite, dorsal view; D-F, genital double-somite, ventral view; G, fifth leg. Male, H, distal exopodal segment of left fifth leg; I, J, medial spine and blunt projection on distal exopodal segment of left fifth leg. Scale bars: A, B = 20 μm; B, D = 10 μm.

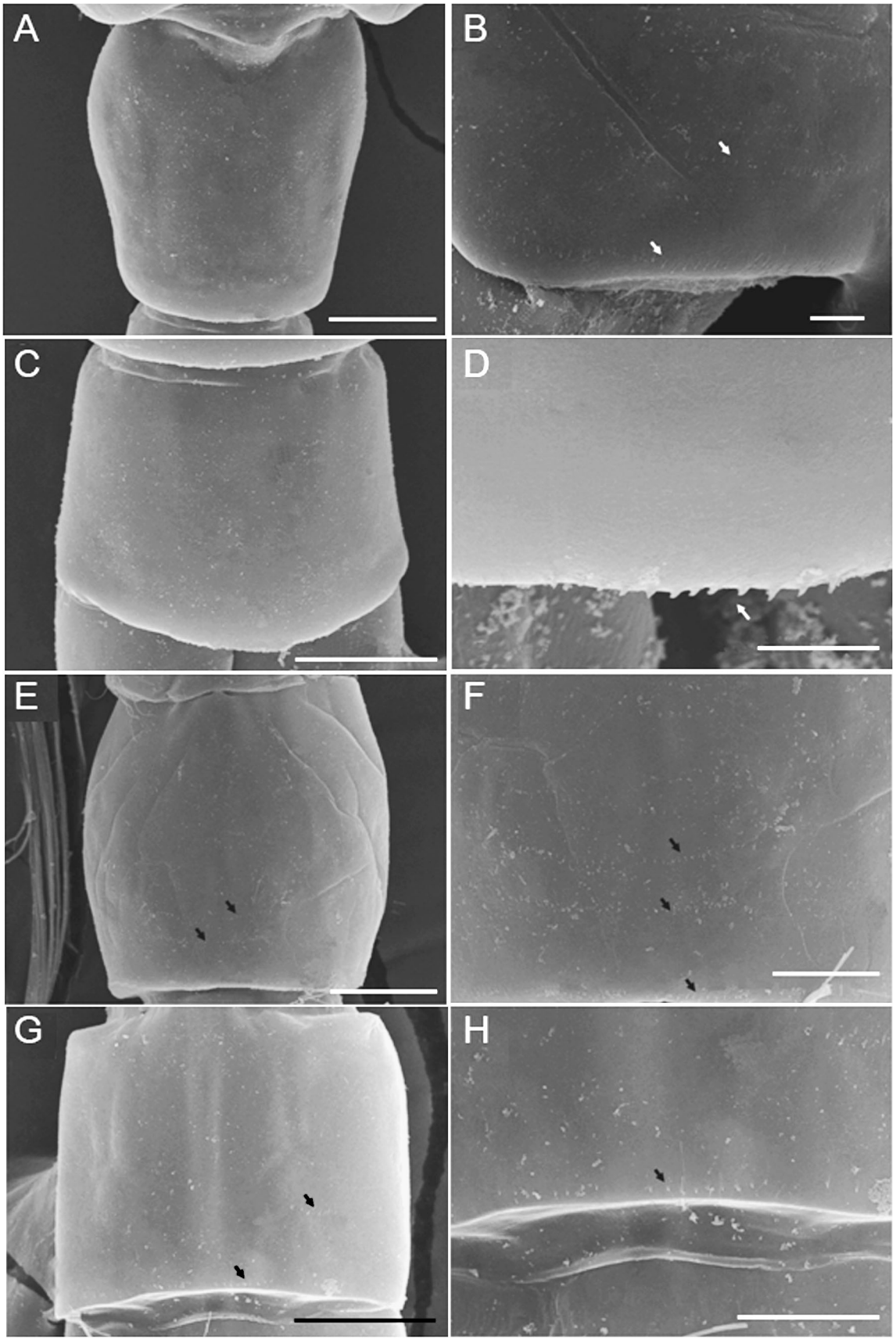

Fig. 6. Scanning electron micrographs of variants of *Acartia forticrusa* n. sp., female. A, B, genital double-somite, dorsal view; C, D, first urosomite, dorsal view. Male, E-H, urosome, dorsal view. Scale bars: A, C, E, G = 20 μm; B, D, F, H = 5 μm. Arrows indicate rows of setules.

Table 1. Comparison of morphological features between *A. forticrusa* n. sp. and its congeners *A. southwelli* and *A. sarojus.*

	A. forticrusa n. sp.	*A. southwelli* (Sewell, 1914)	*A. sarojus* Madhupratap and Haridas, 1994
Female			
Proportion of length to width of caudal ramus	ca. 1.40 (0.5:0.7)	ca. 1.11[a] (0.9:1.0)	ca. 1.25[b] (0.8:1.0)
Basis of 5th leg	Round	Square	Inner-medially swollen medial
Proportion of length of basis to exopod	<ca. 2	>ca. 2	>ca. 2
Exopodal segment of 5th leg	Short and without suture at midlength	Long and with suture at midlength	Long and without suture at midlength
Male			
Lobe on basis of left 5th leg	Presence	Absence	Absence
Outer process on distal segment tip of left 5th leg	Long	Long	Short
Inner side of basis of right 5th leg	Slightly protruded	Flated	Slightly protruded
Inner process on second exopodal segment of right 5th leg	Well grooved	Slightly grooved	More rounded

[a] Based on Sewell (1914).
[b] Based on Madhupratap and Haridas (1994).

Table 2. Pairwise percentage differences for mtCOI sequences between individual females of *Acartia forticrusa* n. sp. and *A. southwelli*.

		1	2	3	4	5	6	7	8
1.	*Acartia forticrusa* No. 1 (GenBank accession No. JX982260)								
2.	*Acartia forticrusa* No. 2 (GenBank accession No. JX982261)	2.4							
3.	*Acartia forticrusa* No. 3 (GenBank accession No. JX982262)	2.5	0.5						
4.	*Acartia forticrusa* No. 4 (GenBank accession No. JX982263)	2.2	0.2	0.3					
5.	*Acartia forticrusa* No. 5 (GenBank accession No. JX982264)	2.5	0.7	0.8	0.5				
6.	*Acartia forticrusa* No. 6 (GenBank accession No. JX982265)	2.5	0.5	0.7	0.3	0.8			
7.	*Acartia southwelli* No. 1 (GenBank accession No. JX982266)	22.1	22.8	23.3	23.0	23.5	23.0		
8.	*Acartia southwelli* No. 2 (GenBank accession No. JX982267)	21.6	22.3	22.8	22.5	23.0	22.5	0.5	
9.	*Acaria omorri* JM1-Kr (GenBank accession No. JX982268)	22.5	22.5	22.5	22.3	22.0	22.0	24.5	24.7

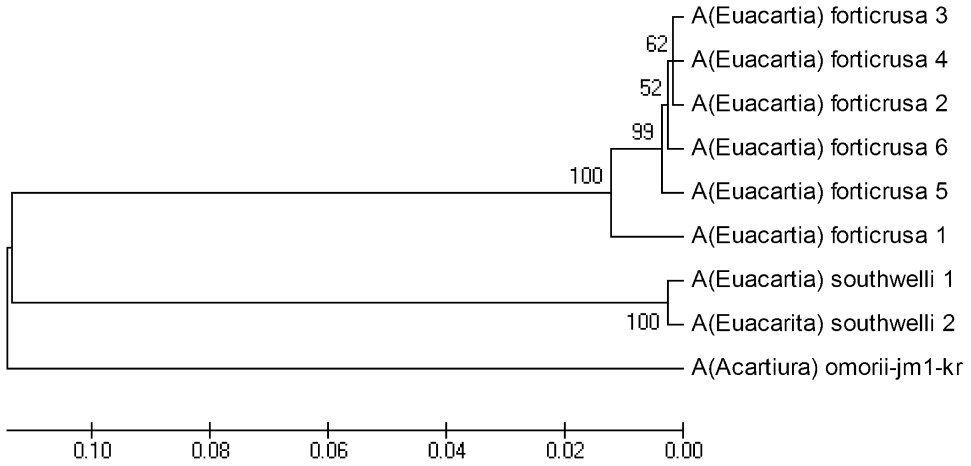

Fig. 7. Gene tree for mtCOI showing proportional differences between individual females of *Acaria forticrusa* n. sp. from Korean waters and *A. southwelli* from Pulicat Lake, India. Numbers at branch points are bootstrap values (i.e., percentage of trees with that branch point among 1000 subreplicates). The gene sequence for *A. omorii*, collected from Jangmok bay, southern Korea was used as an out-group. The specimen number corresponds to that in Table 2.

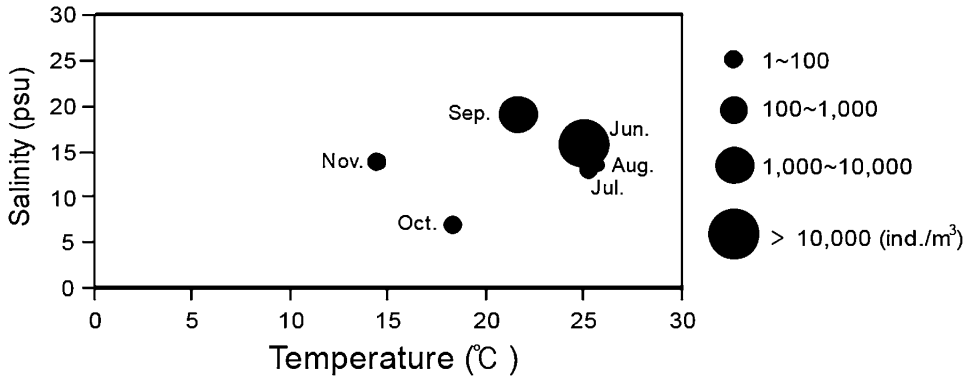

Fig. 8. Temperature-salinity-abundance (ind./m^3) diagram for *A. forticrusa* n. sp.

India, in salinities of 60 to 70 (Madhupratap and Haridas, 1994). *Acartia forticrusa* predominantly occurs in brackish waters of southern Korea of salinity of 5 to 18 in June and September (Park, 2005; present study). The distribution of *A. forticrusa* extends to Chinese brackish waters (Shen and Song, 1979). However, the geographical characteristic of *A. southwelli* is restricted in the western part of the Indo-Malayan region. The speciation within *Euacartia* might go through similar evolutionary processes as in the speciation of the tropical pseudodiaptomids in the Indo-Malayan region (Walter et al., 2002; Nishida and Rumengan, 2005), i.e., low sea-level stands around the Indo-Australian region during glacial periods could have produced barriers between the Indian and the West Pacific populations. As a result, the species could have become isolated from the parental population and speciated allopatrically or parapatrically. Chen

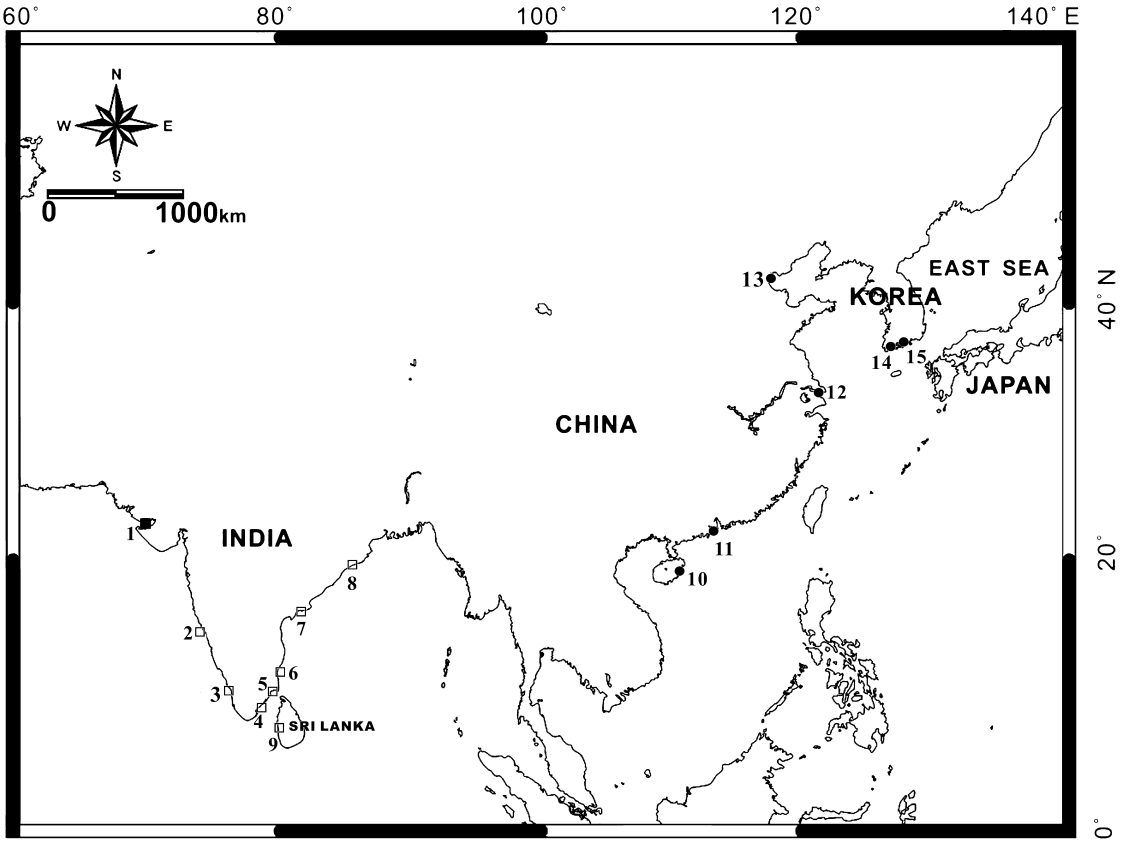

Fig. 9. Schematic illustration on the geographical distribution of the sibling species, *Acartia sarojus* Madhupratap and Haridas, 1994 (black square), *A. southwelli*, (white square) and *A. forticrusa* (black circle) in the brackish and coastal waters of Asia: 1, Gulf of Kutch, Gujarat; 2, Mandovi-Zurai estuaries, Goa; 3, Cochin Harbor, Kochi; 4, Tuticorin, Tamil Nadu; 5, Kilakarai, Tamil Nadu; 6, Porto Novo, Tamil Nadu; 7, Kakinada Bay, Andhra Pradesh; 8, Chilka Lake, Orissa; 9, Sri Lanka; 10, Wenchang River, Hainan; 11, Pearl (Zhu) River, Gwangtung; 12, Taiping Lake, Zhangtsui; 13, Tianjin estuary, Tianjin; 14, Beolgyo Stream, Jeollanamdo; 15, Seomjin River estuary, Jeollanamdo. Number 1 is recorded from Madhupratap and Haridas (1994) and Numbers 2-9 are recorded from Sewell (1914, 1924, 1932), Steuer (1915, 1923), Tranter and Abraham (1971), Madhupratap and Haridas (1994), and the present study, respectively. Numbers 10-15 are cited from Shen and Song (1979) and the present study.

and Hare (2008, 2011) also suggested that a reproductive isolation can occur in the mesoscale (10s-100s of km), especially in *Acartia*. Considering this fact, *A. forticrusa* from Chinese waters and *A. southwelli* from Indian waters might include a cryptic species, although recent study can't find any morphological differences in the species level between their local populations. However, there are no data for any occurrences of the subgenus *Euacartia* in the Indo-Malayan region. More detailed research on the speciation and the geographic distribution is necessary for a better understanding of the evolutionary history of these copepods as suggested by Nishida and Rumengan (2005).

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REFERENCES

Barthélémy, R. M. 1999. Functional morphology and taxonomic relevance of the female genital structures in Acartiidae (Copepoda: Calanoida). Journal of the Marine Biological Association of the United Kingdom 79: 857-870.

Boxshall, G. A., and S. H. Halsey. 2004. An Introduction to Copepod Diversity. The Ray Society, London, 966 pp.

———, and R. Huys. 1998. The ontogeny and phylogeny of copepod antennules. Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences 353: 765-786.

Bucklin, A., and B. W. Frost. 2009. Morphological and molecular phylogenetic analysis of evolutionary lineages within *Clausocalanus* (Copepoda: Calanoida). Journal of Crustacean Biology 29: 111-120.

———, and P. H. Wiebe. 1998. Low mitochondrial diversity and small effective population sizes of the copepods *Calanus finmarchicus* and *Nannocalanus minor*: possible impact of climatic variation during recent glaciation. American Genetic Association 89: 383-392.

Chen, G., and M. P. Hare. 2008. Cryptic ecological diversification of a planktonic estuarine copepod, *Acartia tonsa*. Molecular Ecology 17: 1451-1468.

———, and ———. 2011. Cryptic diversity and comparative phylogeography of the estuarine copepod *Acartia tonsa* on the US Atlantic coast. Molecular Ecology 20: 2425-2441.

Eyun, S., Y. Lee, H.-L. Suh, S. Kim, and H. Y. Soh. 2007. Genetic identification and molecular phylogeny of *Pseudodiaptomus* species (Calanoida, Pseudodiaptomidae) in Korean waters. Zoological Science 24: 265-271.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Folmer, O., M. Black, W. Hoen, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294-299.

Huys, R., and G. A. Boxshall. 1991. Copepod Evolution. The Ray Society, London, 468 pp.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, and D. G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948.

Madhupratap, M., and P. Haridas. 1994. Description of *Acartia* (*Euacartia*) *southwelli* Sewell 1914 and *Acartia* (*Euacartia*) *sarojus* n. sp. from India and status of the subgenus *Euacartia* Steuer 1923. Hydrobiologia 292/293: 67-74.

Nishida, S., and I. F. M. Rumengan. 2005. A new species of *Pseudodiaptomus* (Copepoda: Calanoida: Pseudodiaptomidae) from the coastal waters of Sulawesi, Indonesia. Plankton Biology & Ecology 52: 27-32.

Park, E. O. 2005. Seasonal change of copepod populations in the brackish water of the Seomjin River and Mankyung River. Ph.D. Thesis, Chonnam National University, Yeosu, 63 pp. (In Korean with English abstract.)

Razouls, C., F. de Bovée, J. Kouwenberg, and N. Desreumaux. 2005-2012. Diversity and geographic distribution of marine planktonic copepods. Available online at http://copepodes.obs-banyuls.fr/en (accessed September 23, 2012).

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology Evolution 4: 406-425.

Sakaguchi, S. O., and H. Ueda. 2010. A new species of *Pseudodiaptomus* (Copepoda: Calanoida) from Japan, with notes on the closely related *P. inopinus* Burckhardt, 1913 from Kyushu Island. Zootaxa 2623: 52-68.

Shen, D. J., and D. X. Song. 1979. Fauna Sinica, Crustacea, Freshwater Copepoda. Institute of Zoology, Academia Sinica, Beijing, 450 pp.

Soh, H. Y., S. W. Kwon, W. Lee, and Y. H. Yoon. 2012. A new *Pseudodiaptomus* (Copepoda, Calanoida) from Korea supported by molecular data. Zootaxa 3368: 229-244.

Sewell, R. B. S. 1914. Notes on the surface Copepoda of the Gulf of Mannar. Spolia Zeylanica 9: 191-262.

———. 1924. Fauna of Chilka Lake. Crustacea Copepoda. Memoirs of the Indian Museum 5: 771-851.

———. 1932. The Copepoda of Indian Seas. Calanoida. Memoirs of the Indian Museum 10: 223-407, 3 pls.

Steuer, A. 1915. Revision der Gattung *Acartia* Dana. Zoologischer Anzeiger 45: 392-397.

———. 1923. Bausteine zu einer monographie der copepodengattung *Acartia*. Arbeiten aus dem Zoologisches Institute der Universität Innsbrűck 1(5): 91-144.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. Mega 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology Evolution 24: 1596-1599.

Tranter, D. J., and S. Abraham. 1971. Coexistence of species of Acartiidae (Copepoda) in the Cochin Backwaters, a monsoonal estuarine lagoon. Marine Biology 11: 222-241.

Ueda, H., and A. Bucklin. 2006. *Acartia* (*Odontacartia*) *ohtsukai*, a new brackish-water calanoid copepod from Ariake Bay, Japan, with a redescription of the closely related *A. pacifica* from the Seto Inland Sea. Hydrobiologia 560: 77-91.

Walter, T. C., S. Ohtsuka, S. Putchakarn, K. Pinkaew, and S. Chullasorn. 2002. Redescription of two species of *Pseudodiaptomus* from Asia and Australia (Crustacea: Copepoda: Calanoida: Pseudodiaptomidae) with discussion of the female genital structure and zoogeography of Indo-West Pacific species. Proceedings of the Biological Society of Washington 115: 650-669.

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