ILL Request

REG-10094345

MDUNIH

NLM -- W1 ZO616G (Gen)

National Institutes of Health (NIH)
NIH Library
10 Center Drive, MSC 1150
Building 10 Room B1L306
Bethesda, MD 20892-1150

ATTN:
PHONE: 301-594-6476
FAX: 301-
E-MAIL: edelivery@nih.gov

SUBMITTED: 2017-02-10 11:02:12
PRINTED: 2017-02-10 16:00:47
REQUEST NO.: REG-10094345
SENT VIA: DOCLINE
EXTERNAL NO.: 5911

REG NORMAL Copy Journal

TITLE:	ZOOTAXA
PUBLISHER/PLACE:	Magnolia Press Auckland, N.Z. :
VOLUME/PAGES:	2016 Sep 21;4169(3):515-539. doi: 10. 11646/zootaxa.4169.3.6 515-539
DATE:	2016
AUTHOR OF ARTICLE:	Hiruta SF;Kakui K
TITLE OF ARTICLE:	THREE NEW BRACKISH-WATER THALASSOCYPRIDINE SPECIES (CRUSTACE
ISSN:	1175-5326
OTHER NUMBERS/LETTERS:	NLM Unique ID: 101179386 39721369 27701290
SOURCE:	PubMed
MAX COST:	$0.00
COPYRIGHT COMP.:	Guidelines
CALL NUMBER:	W1 ZO616G (Gen)

NLM Collection Access Section, Bethesda, MD

ILL Request

REG-10094345

REQUESTER INFO:	Ciufo, Stacy - TN: 5911
DELIVERY:	Odyssey: 206.107.44.214
REPLY:	E-mail: edelivery@nih.gov

This document contains 25 pages. This is NOT an invoice.

Questions? Contact NLM: www.nlm.nih.gov/illcontact

NLM Collection Access Section, Bethesda, MD

Zootaxa 4169 (3): 515–539
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://doi.org/10.11646/zootaxa.4169.3.6
http://zoobank.org/urn:lsid:zoobank.org:pub:0794F234-5201-4C55-AEE1-D58ACF678F72

Three new brackish-water thalassocypridine species (Crustacea: Ostracoda: Paracyprididae) from the Ryukyu Islands, southwestern Japan

SHIMPEI F. HIRUTA[1] & KEIICHI KAKUI[2]

[1]*Center for Molecular Biodiversity Research, National Museum of Nature and Science, Tsukuba 305-0005, Japan. E-mail: s-hiruta@kahaku.go.jp*
[2]*Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan. E-mail: kakui@mail.sci.hokudai.ac.jp*

Abstract

We describe three new species of brackish-water ostracods representing two genera in the ostracod tribe Thalassocypridini from mangrove forests in the Ryukyu Islands, subtropical southwestern Japan, and provide their barcoding sequences for the mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene. *Mangalocypria ryukyuensis* **sp. nov.** was found on Okinawa Island. We also found a *Mangalocypria* population on Ishigaki Island that was morphologically identical to *M. ryukyuensis* on Okinawa, but an individual differed by 4.7% in *COI* sequence (K2P distance) from an individual from Okinawa. This is the first record for Japan of a species in *Mangalocypria*. *Paracypria longiseta* **sp. nov.**, obtained from Okinawa Island, is similar to *Pontoparta hartmanni*. *Paracypria plumosa* **sp. nov.** from Ishigaki Island is similar to *Pa. adnata* described from Yakushima Island, Japan. The *COI* genetic distance between individuals of *Pa. longiseta* and *Pa. plumosa* was roughly as large as that between either of these species and individuals in the *Mangalocypria* populations. Our study underscores that genera in Thalassocypridini may not represent natural groups, and that this tribe needs taxonomic revision.

Key words: Ostracoda, Paracyprididae, brackish species, Okinawa Island, Ishigaki Island, *COI*

Introduction

Ostracods of the tribe Thalassocypridini live mostly in brackish environments and have well-developed natatory setae and a relatively small adult body size (ca. 0.5–0.7 mm). Although this tribe is diverse and representative species are abundant in brackish-water environments at low latitudes, the group is relatively poorly known. Thus far, 49 species in seven genera have been recorded worldwide, mainly in tropical regions (Maddocks 2005; Smith & Kamiya 2006; Hu & Tao 2008). Three species, representing two genera, have been reported from Japan: *Dolerocypria mukaishimensis* Okubo, 1980; *Paracypria inujimensis* (Okubo 1980), redescribed by Smith & Kamiya (2003); and *Paracypria adnata* Smith & Kamiya, 2006. Previous studies in Japan have reported ostracods of the family Paracyprididae from rock pool or tidelands on both the eastern and western coasts (Okubo 1980; Nakao & Tsukagoshi 2002; Smith & Kamiya 2006).

Since Hebert *et al.* (2003) introduced and evaluated *COI* (mitochondrial cytochrome *c* oxidase subunit I gene) partial sequences as a taxonomic tool, thousands of *COI* sequences have been determined across a broad range of eukaryotic organisms (Coissac *et al.* 2016). Hebert *et al.* (2003) proposed a Kimura 2-parameter (K2P) genetic distance of around 3% in the *COI* sequence as a rough criterion for differentiation at the interspecific level. This criterion was tested in many taxa, and although there are exceptions (e.g., Waugh 2007; Bucklin *et al.* 2011), a *COI* distance of 3% indicates differentiation at the species level in most metazoans (Ratnasingham & Hebert 2013).

Matzen da Silva *et al.* (2011) examined inter- and intraspecific levels of *COI* differentiation in crustaceans, mainly decapods, and found that most of the genetic distances within species were less than 2%. Among ostracods, various studies on the genetic structure of putatively cosmopolitan species (e.g., Bode *et al.* 2010; Koenders *et al.* 2012; Schön *et al.* 2012) have detected multiple cryptic species, but have also provided information on intra- and interspecific *COI* distances. A phylogeographic study of *Macroscapha* species in the Antarctic (Brandão *et al.* 2010) showed genetic distances of 4.7–13.5% between species, but 0.0–3.5% within species.

Accepted by R. Matzke-Karasz: 3 Aug. 2016; published: 21 Sept. 2016

Complementary to the use of divergence levels to distinguish species, DNA barcoding is a means of identifying individuals to known species by sequence similarity, with *COI* the gene most commonly used for this purpose (e.g., Hebert & Gregory 2005; Bucklin *et al.* 2011). In this approach, the *COI* sequence from an unidentified specimen can be searched against a DNA database, with species identity indicated by database sequences less than ca. 3% divergence. The converse outcome, lack of a similar match in the database, is ambiguous, as the unidentified specimen may belong either to a previously undescribed species, or simply to a previously described species for which no barcoding sequence has been deposited in the database.

During short collecting trips to the mangrove forests on Okinawa and Ishigaki Islands in the subtropical Ryukyu Archipelago in southwestern Japan, we found three undescribed species representing two genera in Thalassocypridini. In this paper, we describe these species and provide their *COI* barcode sequences (accession numbers AB920554–AB920557).

Material and methods

Our study area comprised two sampling sites, both located in the Ryukyu Archipelago and belonging to the same subtropical climate zone. Each side was situated in a bay in mangrove habitat near a river mouth. The mangrove forest in Oura Bay, Okinawa Island, is composed mainly of *Bruguiera gymnorhiza* and *Kandelia obovata* (Wakushima *et al.* 1994; Sheue *et al.* 2003). The river bottom at the mouth consists of rock, with interstices filled in with silt and organic material. The mangrove forest in Kabira Bay, Ishigaki Island, is composed mainly of *Avicennia marina*, *Bruguiera gymnorhiza*, and *Rhizophora mucronata* (Wakushima *et al.* 1994). The river bottom at the mouth is sandy silt with some organic material. The salinity at the sampling site ranged from as low as 2‰ at low tide up to around 25‰ at high tide.

Material was collected from Oura Bay, Okinawa, Japan, on 26 February 2011 by K. Kakui and on 22 November 2013 by S.F. Hiruta, and from Kabira Bay, Ishigaki Island, on 28 March 2013 by S.F. Hiruta. All samples were collected by hand at low tide. Bottom substrate was washed with water through a 0.1-mm-mesh sieve; specimens were then sorted under a dissecting microscope and preserved in 99% ethanol. Specimens from Kabira Bay were maintained alive for six months in the laboratory. For identification and illustration, specimens were dissected and appendages were mounted in Hoyer's solution on glass slides; line drawings were made with the aid of a camera lucida. The software CombineZP (Hadley 2010) was used to make stacked images of male and female genital organs. Selected carapaces were pasted onto microfossil slides with a tragacanth gum solution. For observation by scanning electron microscope (SEM), carapaces and soft parts were treated with hexamethyldisilazane (Nation 1983) and examined with a S-3000N instrument (Hitachi High Technologies) at 15–20 kV accelerating voltage. The material used in this study has been deposited in the invertebrate collection of the Hokkaido University Museum, Sapporo, with catalog numbers bearing the prefix ICHUM.

The chaetotaxic notation follows that of Broodbakker & Danielopol (1982), as revised for the antennae by Martens (1987) and for the thoracopods by Meisch (1996). Hemipenis terminology follows that of Danielopol (1969). The abbreviations for limbs are those of Meisch (2000): A1, antennule; A2, antenna; Md, mandible; Mx, maxillula; L5, maxilliped; L6, walking leg; L7, cleaning leg; Hp, hemipenis; *ya*, aesthetasc of antennule; *Y*, *y2*, *y3*, aesthetascs on antenna; *t1–4*, mid-ventral setae on third podomere of antenna; *G1–3*, inner apical claws/setae on penultimate segment of antenna; *z1–3*, outer apical claws/setae on penultimate segment of antenna; *GM*, *Gm*, apical claws on terminal segment of antenna; *E*, endopod; *Pr*, protopod; *Exo*, exopodite; *Ga*, anterior terminal claw on uropod; *Gp*, posterior terminal claw on uropod; *Sa*, anterior seta on uropod; *Sp1–2*, posterior setae on uropod. Abbreviations for structures of the copulatory organs follow the usage of Wouters (1998) for *Mangalocypria* (*a*, dorsal lobe of hemipenis; *b*, median lobe of hemipenis; *c*, ventral lobe of hemipenis) and Smith & Kamiya (2006) for *Paracypria* (*p*, bursa copulatrix of hemipenis; *r*, prelabyrinthal wide spermiduct of hemipenis; *t*, outer process of hemipenis).

Total genomic DNA was extracted from whole individuals by means of a DNeasy Blood & Tissue Kit (Qiagen), with modifications from Johnson *et al.* (2004). Specimens were incubated for at least 48 h to lyse the tissue. Before the lysis mixture from a specimen was pipetted into the spin column, the exoskeleton and carapace were retrieved and mounted in Hoyer's solution on a glass slide, as a voucher specimen.

Part of the mitochondrial *COI* gene was amplified by using universal primers LCO1490 and HCO2198

(Folmer *et al.* 1994). PCRs were performed in an Applied Biosystems 2720 thermal cycler in 10 µl volumes containing 0.5 µl of template solution, 2 mM $MgCl_2$, 2.5 mM each dNTP, 10 pmol each primer, and 0.25 U Ex *Taq* polymerase (Takara) in 1× buffer provided by the manufacturer. Amplification conditions were 95°C for 2 min; 35 cycles of 95°C for 30 sec, 50°C for 30 sec, and 72°C for 1 min; and 72°C for 7 min. Amplification products were purified by the method of Boom *et al.* (1990). All nucleotide sequences were determined by direct sequencing using a BigDye Terminator Cycle Sequencing Kit ver. 3.1 with an ABI 3730 Avant automated sequencer (Applied Biosystems). The amplification primers were also used for sequencing. Sequences have been deposited in GenBank under accession numbers AB920554–AB920557.

Nucleotide sequences were assembled and edited with MEGA5 (Tamura *et al.* 2011). K2P distances for *COI* in pairwise comparisons among individuals from two populations of *Mangalocypria ryukyuensis* **sp. nov.** and two *Paracypria* species were calculated with MEGA5.

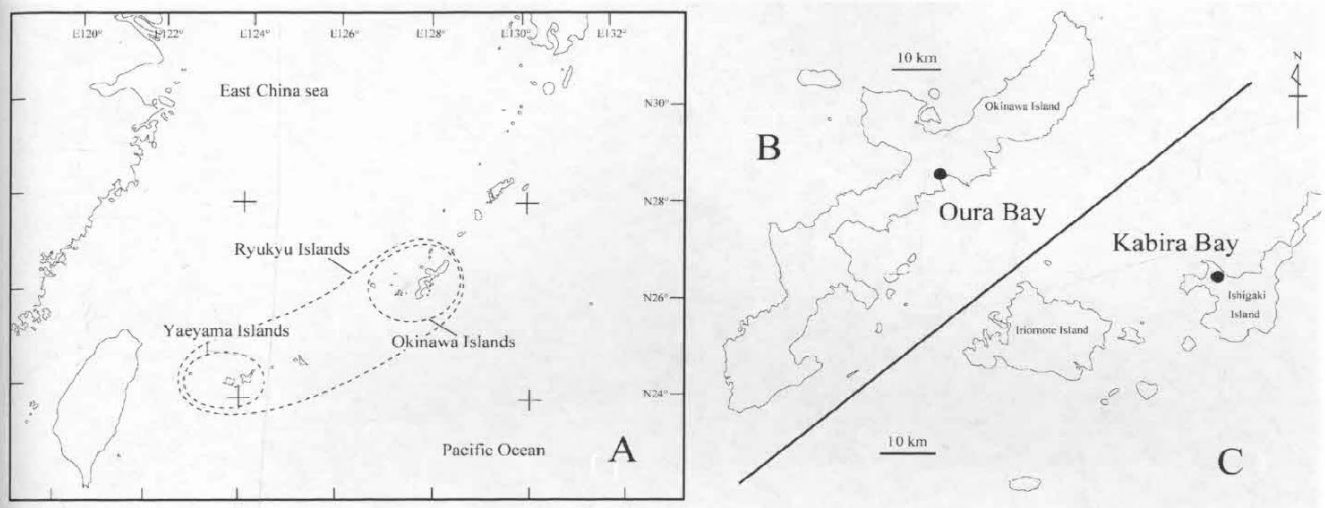


FIGURE 1. (A) Map showing the location of Ryukyu Islands. (B, C) Enlargements showing Okinawa Island (B) and the Yaeyama Islands (C), with the sampling sites indicated with filled circles.

Taxonomic descriptions

Family Paracyprididae Sars, 1923

Tribe Thalassocypridini Hartmann & Puri, 1974

Genus *Mangalocypria* Wouters, 1998

***Mangalocypria ryukyuensis* sp. nov.**
(Figures 2–7)

Material examined. Type material. Holotype: ICHUM 4895 (male), 26°33′36″N, 128°2′37″E, Oura River, Okinawa Island, Japan, 26 February 2011, soft parts mounted on 19 slides, carapace mounted on a microfossil slide. Allotype: ICHUM 4897 (female), collection data same as for holotype, softparts mounted on 17 slides, carapace mounted on a microfossil slide. Paratypes: ICHUM 4896 (male), 4898–4899 (two females), collection data same as for holotype, soft parts mounted on glass slides, carapace mounted on a microfossil slide; ICHUM 4904–4905 (two males), 4906–4907 (two females), collection data same as for holotype, mounted on stubs for SEM observation; ICHUM 4902 (female), collection data same as for holotype, exoskeleton and carapace after DNA extraction mounted on a glass slide.

Additional material. ICHUM 4900 (male) and 4901 (female), 24°26′32″N, 124°8′21″E, Kabira Bay, Ishigaki Island, Japan, 28 March 2013, softparts mounted on glass slides, carapace mounted on a microfossil slide; ICHUM

4903 (female), 24°26′32″N, 124°8′21″E, Kabira Bay, Ishigaki Island, Japan, 28 March 2013, exoskeleton and carapace after DNA extraction mounted on a glass slide.

Etymology. The specific epithet is an adjective derived from Ryûkyû, the geographical name for the archipelago extending from Okinawa Island to Yonaguni Island, in combination with the Latin suffix *-ensis*.

Diagnosis. Medium-sized valves, with evenly convex dorsal margin. Carapace spindle-shaped in dorsal view, widest in the middle. Rome organ of A1 without distal expansion. Natatory setae of A2 reaching slightly beyond tip of claw. Distal setae on third and fourth segments of L6 short. Hook of male clasping apparatus with long apical seta. Dorsal lobe of Hp beaked, median lobe large and curved, ventral lobe strongly developed and widely curved. Uropodal ramus with very short, fine *Sp1* and *Sp2*.

COI barcoding sequences. AB920556 (658 bp) from ICHUM 4902 (paratype) collected in the type locality on Okinawa Island; AB920557 (658 bp) from ICHUM 4903 collected on Ishigaki Island.

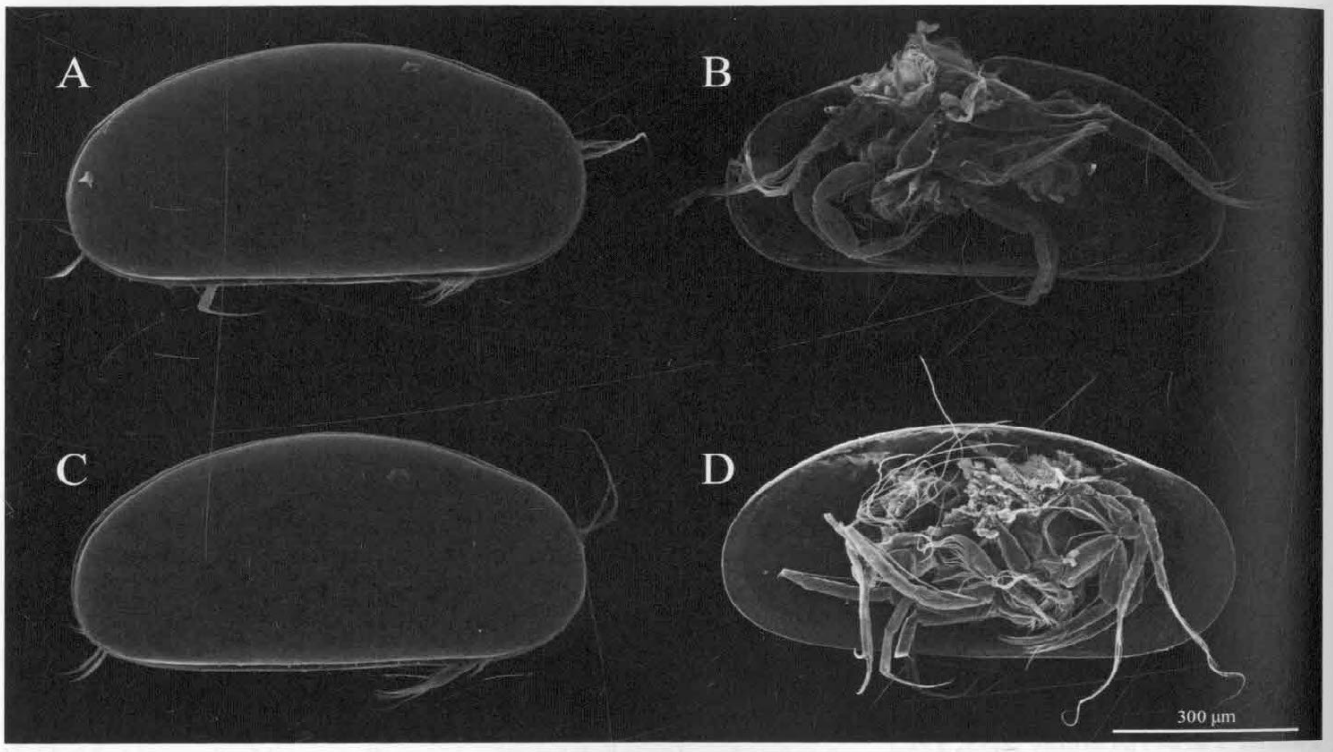


FIGURE 2. *Mangalocypria ryukyuensis* **sp. nov.**, SEM images. (A) Paratype, ICHUM 4904, lateral view of male. (B) Paratype, ICHUM 4905, lateral view of male with LV removed; (C) Paratype, ICHUM 4906, lateral view of female. (D) Paratype, ICHUM 4907, lateral view of female with RV removed.

Description of male. Carapace (Figures 2A, C; 3B) 0.76–0.80 mm long, 0.37–0.39 mm high (n = 4); 0.76 mm long, 0.37 mm high in holotype; elongate in lateral view, highest just behind mid-length, broadly rounded at anterior and posterior ends, slightly convex ventrally; conspicuously narrow in dorsal view, pointed at anterior and posterior ends. Valves very thin, transparent, with light-brownish epidermal pigment; surface very smooth, with short, fine setae.

A1 (Figure 3B) seven-segmented. First two podomeres fused, with one dorsal seta and two long apico-ventral setae Wouters organ not observed. Third podomere trapezoidal, with apico-dorsal seta and short, flat-end Rome organ; Fourth podomere rectangular, with apico-dorsal seta and apico-ventral seta. Fifth and sixth podomeres both rectangular, each with one apico-dorsal seta and two long apico-ventral setae. Seventh podomere with four long apical setae and one shorter apico-dorsal seta. Eighth podomere elongate and slender, with two long setae, one shorter seta, and long aesthetasc *ya*.

A2 (Figure 3C) five-segmented. First podomere (*Pr*) with one long apico-ventral seta and one antero-proximal seta. Second podomere (*EI*) with one normal, one fine apico-ventral setae, aesthetasc *Y*, and *Exo*, consisting of one long and two short setae; 4 + 1 natatory setae extending to the tip of A2 terminal claw. Third podomere (*EII*) with

two mid-apical male bristles (*t2* and *t3*), one apico-ventral and two apico-dorsal setae, and mid-ventral aesthetasc *y2*. Fourth podomere (*EIII*) with claws *G1*, short *G2*, *z1*, *z2*, seta *z3*, and apico-ventral setae. Fifth podomere (*EIV*) with *GM* and slender, short *Gm*, one apical seta, and aesthetasc *y3*.

Md (figure 3E) consisting of coxal plate and four-segmented palp. Coxal plate with antero-lateral seta and six stout teeth. Palp with smooth alpha, smooth long gamma setae, and plumed very short beta seta. First podomere with exopodal plate (*Exo*). Terminal podomere of palp with three claws and two setae.

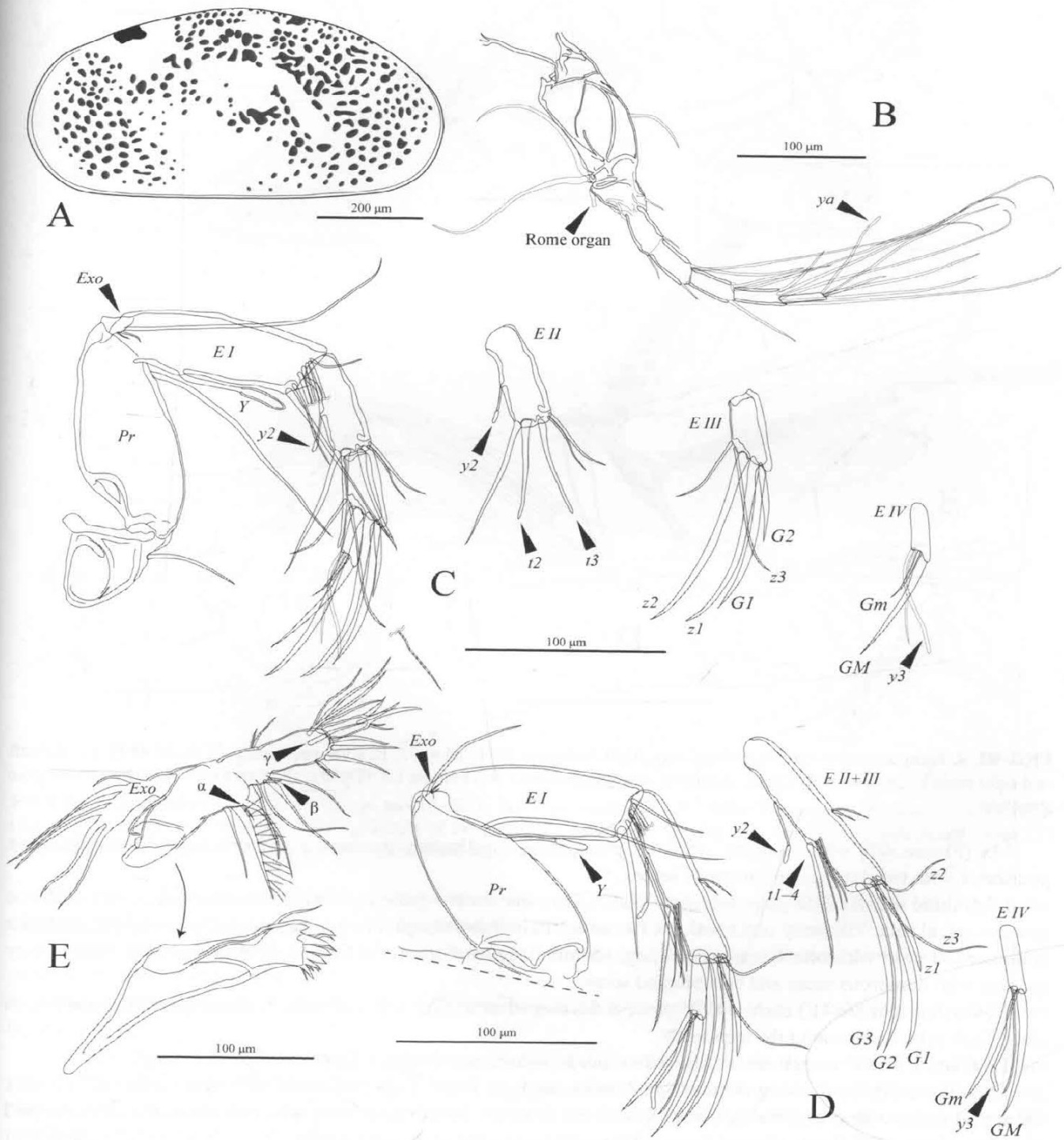


FIGURE 3. *Mangalocypria ryukyuensis* **sp. nov.** A–C and E, holotype, male, ICHUM 4895; D, allotype, female, ICHUM 4897. (A) Lateral view of RV. (B) A1. (C) Male A2 (inset, details of third to fifth podomeres). (D) Female A2 (inset details of terminal two podomeres). (E) Md.

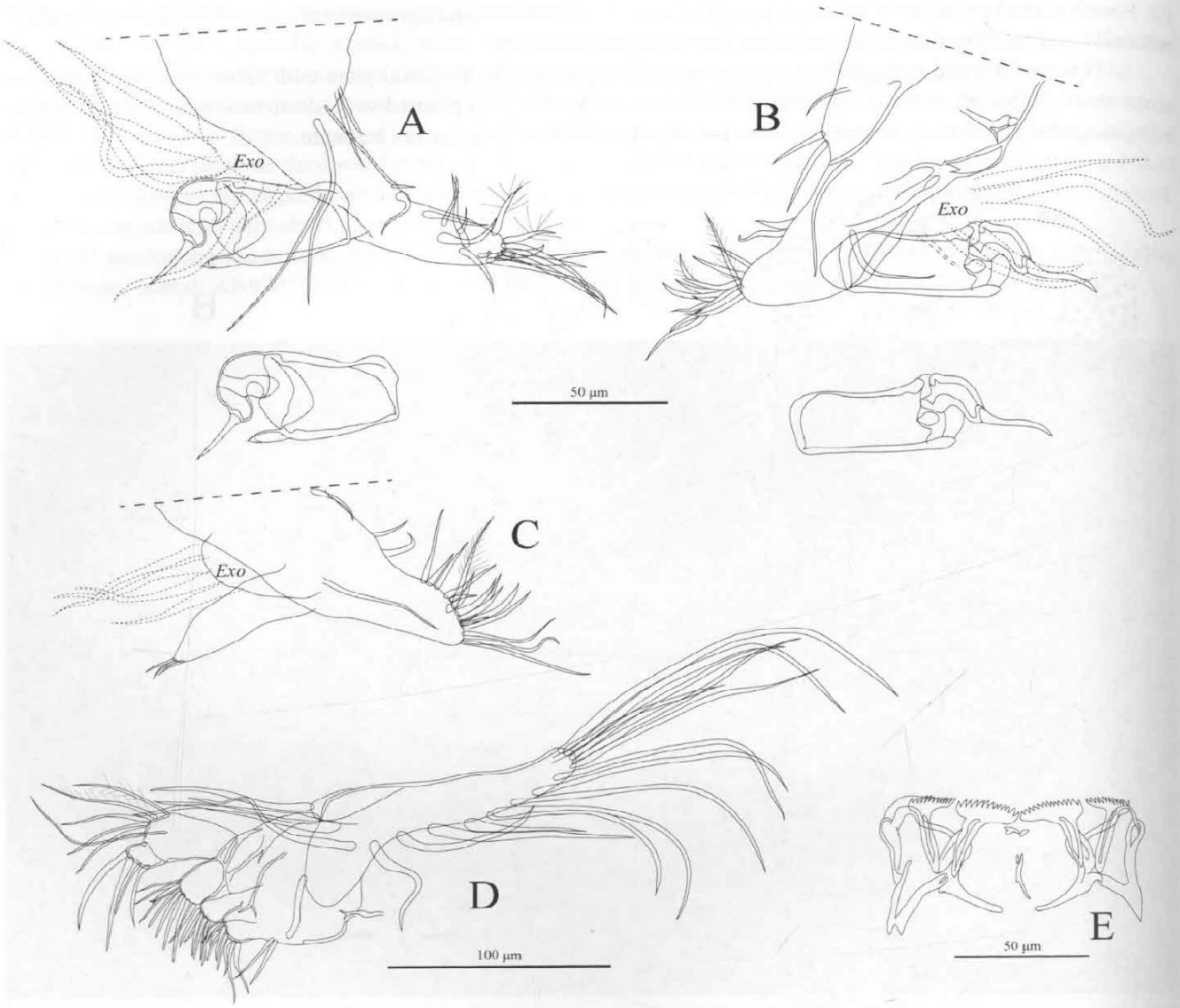


FIGURE 4. *Mangalocypria ryukyuensis* **sp. nov.** A–D, holotype, ICHUM 4895; E, allotype, female, ICHUM 4897. (A, B) Left and right male L5, respectively (inset, details of clasping process). (C) Female L5. (D) Mx. (E) Female lower lip with rake-like structure.

Mx (Figure 4D) with elongate vibratory plate, three masticatory processes, and two-segmented palp. First podomere with two long apical plumose setae.

L5 (Figure 4A, B) with palp, vibratory plate (*Exo*), one antero-proximal seta, one antero-apical seta, and one postero-apical seta. Vibratory plate with six filaments. Palp transformed into two-segmented clasping process; first podomere of palp with one apico-ventral peg; terminal segment stout and curved, with apical seta. Masticatory process with numerous setae and two plumed setae.

Lower lips (Figure 4E) each with V-process developed as a rake. Arms of both, V-process and T-process with slots. Each rake with around thirteen teeth.

L6 (Figure 5A) five-segmented. Second to fourth podomeres elongate. Terminal claw (*h2*) long.

L7 (Figure 5B, C) four-segmented. Penultimate segment fused. First podomere with three setae (*d1*, *d2*, *dp*). Third podomere with one apical (*g*), one mid-ventral setae (*f*). Fourth podomere with two short (*h1*, *h2*) setae and one long, recurved (*h3*) seta plumed from the tip to three-fourths of its total length.

Zenker organ (Figure 6) with 5 + 2 internal rings of spines. Entrance to organ formed as a small star-shaped hole in a spherically shaped proximal end.

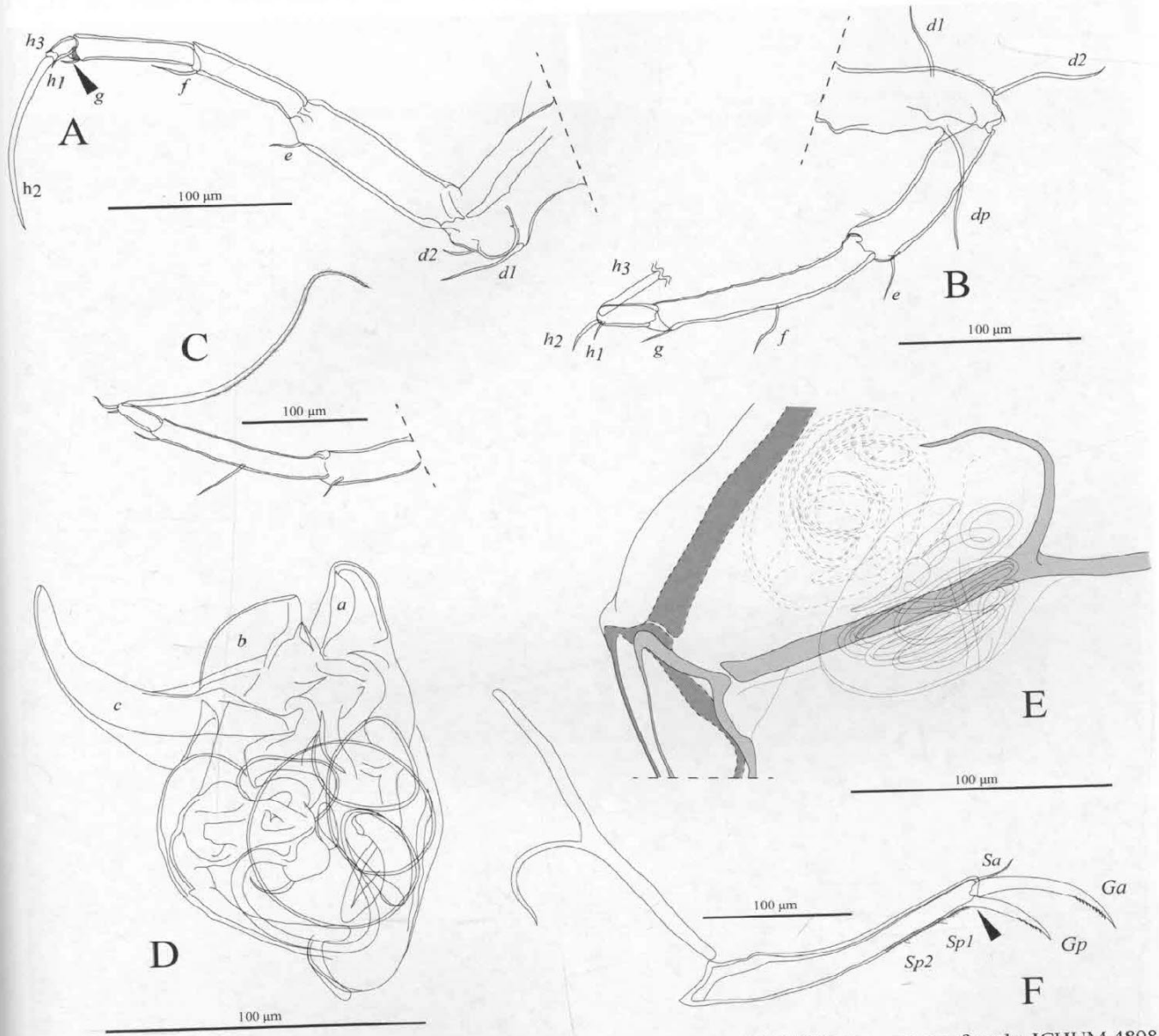


FIGURE 5. *Mangalocypria ryukyuensis* **sp. nov.** A–D, F, holotype, male, ICHUM 4895; E, paratype, female, ICHUM 4898. (A) L6. (B) L7. (C) Enlargement showing details of the terminal setae on L7. (D) Hp. (E) Genital lobe with spiral canal. (F) Uropod; arrowhead shows where joint of postero-distal claw is lacking.

Hp (Figure 5D) distally with three lobes. Dorsal lobe (*a*) distally beak-shaped. Median lobe (*b*) large, outer edge rounded. Ventral lobe (*c*) conspicuously extruding, hook-like, widely curved.

Uropod (Figure 5F) with one *Sa*; with reduced *Sp1* and *Sp2*; *Ga* and *Gp* with tiny denticles. *Gp* without movable joint (arrowhead, Figure 5F).

Description of female. Carapace 0.75–0.82 mm long, 0.37–0.41 mm high (n = 3); 0.77 mm long, 0.38 mm high in allotype.

A2 (Figure 3D) four-segmented. First (*Pr*) and second (*EI*) podomeres similar to those of male. Third podomere (*EII* + *EIII*) with claws *G1*, *G2*, *G3* and *z1*, setae *z2* and *z3*, one mid-ventral aesthetasc *y2*, one long, plumed mid-ventral seta (*t1*), and three mid-ventral setae (*t2–t4*). Fourth podomere (*EIV*) slender and elongate; *Gm* longer than that of male.

Palp of L5 (Figure 4C) simple, non-segmented, with three short apical setae.

Genital lobe with conspicuous, well-developed spiral canal (Figures 5E; 7A–D, Supplementary Data 1).

Vaginal opening rimmed with well-developed sclerotized ring, and forming claw-like process (Figures 5E; 7E, F). In lateral view, vaginal opening situated at the center of the spiral canal inside genital lobe.

In other characters, female similar to male.

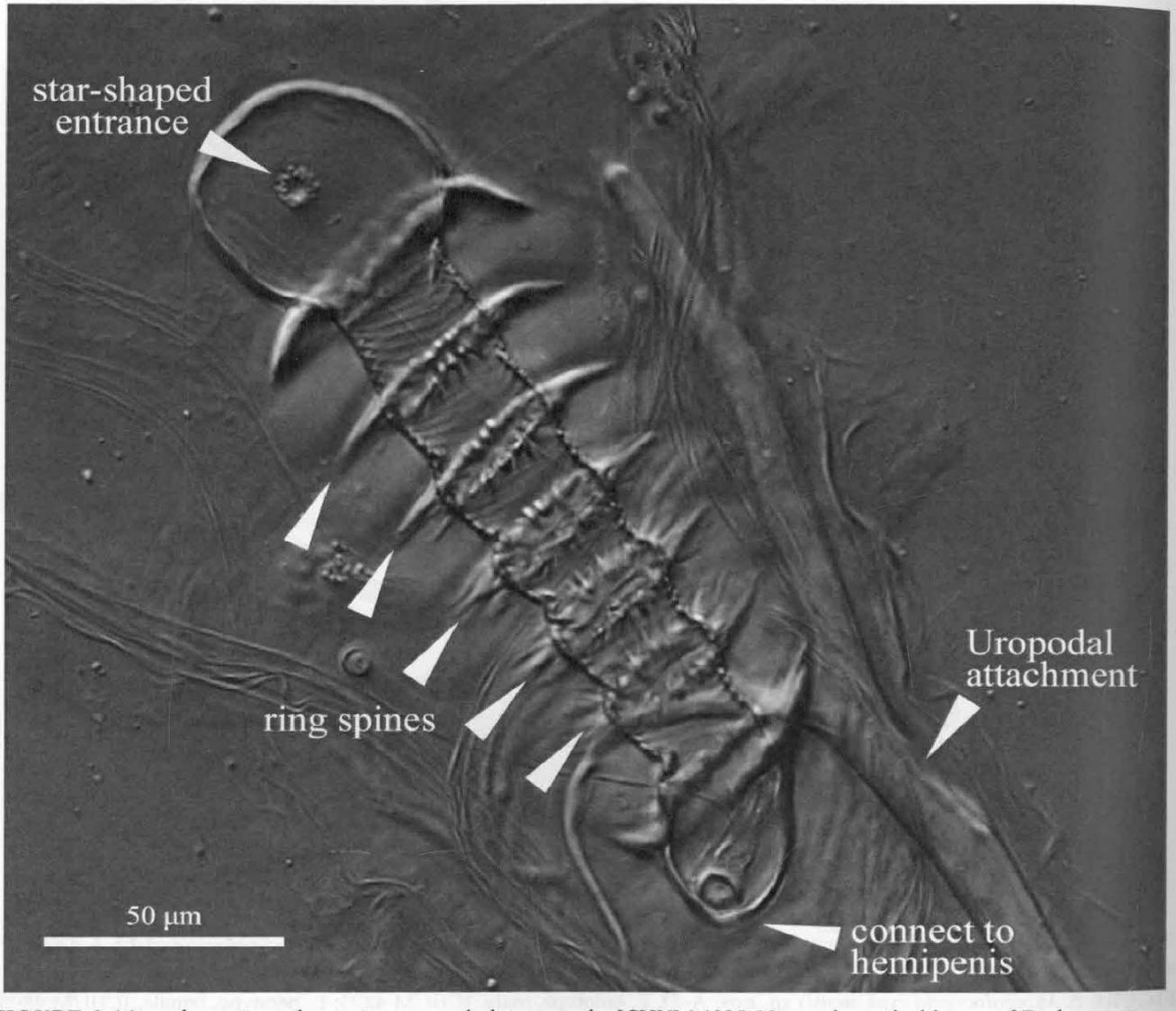


FIGURE 6. *Mangalocypria ryukyuensis* **sp. nov.**, holotype, male, ICHUM 4895. Nomarsky optical image of Zenker organ.

Remarks. This species clearly belongs in genus *Mangalocypria* due to the presence of a V-process developed as a rake on the lower lips, and a fixed postero-dorsal claw on the uropodal ramus. The genus *Mangalocypria* Wouters, 1998 contains three species: *M. africana* (Hartmann, 1974); *M. appendix* Wouters, 1998; and *M. eleotridis* (Harding, 1962). *Mangalocypria africana* was originally described from Mozambique (Hartmann 1974), and Wouters (1998) also reported it from Papua New Guinea, Indonesia, and the Comoros Islands. Meisch et al. (2007) mention it from Palau, Mecherchar Island (Jellyfish Lake). *Mangalocypria appendix* was originally described from Papua New Guinea. These two species differ from *M. ryukyuensis* **sp. nov.** in the genital organs. *Mangalocypria ryukyuensis* is generally similar to *M. eleotridis*, redescribed by Wouters (1998), in having a conspicuous, well-developed, curved ventral lobe on Hp. These two species differ, however, in the shape of the median lobe (Figure 5D; b) of Hp (large with the outer side rounded in *M. ryukyuensis*; small in *M. eleotridis*), the two posterior setae on the uropodal rami (setae very short in *M. ryukyuensis*; longer in *M. eleotridis*), the terminal segment of the clasping process (stout and elongate in *M. ryukyuensis*; shorter in *M. eleotridis*), and carapace size (0.76 mm in the holotype of *M. ryukyuensis*; 0.98 mm in *M. eleotridis*).

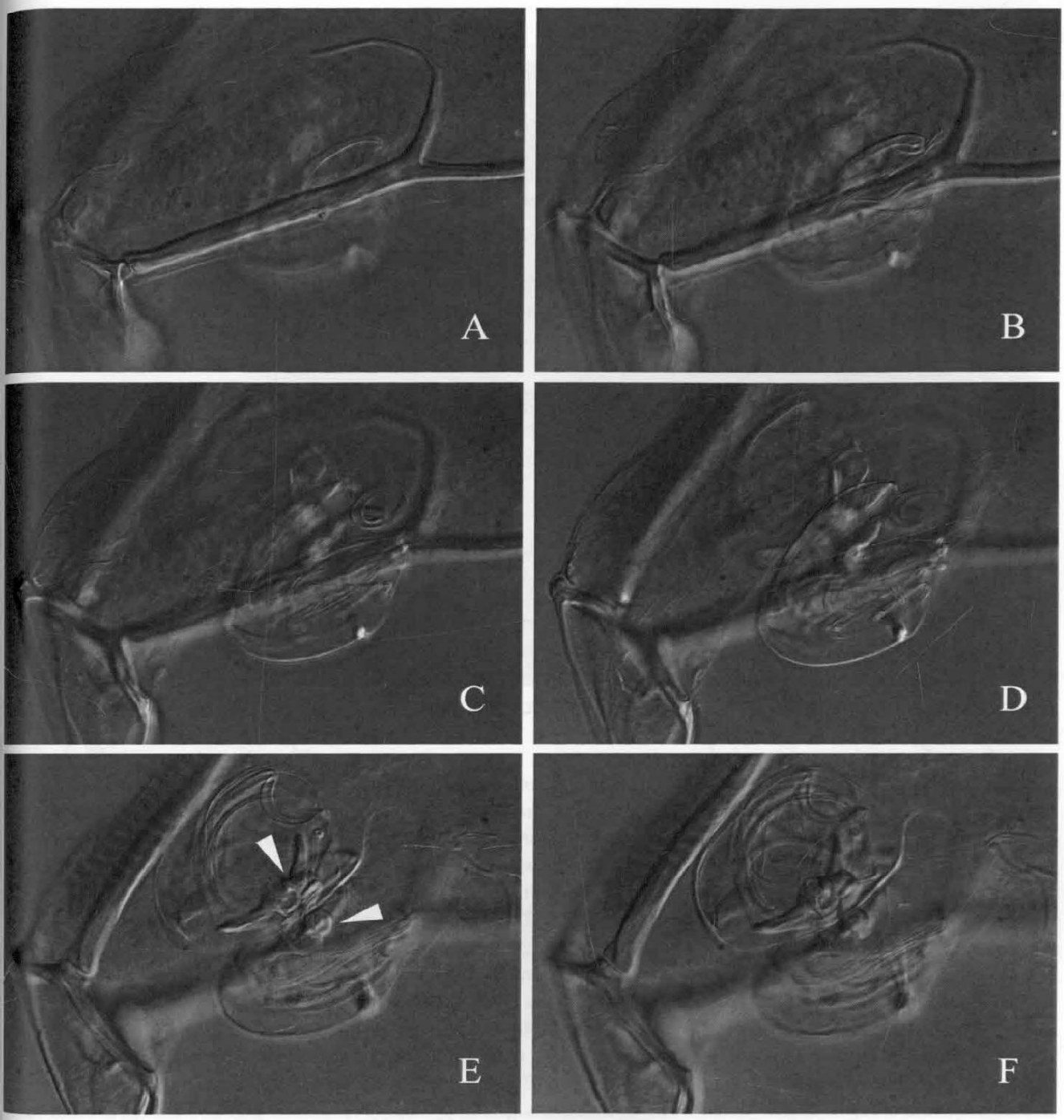


FIGURE 7. *Mangalocypria ryukyuensis* **sp. nov.** A–F, allotype, female, ICHUM 4898. Serial optical microscopic images of female genital organs. Arrowhead shows vaginal opening rimmed with sclerotized ring.

The *Mangalocypria* population on Ishigaki Island was morphologically indistinguishable from the *Mangalocypria ryukyuensis* population on Okinawa Island, but *COI* sequences (658 bp) between the two showed a K2P genetic distance of 4.7%. While this seems to exceed typical levels of intraspecific divergence (see Introduction), we sequenced *COI* for only one individual from each population, and the range of variation and haplotype distributions within and between the populations remain unknown. For this reason, we have included only specimens from the Okinawa population in the type series and regard the identity of the Ishigaki population with *Mangalocypria ryukyuensis* as requiring confirmation.

Genus *Paracypria* Sars, 1910

Paracypria longiseta sp. nov.
(Figures 8–12)

Material examined. Holotype: ICHUM 4908 (male), 26°33′36″N, 128°2′37″E, Oura River, Okinawa Island, Japan, 26 February 2011, soft parts mounted on 18 slides, carapace mounted on a microfossil slide. Allotype: ICHUM 4909 (female), collection data same as for holotype, soft parts and carapace mounted on two glass slides. Paratypes: ICHUM 4910 (female), collection data same as for holotype, soft parts mounted on a glass slide, carapace mounted on a microfossil slide; ICHUM 4911 (male) and 4912 (female), same locality as holotype, 22 November 2013, soft parts and carapace mounted on glass slides, carapace mounted on a microfossil slide; ICHUM 4914 (female), collection data same as for ICHUM 4911, mounted on stubs for SEM observation; ICHUM 4913, collection data same as for holotype, exoskeleton and carapace after DNA extraction mounted on a glass slide.

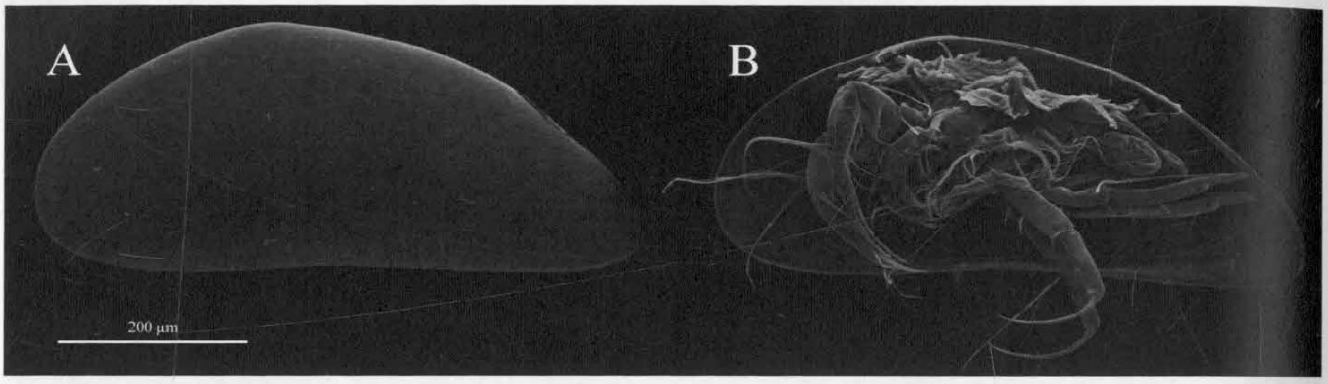


FIGURE 8. *Paracypria longiseta* **sp. nov.** Paratype, female, ICHUM 4914, SEM images. (A) Lateral view of LV. (B) Lateral view with LV removed.

Etymology. The specific name is a noun in apposition, derived from the combination of the Latin *longi* (long) and *seta*, referring to the long, filament-like rays on the male L5 palp.

Diagnosis. Carapace with maximum height anterior of mid-length, ventral margin straight, anterior margin broadly rounded, posterior margin sharply rounded. A1 with Rome organ and Wouters organ. Final segment of Md palp with two long claws. Male L5 clasping process symmetrical, with three long mid-ventral rays and one shorter apical filament-like peg protruding from palp. Female L5 with slender palp and very short *h1–3* setae. Zenker organ with 5 internal rosettes. Hp small, with protruding, rounded distal lobe. Uropodal ramus with short *Sa*, fine and very short *Sp1–2* setae, and claws *Ga* and *Gp* with denticles distally.

COI barcoding sequence. AB920554 (658 bp) from ICHUM 4913 (paratype).

Description of male. Carapace (Figures 8A; 9A, B) 0.68–0.70 mm long, 0.29–0.30 mm high (n = 3); 0.69 mm long, 0.30 mm high in holotype; elongate in lateral view, maximum height anterior of mid-length, broadly rounded at anterior end, posterior margin sharply rounded, slightly concave ventrally; spindle-shaped in dorsal view, with maximum width anterior of mid-length. Valves transparent, with brownish epidermal pigment except around fused median eyes; fine setae present on smooth surface.

A1 (Figure 9C) seven-segmented. First two podomeres fused, with two dorsal setae and two long apico-ventral plumose setae, and Wouters organ. Third podomere wider than long, with apico-dorsal seta and Rome organ. Fourth podomere rectangular, with long apico-dorsal seta. Fifth and sixth podomeres both rectangular, each with two long apico-dorsal setae. Seventh podomere with four long apical setae. Eighth podomere elongate, slender, with two long setae, one shorter seta, and aesthetasc *ya*.

A2 (Figure 9D) five-segmented. First podomere (*Pr*) with one long apico-ventral and one antero-proximal setae. Second podomere (*EI*) with one normal and one finer apico-ventral setae, aesthetasc *Y*, and *Exo* which consists of one long and two short setae; 4 + 1 natatory setae extending to tip of A2 terminal claw. Third podomere (*EII*) with two mid-apical male bristles (*t2* and *t3*), one long apico-ventral (*t1*) and one apico-dorsal (*t4*) setae.

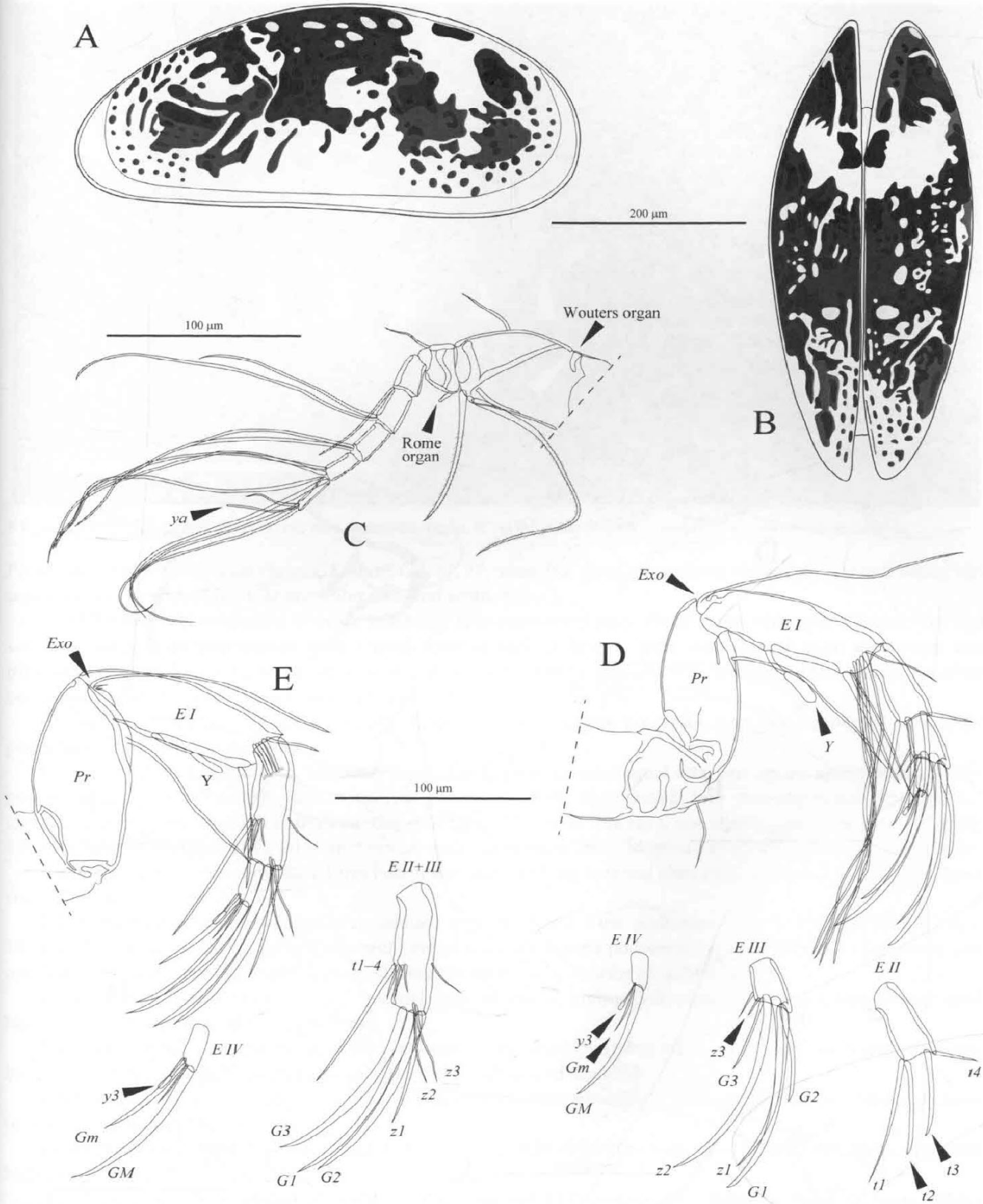


FIGURE 9. *Paracypria longiseta* **sp. nov.** A–D, holotype, male, ICHUM 4908; E, allotype, female, ICHUM 4909. (A) Lateral view of RV. (B) Dorsal view of valves. (C) A1. (D) Male A2 (inset, details of third to fifth podomeres). (E) Female A2 (inset, details of terminal two podomeres).

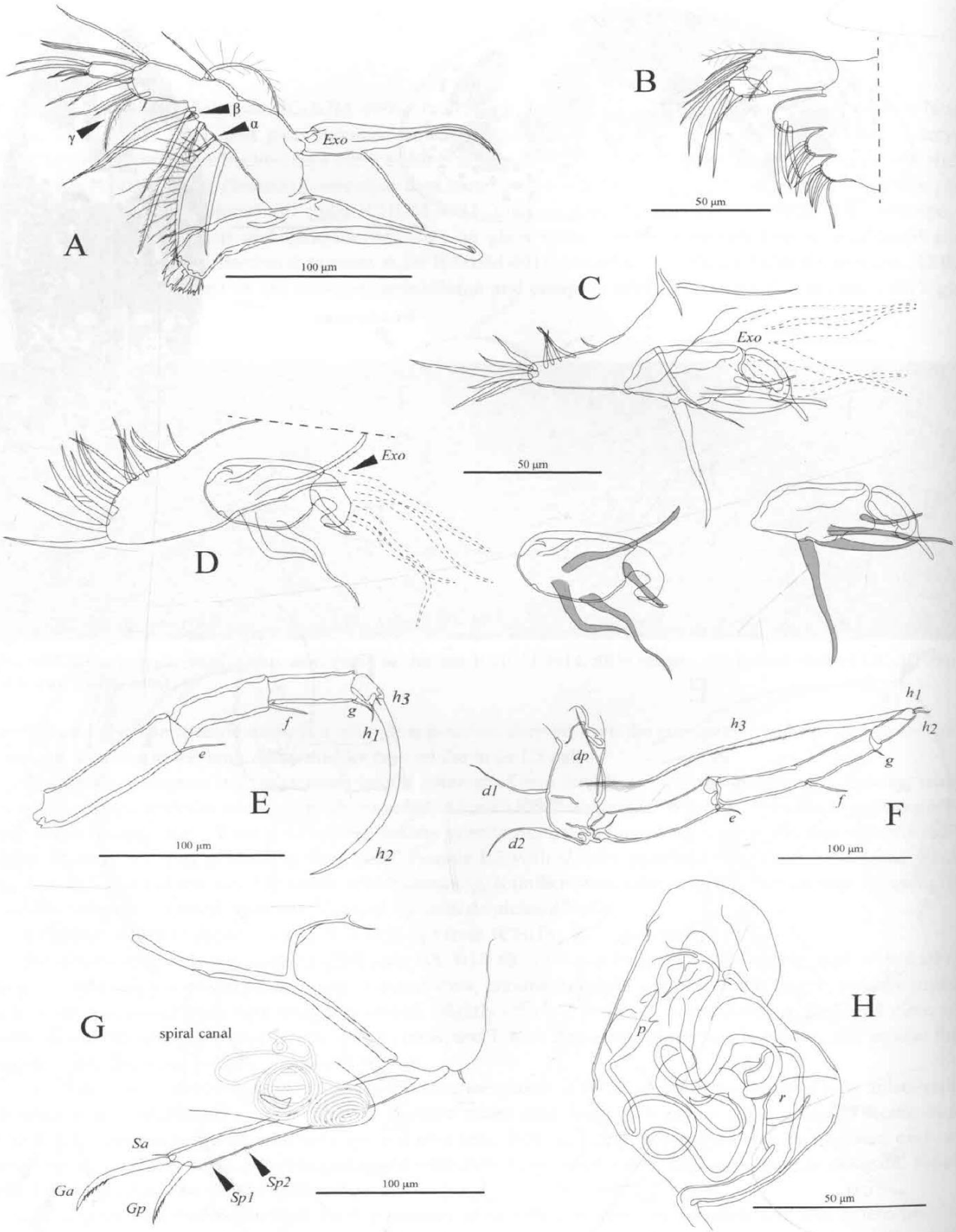


FIGURE 10. *Paracypria longiseta* **sp. nov.** A–F and H, holotype, male, ICHUM 4908; G, allotype, female, ICHUM 4909. (A) Md. (B) Mx. (C, D) Left and right male L5, respectively (inset, details of male clasping processes). (E) L6 (first podomere not illustrated). (F) L7. (G) Uropod and genital lobe with spiral canal. (H) Hp.

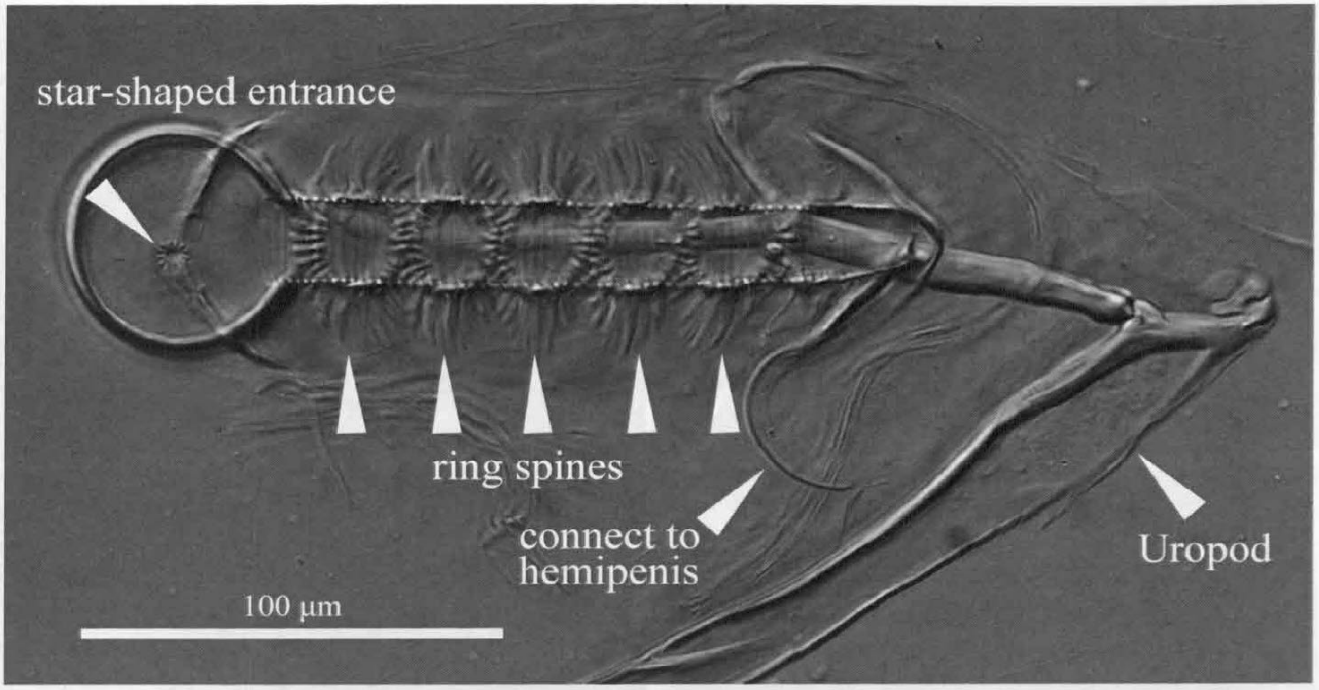


FIGURE 11. *Paracypria longiseta* **sp. nov.** holotype, male, ICHUM 4908. Nomarsky optical image of Zenker organ.

Fourth podomere (*EIII*) with claws *G1*, short *G2*, *z1*, *z2*, setae *G3*, short *z3*, and one short apico-ventral seta. Fifth podomere (*EIV*) with claws *GM* and short *Gm*, and aesthetasc *y3*.

Md (Figure 10A) consisting of coxal plate and four-segmented palp. Coxal plate with antero-lateral seta and six stout teeth, latter interspersed with several setae of various lengths. Palp with smooth short alpha seta, and plumed very short beta seta, and plumed long gamma setae. First podomere with exopodal plate (*Exo*). Terminal podomere of palp with two long claws and three setae.

Mx (Figure 10B) with elongate vibratory plate, three masticatory processes, and two-segmented palp. First podomere with two plumose setae.

L5 (Figure 10C, D) with palp, vibratory plate (*Exo*), one antero-proximal seta, one antero-apical seta, and one postero-apical seta. Vibratory plate with four filaments. Palp transformed into two-segmented symmetrical clasping process; first segment with three long mid-ventral filament-like rays, one shorter apical filament-like peg. Terminal segment (Figure 10C, D) stout, curved, with one terminal seta. Masticatory process with numerous setae.

L6 (Figure 10E) five-segmented. Fifth podomere with one long terminal claw (*h2*) one short, and one indistinct short, fine setae (*h1*, *h3*).

L7 (Figure 10F) four-segmented. Penultimate segment fused. First podomere with three setae (*d1*, *d2*, *dp*). Third podomere with one apical (*g*), one mid-ventral setae (*f*). Fourth podomere with two short (*h1*, *h2*) setae and one long, terminally curved seta (*h3*) plumed from the tip to three-fourths of its total length.

Zenker organ (Figure 11) with 5 + 2 internal rings of spines. Entrance to organ formed as a small star-shaped hole in a spherically shaped proximal end.

Hp (Figure 10H) with large, round terminal outer lobe. Prelabyrinthal inner spermiduct (*r*) forms relatively large, thin-walled chamber. Bursa copulatrix (*p*) distally stout and rounded.

Uropod with one anterior seta (*Sa*) and two reduced posterior setae (*Sp1*, *Sp2*). Tips of the two terminal claws (*Ga*, *Gp*) with tiny denticles.

Description of female. Carapace 0.71–0.76 mm long, 0.31–0.33 mm high (n = 3); 0.75 mm long, 0.33 mm high in allotype.

A2 (Figure 9E) four-segmented. First (*Pr*) and second (*EI*) podomeres similar to those of male. Third podomere (*EII* + *EIII*) with claws *G1 G2*, *G3*, and short *z1*, setae *z2*, *z3*, one mid-dorsal seta, and one long, three short mid-ventral setae. Fourth podomere (*EIV*) with *GM*, shorter *Gm*, one apical seta, and aesthetasc *y3*.

Palp of L5 simple, non-segmented, with three apical setae.

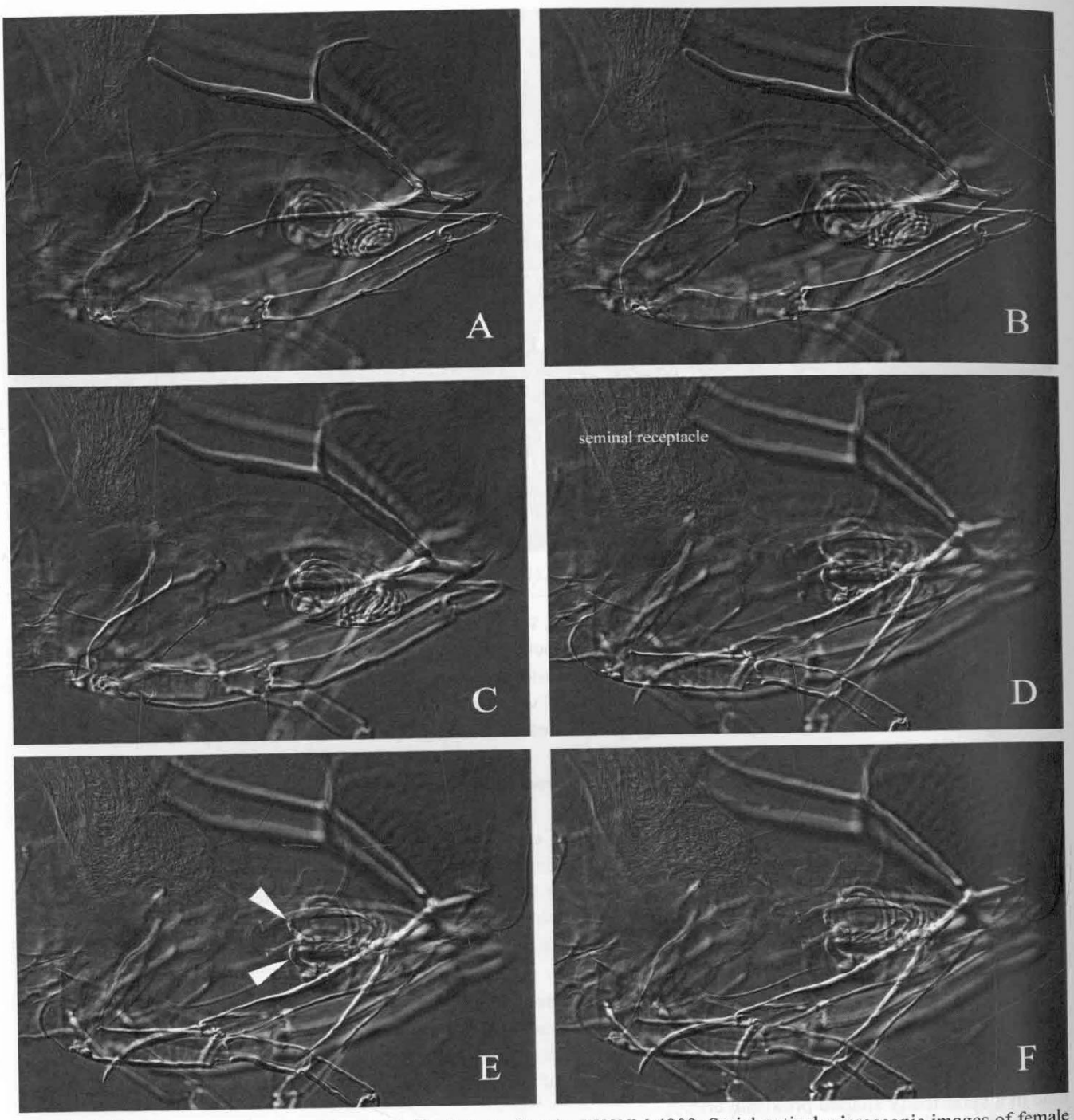


FIGURE 12. *Paracypria longiseta* **sp. nov.** A–F, allotype, female, ICHUM 4909. Serial optical microscopic images of female genital organs. Arrowhead shows vaginal opening rimmed with sclerotized ring. Seminal receptacle is filled with sperms.

Genital lobe protrude posteriorly, with conspicuous, well-developed spiral canal (Figures 10G; 12A–C, Supplementary Data 2). Vaginal opening rimmed with sclerotized ring (Figures 10G; 12D–F). From the lateral view, this opening situated at inner, proximal part of the genital lobes.

In other characters, female similar to male.

Remarks. Although we place this species in the genus *Paracypria*, it is generally similar to *Pontoparta hartmanni* Keyser, 1975. Wouters (1998) noted that there were some ill-defined genera in the tribe Thalassocypridini, including the genus *Pontoparta*. In a statistical analysis of affinities among 40 species representing seven genera in this tribe, based on 49 morphological characters, Maddocks (2005) found that the

genus *Pontoparta* was inadequately diagnosed and needed redescription. The type species, *Po. rara* Várvra, 1901 had been described from one female specimen, and the holotype might be lost (Wouters 1998). A temporary solution to this problem has been to avoid using the genus *Pontoparta* and to transfer species other than *Po. rara to Paracypria* (K. Wouters pers. comm.).

Paracypria longiseta **sp. nov.** is of medium carapace size (around 0.7 mm long) among the species in *Paracypria* and *Pontoparta*. *Paracypria tenuis* Sars, 1905, *Pa. inopinata* (Klie, 1939), *Pa. uberis* Maddocks, 2005, and *Po. hartmanni* Keyser, 1975 are similar in body size. The former three species differ from *Pa. longiseta* in having a distally broad lobe on each Hp. Especially in *Pa. tenuis* and *Pa. uberis*, additional processes are situated at the outer edge of the distal lobe. The shapes of the carapace and appendages are similar between *Pa. longiseta* and *Po. hartmanni*. However, *Po. hartmanni*, reported from Florida Everglades, USA, and redescribed by Maddocks & Iliffe (1993), clearly differs in the shape of the outer lobe shape of Hp (triangular in *Po. hartmanni*; protruding farther and more rounded in *Pa. longiseta*), the male clasping process on L5 (with two relatively short rays in *Pa. hartmanni*; with three long, mid-ventral filament-like rays and one shorter apical filament-like peg in *Pa. longiseta*), and the posterior setae on the uropodal rami (typical for the genus in *Po. hartmanni*; reduced and difficult to find in *Pa. longiseta*).

Paracypria plumosa sp. nov.

(Figures 13–16)

Material examined. Holotype: ICHUM 4915 (male), 24°26′32″N, 124°8′21″E, Kabira Bay, Ishigaki Island, Okinawa, Japan, 28 March 2013, soft parts mounted on 10 slides, carapace mounted on a microfossil slide. Allotype: ICHUM 4920 (female), collection data same as for holotype, soft parts mounted on nine slides, carapace mounted on a microfossil slide. Paratypes (collection data same as for holotype): ICHUM 4916–4919 (males) and 4921–4923 (females), soft parts mounted on glass slides, carapaces mounted on microfossil slides; ICHUM 4926–4928 (females), mounted on stubs for SEM observation; ICHUM 4924–4925 (females), exoskeleton and carapace after DNA extraction mounted on a glass slide.

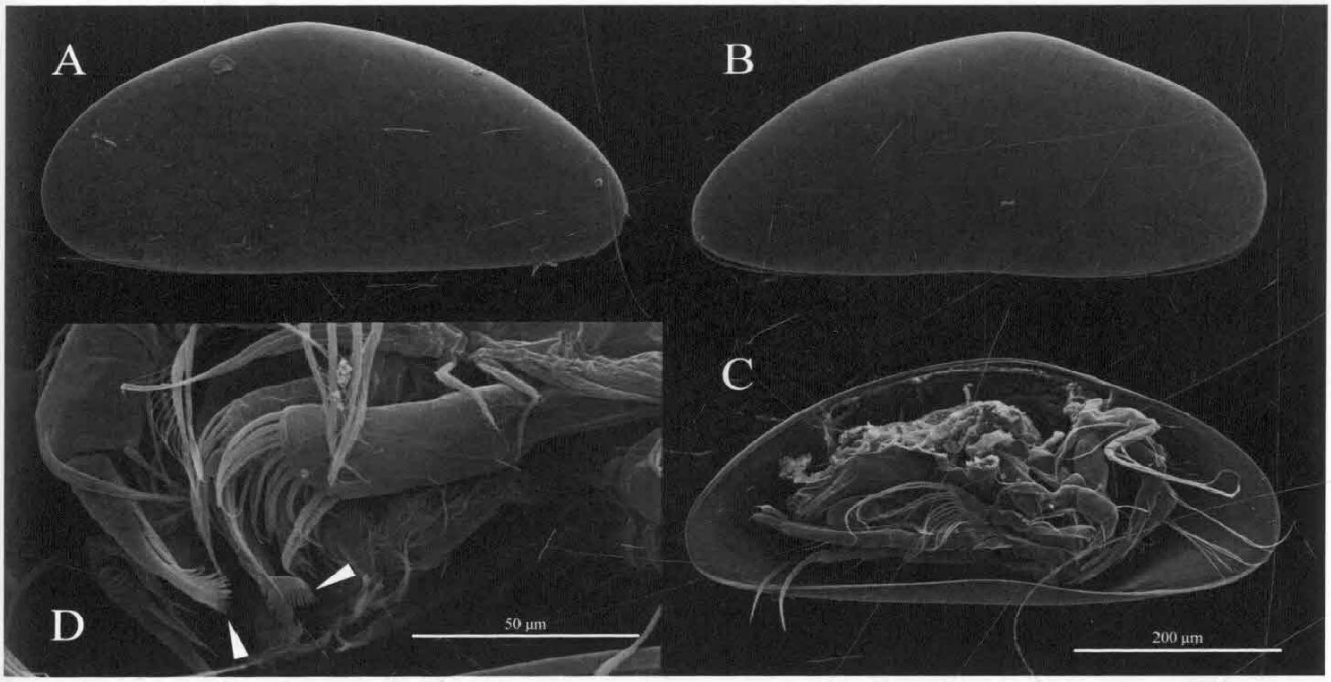


FIGURE 13. *Paracypria plumosa* **sp. nov.** Females, SEM images; A, B, paratype, ICHUM4926; C, paratype, ICHUM 4927; D, paratype, ICHUM 4928. (A, B) Lateral views of LV and RV, respectively. (C) Lateral view of female with RV removed. (D) Enlarged image of masticatory apparatuses; arrowheads indicate brush-like fused claw on Md palp, and comb-like structure on terminal tooth of coxal plate.

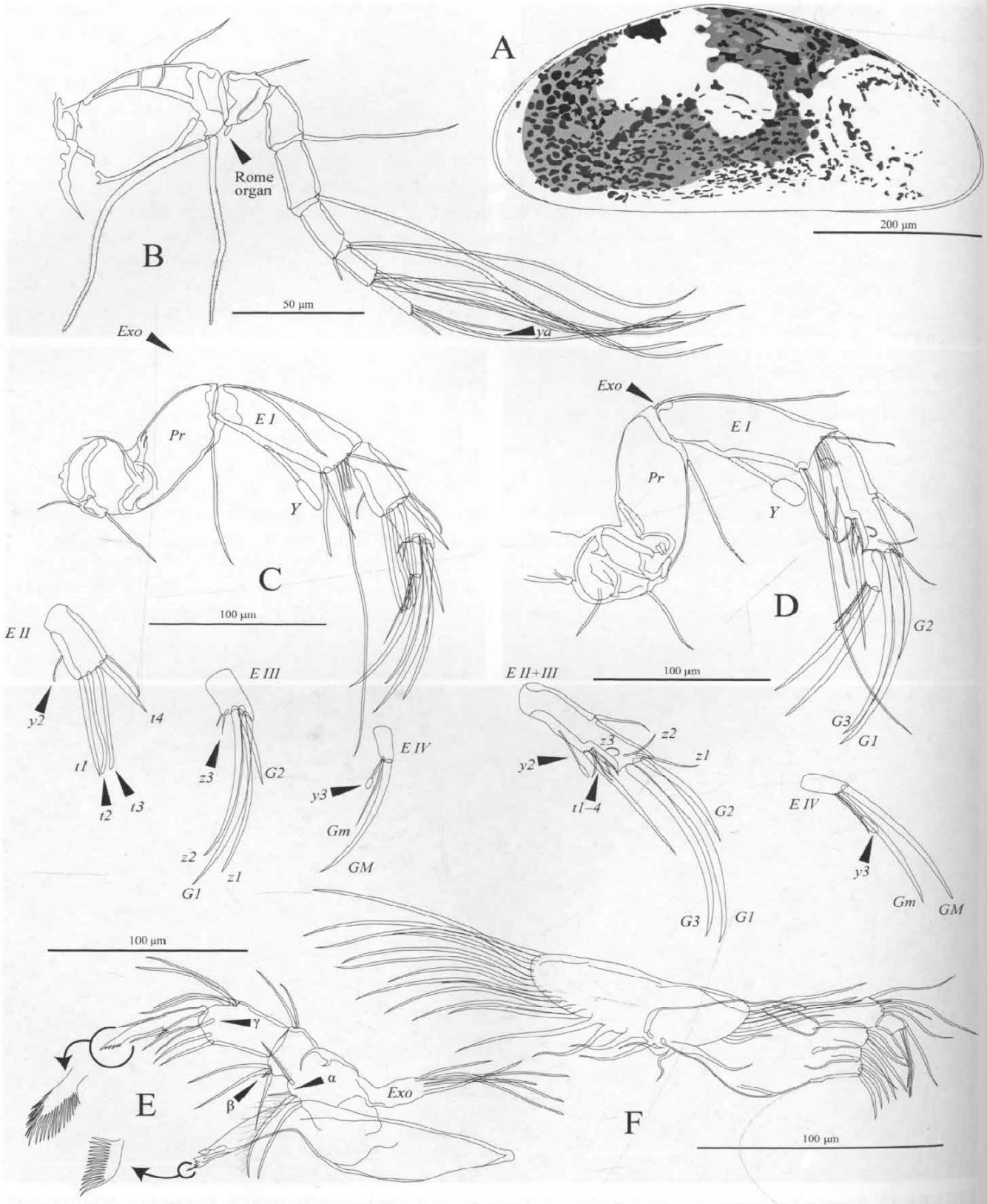


FIGURE 14. *Paracypria plumosa* **sp. nov.** A–C, E, F, holotype, male, ICHUM 4915; D, allotype, ICHUM 4920. (A) Lateral view of LV. (B) A1. (C) Male A2 (inset, details of third to fifth podomeres). (D) Female A2 (inset, details of terminal two podomeres). (E) Md (inset, enlarged details of comb-like process of Md coxa and fused brush-like terminal claw of Md palp). (F) Mx.

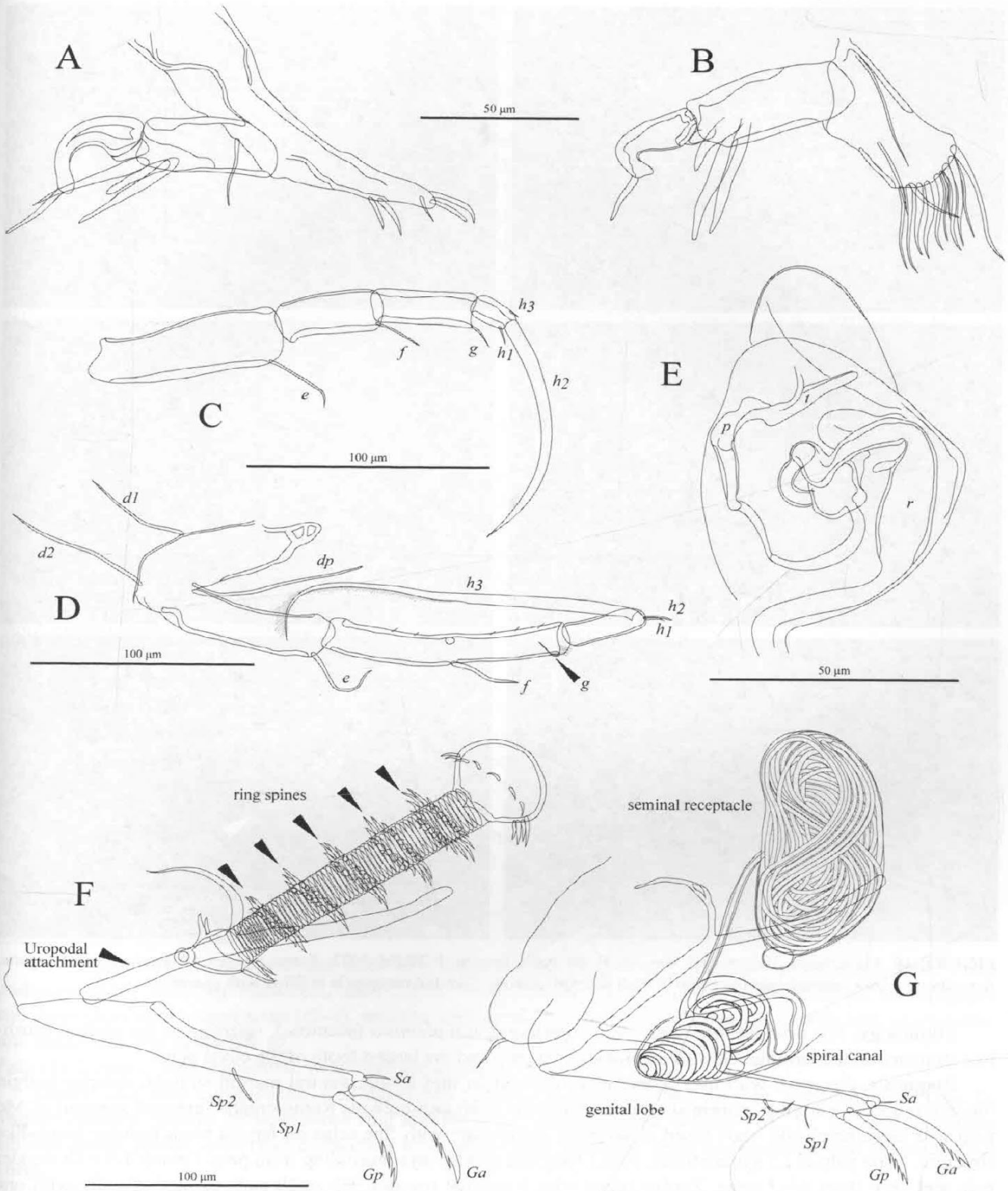


FIGURE 15. *Paracypria plumosa* **sp. nov.** A–F, holotype, male, ICHUM 4915; G, allotype, ICHUM 4922. (A, B) Left and right male L5, respectively. (C) L6 (first podomere not illustrated). (D) L7. (E) Hp. (F) Uropod and Zenker organ. (G) Uropod and genital lobe with spiral canal.

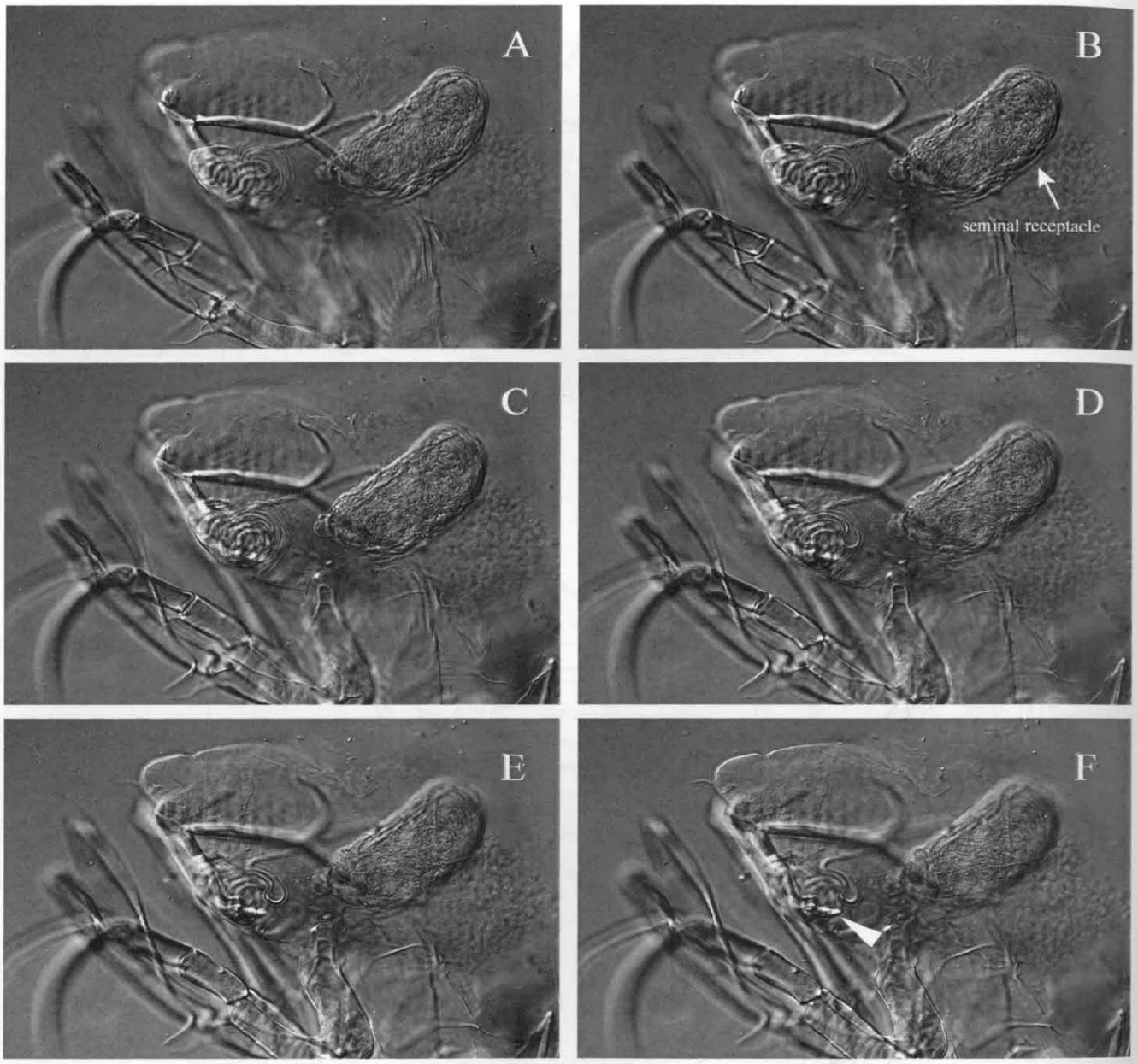


FIGURE 16. *Paracypria plumosa* **sp. nov.** A–F, allotype, female, ICHUM 4922. Serial images of female genital organs. Arrowhead shows vaginal opening rimmed with sclerotized ring. Seminal receptacle is filled with sperms.

Etymology. The new specific name is an adjective, Latin *plumosa* (plumose), referring to the plumed brush-like structure on the terminal claw of the mandibular palp and the largest tooth of the coxal plate.

Diagnosis. Carapace with maximum height anterior of mid-length, ventral margin straight, anterior margin broadly rounded, posterior margin sharply rounded. A1 with non-bulbous Rome organ. Terminal segment of Md palp with large brush-like claw fused to segment, coxal plate with fine setae on largest tooth forming comb-like structure. Male palp of L5 symmetrical, with 2 long one shorter rays protruding from palp. Female L5 with slender palp and very short *h1–3* setae. Zenker organ with 5 internal rosettes. Hp small and rounded, distally with one round lobe. Uropodal ramus with small *Sa*, fine *Sp1*, longer *Sp2* setae, and claws *Ga* and *Gp* with robust spines distally.

COI barcoding sequence. AB920555 (658 bp), from ICHUM 4924 (paratype).

Description of male. Carapace (Figures 13A, B; 14A) 0.57–0.60 mm long, 0.27–0.28 mm high (n = 4); 0.59 mm long, 0.27 mm high in holotype; elongate in lateral view, maximum height anterior of mid-length, broadly

rounded at anterior end, posterior margin sharply rounded, slightly concave ventrally; spindle-shaped in dorsal view, with maximum width anterior of mid-length. Valves transparent, with brownish epidermal pigment except around fused median eyes; fine setae present on smooth surface.

A1 (Figure 14B) seven-segmented. First two podomeres fused, with one dorsal and two long apico-ventral plumose setae. No Wouters organ present. Third podomere wider than long, with apico-dorsal seta, and Rome organ. Fourth podomere quadrate, with apico-dorsal seta. Fifth podomere quadrate, with one long apico-dorsal seta. Sixth podomere quadrate, with two long apico-dorsal and one apico-ventral setae. Seventh podomere with four long apical setae. Eighth podomere elongate, slender, with one long, one normal, and one short setae, and aesthetasc *ya*.

A2 (Figure 14C) five-segmented. First podomere (*Pr*) with one long, apico-ventral seta, one medium-long, antero-proximal seta, and one latero-proximal seta. Second podomere (*EI*) with two apico-ventral setae, aesthetasc *Y*, and *Exo,* which consists of one long and two short inconspicuous setae; 4 + 1 natatory setae extending to the tip of A2 terminal claw. Third podomere (*EII*) with two mid-apical male bristles (*t2* and *t3*), one apico-ventral seta (*t1*), one stout apico-dorsal (*t4*) and one normal setae, and aesthetasc *y2*. Fourth podomere (*EIII*) with claws *G1* short *G2*, *z1* and *z2*, setae short *z3*, and one short apico-ventral seta. Fifth podomere (*EIV*) with *GM* and short, fine *Gm*, one apical seta, and aesthetasc *y3*.

Md (Figure 14E) consisting of coxal plate and four-segmented palp. Coxal plate with antero-lateral plumose seta and five stout teeth, latter interspersed with several setae of various lengths. Largest teeth with comb-like process (Figures 13D; 14E). Palp with smooth alpha seta; smooth, long gamma setae; and plumed, very short beta seta. First podomere with exopodal plate (*Exo*). Terminal podomere of palp fused with terminal claw to form distally plumed claw (Figures 13D; 14E); with two apical setae and one shorter apical seta.

Mx (Figure 14F) with elongate vibratory plate, three masticatory processes, and two-segmented palp.

L5 (Figure 15A, B) with palp, vibratory plate (*Exo*; not illustrated), one antero-proximal seta, one antero-apical seta, and one postero-apical seta. Vibratory plate with four filaments. Palp transformed into two-segmented clasping process; first segment with two long and one shorter apico-ventral filament-like rays; terminal segment stout, curved, with one terminal seta. Masticatory process with numerous setae.

L6 (Figure 15C) five-segmented. Fifth podomere with one long terminal claw (*h2*) and one short, one indistinct short fine setae (*h1*, *h3*).

L7 (Figure 15D) four-segmented. Penultimate segment fused. First podomere with three setae (*d1*, *d2*, *dp*). Third podomere with one short, curled, reflexed apical (*g*), one mid-ventral setae (*f*). Fourth podomere with two short setae (*h1*, *h2*) and one long, curved seta (*h3*) plumed from the tip to half its total length.

Zenker organ (Figure 15F) with 5 + 2 internal rings of spines. Proximally with spherical lobe.

Hp (Figure 15E) with one distally rounded ventral lobe. Prelabyrinthal inner spermiduct (*r*) wide, forming thin-walled chamber. Bursa copulatrix (*p*) simple, with chitinous process (*t*).

Uropod (Figure 15F) with one anterior seta (*Sa*) and one fine (*Sp1*), one normal (*Sp2*) posterior setae. Tips of the two terminal claws (*Ga*, *Gp*) with robust spines.

Description of female. Carapace (Figure 13A–C) 0.57–0.60 mm long, 0.26–0.28 mm high (n = 3); 0.59 mm long, 0.28 mm high in allotype.

A2 (figure 14D) four-segmented. First (*Pr*) and second (*EI*) podomeres similar to those of male. Third podomere (*EII* + *EIII*) with claws *G1*, short *G2*, *G3*, setae *z1*, *z2*, *z3*, one mid-ventral aesthetasc *y2*, one long (*t1*) and three short mid-ventral setae (*t2–4*), and one mid-dorsal seta. Fourth podomere (*EIV*) with *GM*, shorter *Gm*, one apical seta, and aesthetasc *y3*.

Palp of L5 simple, non-segmented, with three apical setae.

Genital lobe with conspicuous, well-developed spiral canal (Figures 15G; 16A–D, Supplementary Data 3). Vaginal opening rimmed by sclerotized ring and forming bulbous process (Figure 16E, F). Seminal receptacle situated above genital lobe; duct connecting with spiral canal protrudes from mid-posterior part (Figures 15G; 16B).

In other characters, female similar to male.

Remarks. Among congeners, *Paracypria plumosa* **sp. nov.** is relatively small in body size, though *Paracypria minuta* McKenzie, 1968 and *Pa. adnata* Smith & Kamiya, 2006 are similar in body size. *Paracypria minuta* differs from *Pa. plumosa* in having a distally pointed lobe on Hp. The new species is generally similar to *Pa. adnata* from Yakushima Island, Japan, in the size of the carapace, in the shape of Hp, in having a stout claw fused to the terminal

podomere of the Md palp, and in lacking a Wouters organ (Smith & Matzke-Karasz 2008). However, these two species differ in the mid-ventral seta on the penultimate segment in female A2 (long and stout in *Pa. plumosa*; fine and short in *Pa. adnata*); the stout claw on the terminal segment of the Md palp (forming a brush-like tip in *Pa. plumosa*; typical for the genus in *Pa. adnata*); the largest terminal tooth on the coxal plate (with a comb-like process in *Pa. plumosa*; with denticles along the inner edge in *Pa. adnata*); the number of filament-like setae on the male clasping process (three in *Pa. plumosa*; two in *Pa. adnata*); and *Sp1* on the uropodal rami (long in *Pa. plumosa*; short in *Pa. adnata*).

Discussion

Our study increased the number of thalassocypridine ostracods known in Japan from three to six species in three genera. *Mangalocypria ryukyuensis* **sp. nov.** represents the first record for this genus for Japan.

Our study also underscored taxonomic problems both in the genus *Paracypria* and the tribe Thalassocypridini, which may a consequence of convergent evolution (small body size and compressed carapace) through adaptation to nearshore/brackish environments. The tribe has been relatively poorly studied, and relationships among genera are unclear (Wouters 1998; Maddocks 2005). The K2P distance for *COI* between *Pa. longiseta* **sp. nov.** and *Pa. plumosa* **sp. nov.** was 24.9%, roughly as high as that between these species and *M. ryukyuensis* (21.5–24.6%), which may suggest that *Paracypria* is not a natural group but instead comprises several genera. The presence of the Wouters organ might be a useful taxonomic character in *Paracypria* and *Pontoparta*; at present, only *Pa. adnata* and *Pa. plumosa* are known to lack this organ.

If the populations of *Mangalocypria ryukyuensis* **sp. nov.** on Okinawa and Ishigaki Islands prove to be conspecific, or even sister taxa, this raises the question how these ostracods disperse to islands. Thalassocypridine ostracods in general inhabit marine and brackish-water environments and survive salinity extremes ranging from freshwater to seawater (Maddocks 2005). While they are good swimmers, they also attach themselves to vegetative substrates, and furthermore attach resting eggs to their molted carapaces, which they then attach to vegetative substrates (pers. observation). The wide salinity tolerance and attachment to vegetative substrate may facilitate dispersal to distant islands by rafting (Thiel & Gutow 2005).

Indeed, there are indications of dispersal to islands for some thalassocypridine species. *Pontoparta hartmanni* has been reported from both Florida (Keyser 1975) and Jamaica, 1300 km away (Maddocks & Iliffe 1993), although there is some question about the identity of the Jamaica population (Smith & Kamiya 2006). Around Japan, *Dolerocypria mukaishimensis* has been reported from the Seto Inland Sea, eastern Hokkaido, Chiba Prefecture, and Korea (Okubo 1980; Hiruta & Smith 2001; Nakao & Tsukagoshi 2002; Karanovic & Lee 2012), although Karanovic & Lee (2012) noted that there are some minor morphological differences between the populations in Japan and Korea. The large genetic distance we detected between morphologically indistinguishable *Mangalocypria* populations on Okinawa and Ishigaki Islands, and morphological differences among populations separated by stretches of open water in other cases, raise the possibility that estuarine ostracods may disperse infrequently to islands, probably by rafting, and undergo divergence and eventually allopatric speciation, leading to island endemism. To elucidate the extent to which this is the case will require phylogeographic studies involving larger samples from over broader ranges than in our study.

Acknowledgments

The authers thank two reviewers, Dr Rosalie F. Maddocks and Dr Karel Wouters, for insightful comments and suggestions; Dr Matthew H. Dick (Hokkaido University) and Dr Hiroshi Kajihara (Hokkaido University) for critical comments on the manuscript; and colleagues for advice and discussion.

References

Bode, S.N.S., Adolfsson, S., Lamatsch, D.K., Martens, M.J.F., Schmit, O., Vandekerkhove, J., Mezquita, F., Namiotko, T., Rossetti, G., Schön, I., Butlin, R.K. & Martens, K. (2010) Exceptional cryptic diversity and multiple origins of

parthenogenesis in a freshwater ostracod. *Molecular phylogenetics and evolution*, 54, 542–552. http://dx.doi.org/10.1016/j.ympev.2009.08.022

Boom, R.C.J., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M. & van der Noordaa, J.P.M.E. (1990) Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology*, 28, 495–503. http://dx.doi.org/10.1093/nar/18.8.2195

Brandão, S.N., Sauer, J. & Schön, I. (2010) Circumantarctic distribution in southern ocean benthos? A genetic test using the genus *Macroscapha* (Crustacea, Ostracoda) as a model. *Molecular Phylogenetics and Evolution*, 55, 1055–1069. http://dx.doi.org/10.1016/j.ympev.2010.01.014

Broodbakker, N.W. & Danielopol, D.L. (1982) The chaetotaxy of Cypridacea (Crustacea, Ostracoda) limbs: proposals for a descriptive model. *Bijdragen tot de Dierkunde*, 52, 103–120.

Bucklin, A., Steinke, D. & Blanco-Bercial, L. (2011) DNA barcoding of marine Metazoa. *Annual Review of Marine Science*, 3, 471–508. http://dx.doi.org/10.1146/annurev-marine-120308-080950

Coissac, E., Hollingsworth, P.M., Lavergne, S. & Taberlet, P. (2016) From barcodes to genomes: extending the concept of DNA barcoding. *Molecular Ecology*, 25, 1423–1428. http://dx.doi.org/10.1111/mec.13549

Danielopol, D.L. (1969) Recherches sur la morphologie de l'organe copulateur mâle chez quelques ostracodes du genre *Candona* Baird (Fam. Cyprididae Baird). *In*: Neale, J.W. (Ed.), *The Taxonomy, Morphology and Ecology of Recent Ostracoda*. Oliver and Boyd, Edinburgh, pp. 136–153.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.

Hadley, A. (2010) CombineZP Image Stacking Software. Release date: 6 June 2010. Available from: http://www.hadleyweb.pwp.blueyonder.co.uk (accessed 19 February 2016)

Harding, J.P. (1962) *Mungava munda* and four other new species of ostracod crustaceans from fish stomachs. *Natural History of Rennell Island, British Solomon Islands*, 4, 51–62.

Hartmann, G. (1974) Zur Kenntnis des Eulitorals der afrikanischen Westküste zwischen Angola und Kap der Guten Hoffnung und der afrikanischen Ostküste von Südafrika und Moçambique unter besonderer Berücksichtigung der Polychaeten und Ostracoden. Teil III. Die Ostracoden des Untersuchungsgebietes. *Mitteilungen aus dem hamburgischen zoologischen Museum und Institut*, Supplement 69, 229–520.

Hartmann, G. & Puri, H.S. (1974) Summary of neontological and paleontological classification of Ostracoda. *Mitteilungen aus dem hamburgischen zoologischen Museum und Institut*, 70, 7–73.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & deWaard, J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the royal society B*, 270, 313–321. http://dx.doi.org/10.1098/rspb.2002.2218

Hebert, D.N. & Gregory, R. (2005) The promise of DNA barcoding for taxonomy. *Systematic Biology*, 54, 852–859. http://dx.doi.org/10.1080/10635150500354886

Hiruta, S. & Smith, R.J. (2001) Living freshwater and shallow marine Ostracoda in eastern Hokkaido, Japan. *In*: *Guidebook of Excursion Exc. E: Eastern Hokkaido*. The Organizing Committee of the 14th International Symposium on Ostracoda, Shizuoka, pp. 107–125.

Hu, C.-H. & Tao, H.-J. (2008) Studies on the ostracod fauna of Taiwan and its adjacent seas. *National Taiwan Museum Special Publication Series*, 13, 1–910.

Johnson, K.P., Yoshizawa, K. & Smith, V.S. (2004) Multiple origins of parasitism in lice. *Proceedings of the Royal Society of London B*, 271, 1771–1776. http://dx.doi.org/10.1098%2Frspb.2004.2798

Karanovic, I. & Lee, W. (2012) A review of candonid ostracods (Crustacea: Ostracoda: Podocopida) from East Asia, with descriptions of five new species from South Korea. *Zootaxa*, 3368, 7–49.

Keyser, D. (1975) Ostracoden aus den Mangrovegebieten von Südwest-Florida (Crustacea: Ostracoda: Podocopa). *Abhandlungen und Verhandlungen des Naturwissenschaftlichen Vereins in Hamburg*, 18, 255–290.

Klie, W. (1939) Ostracoden aus den marinen Salinen von Bonaire, Curaçao und Aruba. *Capita Zoologica*, 8, 1–19.

Koenders, A., Martens, K., Halse, S. & Schön, I. (2012) Cryptic species of the *Eucypris virens* species complex (Ostracoda, Crustacea) from Europe have invaded western Australia. *Biological Invasions*, 14, 2187–2201. http://dx.doi.org/10.1007/s10530-012-0224-y

Maddocks, R.F. (2005) New thalassocypridine Ostracoda from anchialine caves of the Loyalty Islands, New Caledonia (Podocopida, Paracypridinae). *Micropaleontology*, 51, 169–216. http://dx.doi.org/10.2113/51.3.169

Maddocks, R.F. & Iliffe, T.M. (1993) Thalassocypridine Ostracoda from anchialine habitats of Jamaica. *Journal of Crustacean Research*, 13 (1), 142–164. http://dx.doi.org/10.2307/1549130

Martens, K. (1987) Homology and functional morphology of the sexual dimorphism in the antenna of *Sclerocypris* Sars, 1924 (Crustacea, Ostracoda, Megalocypridinae). *Bijdragen tot de Dierkunde*, 57, 183–190.

Matzen da Silva, J., Creer, S., Dos Santos, A., Costa, A.C., Cunha, M.R., Costa, F.O. & Carvalho, G.R. (2011) Systematic and evolutionary insights derived from mtDNA *COI* barcode diversity in the Decapoda (Crustacea: Malacostraca). *PLoS One*, 6 (5), e19449.
http://dx.doi.org/10.1371/journal.pone.0019449

McKenzie, K.G. (1968) A new species of *Paracypria* (Ostracoda: Cyprididae) from Victoria, Australia. *Zoologischer Anzeiger*, 180, 384–389.

Meisch, C. (1996) Contribution to the taxonomy of *Pseudocandona* and four related genera, with the description of *Schellencandona* nov. gen., a list of the Candoninae genera, and a key to the European genera of the subfamily (Crustacea, Ostracoda). *Bulletin de la Société des Naturalistes Luxembourgeois*, 97, 211–237.

Meisch, C. (2000) *Freshwater Ostracoda of Western and Central Europe. Süßwasserfauna von Mitteleuropa 8/3*. Spektrum Akademischer Verlag, Heidelberg, Berlin, 522 pp.

Meisch, C., Mary-Sasal, N., Colin, J.-P. & Wouters, K. (2007) Freshwater Ostracoda (Crustacea) collected from the islands of Futuna and Wallis, Pacific Ocean, with a checklist of the non-marine Ostracods of the Pacific Islands. Bulletin de la Société des naturalistes luxembourgeois, 108, 89–103.

Nakano, Y. & Tsukagoshi, A. (2002) Brackish-water Ostracoda (Crustacea) from the Obitsu River Estuary, central Japan. *Species Diversity*, 7 (1), 67–115.

Nation, J.L. (1983) A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. *Stain Technology*, 58, 347–351.
http://dx.doi.org/10.3109/10520298309066811

Okubo, I. (1980) Three new species of the family Candonidae (Ostracoda) from the Inland Sea of Japan. *Proceedings of the Japanese Society of Systematic Zoology*, 18, 18–27.

Ratnasingham, S. & Hebert, P.D.N. (2013) A DNA-based registry for all animal species: The Barcode Index Number (BIN) system. *PLoS ONE*, 8(8), e66213.
http://dx.doi.org/10.1371/journal.pone.0066213

Sars, G.O. (1905) Pacifische Plankton-Crustaceen (Ergebnisse einer Reise nach dem Pacific, Schauinsland 1896-1897). II. Brackwasser-Crustaceen von den Chatham-Inseln. *Zoologisches Jahrbuch, Abteilung für Systematik, Geographie und Biologie*, 21, 399–414.

Sars, G.O. (1910) Zoological results of the third Tanganyika Expedition, conducted by Dr. W.A. Cunnington, F.Z.S., 1904–1905. Reports on the Ostracoda. *Proceedings of the Zoological Society of London*, 65, 732–760.

Sars, G.O. (1923) An account of the Crustacea of Norway with short descriptions and figures of all the species. vol. 9. Ostracoda, *Bergen Museum, Bergen*, 3/4, 33–72.

Schön, I., Pinto, R.L., Halse, S., Smith, A.J., Martens, K. & Birky, Jr. C.W. (2012) Cryptic species in putative ancient asexual darwinulids (Crustacea, Ostracoda). *PLoS ONE*, 7(7), e39844.
http://dx.doi.org/10.1371/journal.pone.0039844

Sheue, C.-R., Liu, H.-Y. & Yong, J.-W. (2003) *Kandelia obovata* (Rhizophoraceae), a new mangrove species from Eastern Asia. *Taxon*, 52 (2), 287–294.
http://dx.doi.org/10.2307/3647398

Smith, R.J. & Kamiya, T. (2003) Three brackish-water ostracods (Crustacea) from Ushimado, Okayama Prefecture, Japan. *Species Diversity*, 8 (1), 79–91.

Smith, R.J. & Kamiya, T. (2006) Six new species of fresh and brackish water ostracods (Crustacea) from Yakushima, Southern Japan. *Hydrobiologia*, 559, 331–355.
http://dx.doi.org/10.1007/s10750-005-0946-2

Smith, R.J. & Matzke-Karasz, R. (2008) The organ on the first segment of the cypridoidean (Ostracoda, Crustacea) antennule: morphology and phylogenetic significance. *Senckenbergiana Lethaea*, 88 (1), 127–140.
http://dx.doi.org/10.1007/BF03043984

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.
http://dx.doi.org/10.1093/molbev/msr121

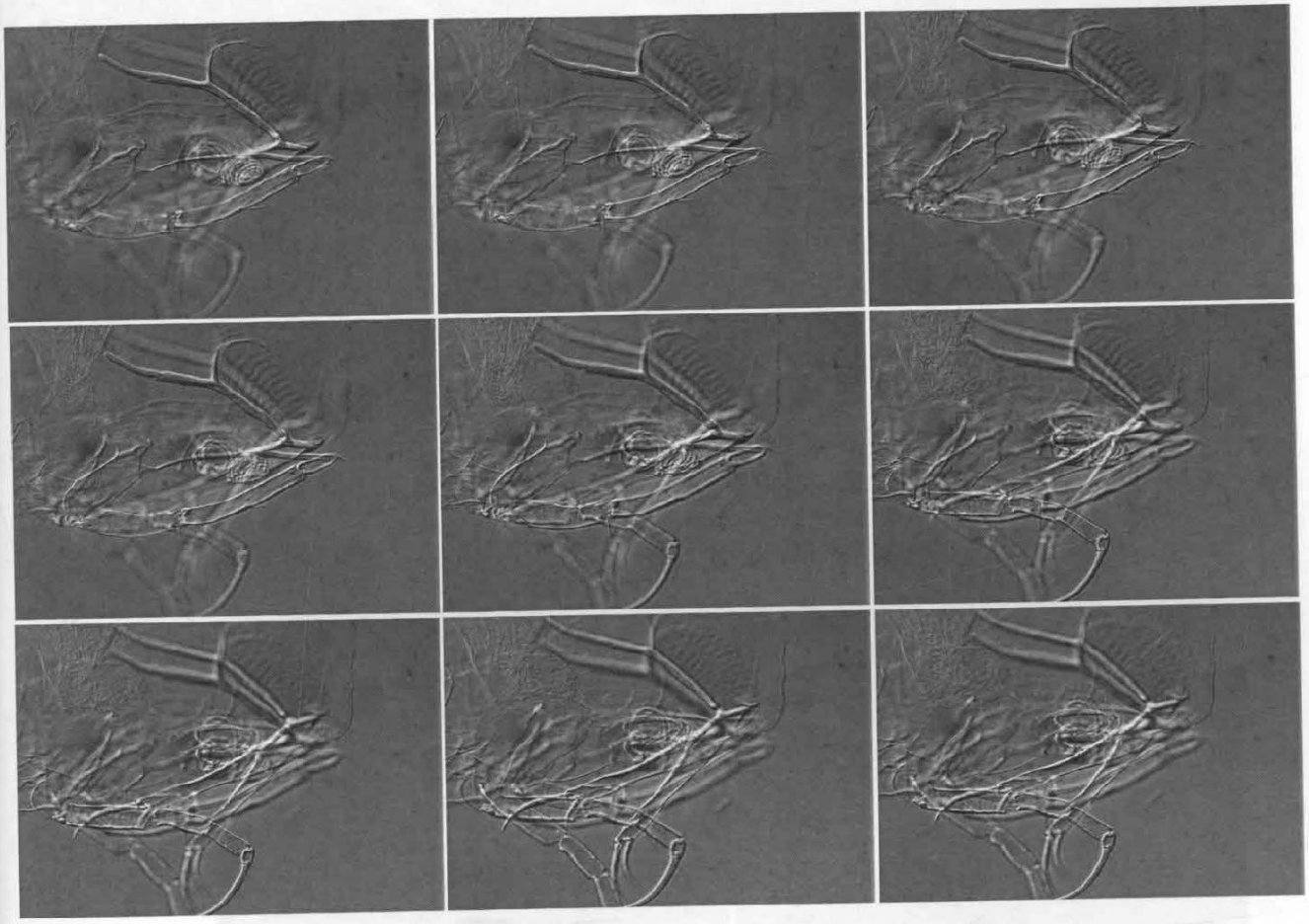
Thiel, M. & Gutow, L. (2005) The ecology of rafting in the marine environment. II. The rafting organisms and community. *Oceanography and Marine Biology: An Annual Review*, 43, 279–418.
http://dx.doi.org/10.1201/9781420037449.ch7

Vávra, W. (1901) Die Ostracoden vom Bismarck-Archipel. Archiv für Naturgeschichte, 61, 179–186.

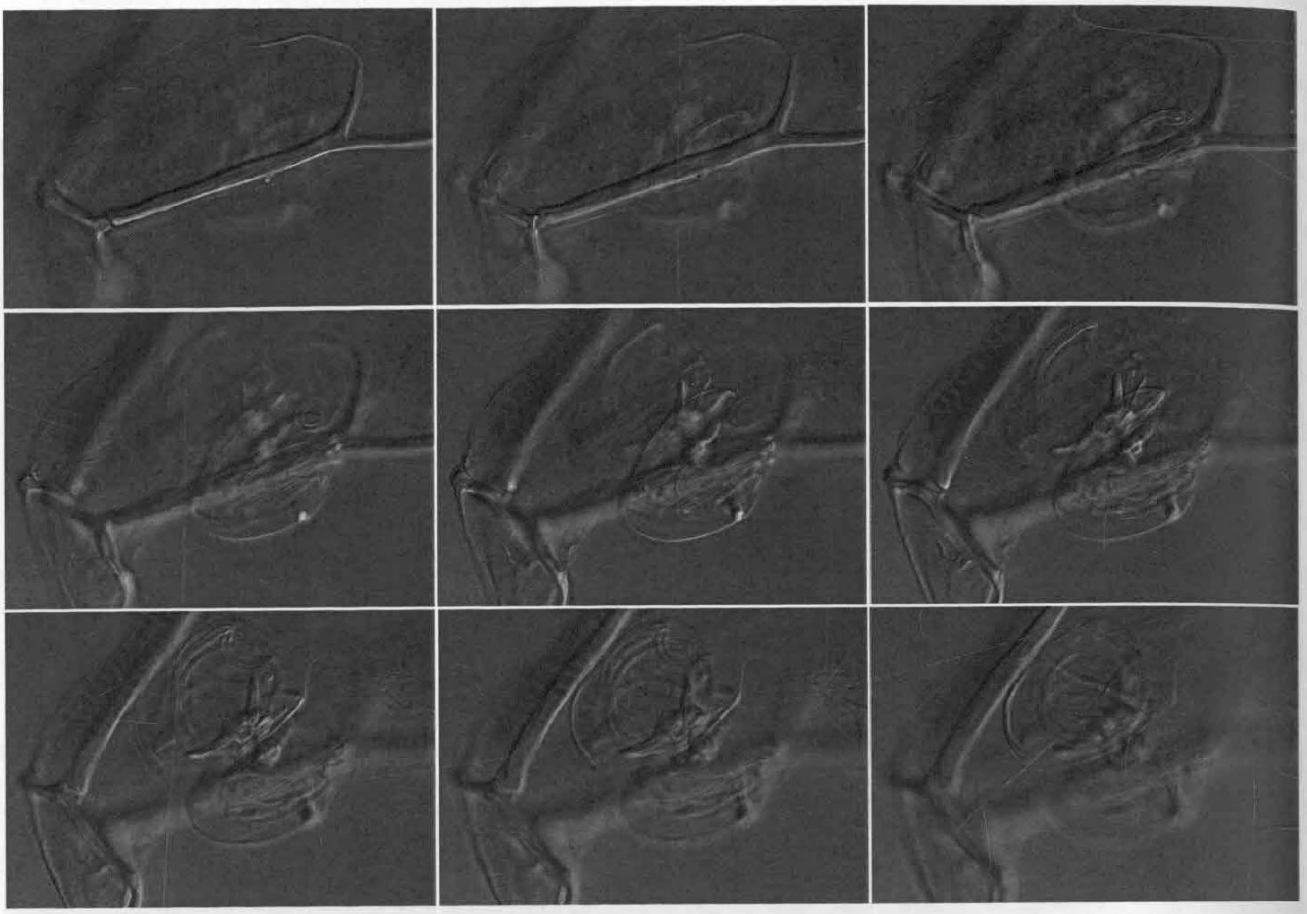
Wakushima, S., Kuraishi, S. & Sakurai, N. (1994) Soil salinity and pH in Japanese mangrove forests and growth of cultivated mangrove plants in different soil conditions. *Journal of Plant Research*, 107 (1), 39–46.
http://dx.doi.org/10.1007/BF02344528

Waugh, J. (2007) DNA barcoding in animal species: progress, potential and pitfalls. *BioEssays*, 29, 188–197.
http://dx.doi.org/10.1002/bies.20529

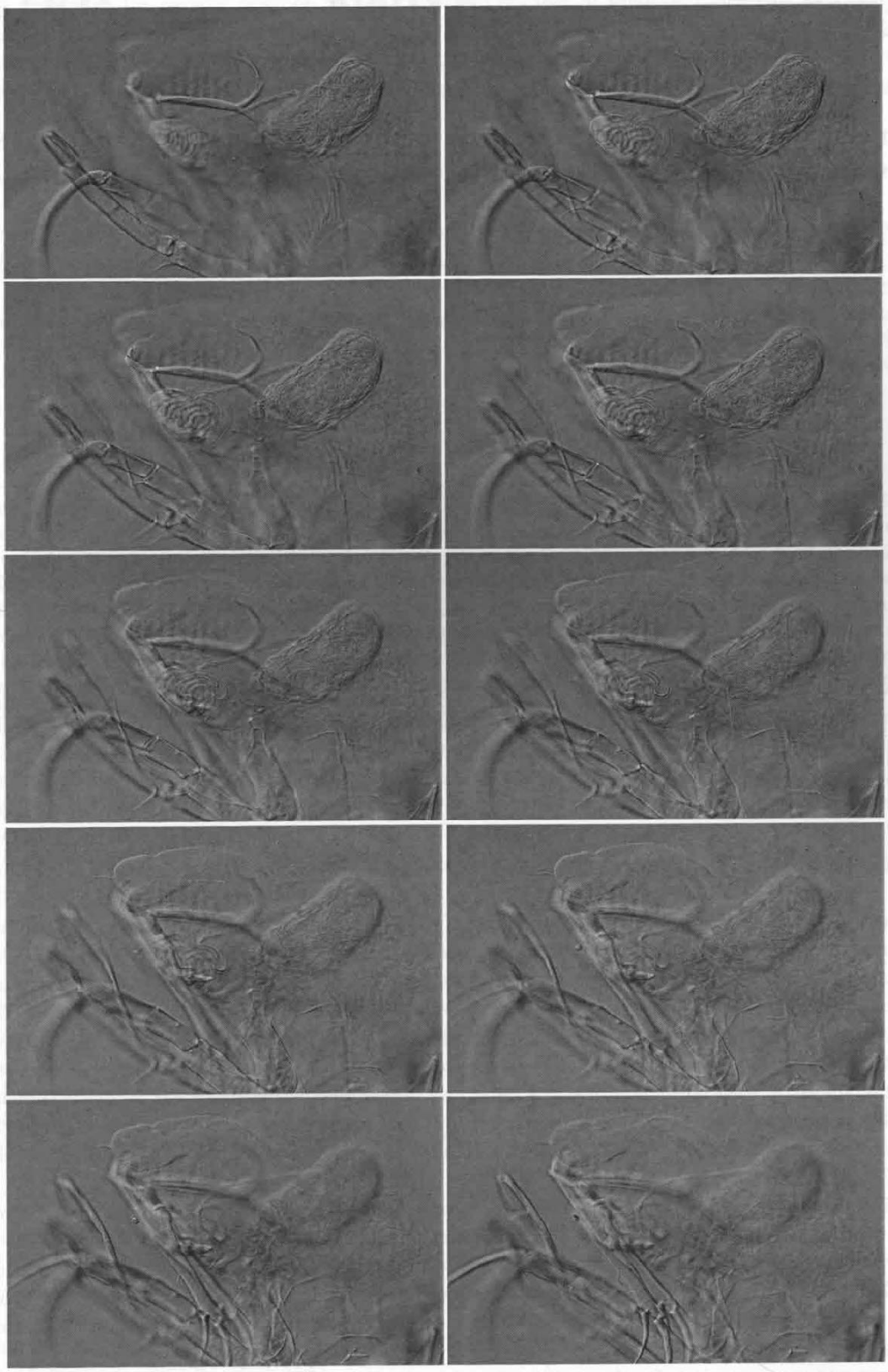
Wouters, K. (1998) A new thalassocypridine genus (Crustacea, Ostracoda) from brackish waters in the Indian and Pacific Oceans, with the description of a new species. *Bulletin de l'Institut royal des sciences naturelles de Belgique*, Biologie, 68, 111–122.



Supplemental Data 1. *Mangalocypria ryukyuensis* **sp. nov.** allotype, ICHUM 4898. Original, serial optical microscopic images of female genital organs.



Supplemental Data 2. *Paracypria longiseta* **sp. nov.** allotype, ICHUM 4909. Original, serial optical microscopic images of female genital organs.



Supplemental Data 3. *Paracypria plumosa* **sp. nov.** allotype, ICHUM 4922. Original, serial optical microscopic images of female genital organs.