

Primary Research Paper

***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**

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Abstract

Acartia ohtsukai sp. nov. (Calanoida, Acartiidae), which has so far been identified as *A. pacifica*, is described from an estuary of Ariake Bay, Japan, with a redescription of *A. pacifica* s. str. from the Seto Inland Sea. The new species is distinguishable from *A. pacifica* by characters of the caudal rami and the antennule in female and those of the urosomal somites and the fifth leg in male. The most closely affinity of *A. ohtsukai* is seen with *A. mertoni* new rank, which was formerly a variety of *A. pacifica* and differs from *A. ohtsukai* by having dorsal spinule ornamentation on male urosome. DNA sequences of two mitochondrial genes differed between individuals of the new species from Ariake Bay and *A. pacifica* from the Seto Inland Sea and supported their designation as distinct species: 23–24% for cytochrome oxidase I (mtCOI) and 28% for 16S rRNA. The population in Ariake Bay is regarded as a continental relict, of which the main population is distributed in brackish waters along the coast of the East Asian continent.

Introduction

A calanoid copepod *Acartia (Odontacartia) pacifica* Steuer has originally described from an oceanic region (32° N, 157° E) in the Northwest Pacific by Steuer (1915). The species has been recorded from various localities of both ocean and neritic waters, even brackish waters, such as the Amur River estuary in Russia (Brodsky, 1948), river estuary in Amoy, China (Zheng et al., 1965) and the Cochin Backwater in India (Wellershous, 1969). In Japan, the species is commonly observed in both high-saline (>30 psu) water in the Seto Inland Sea (Hirota, 1969; present study) and low-saline (<10 psu)

water in Ariake Bay (Ueda et al., 2003, present study). It is unlikely that the same copepod species can propagate successfully in such a wide range of salinity. Morphological and genetic characteristics of specimens *Acartia pacifica* s. l. collected from the Seto Inland Sea and the Rokkaku River estuary, Ariake Bay, Japan, were compared to determine whether or not these were conspecific. The two populations showed clear genetic and morphological differences that are regarded beyond that typical of intra-specific variations. We present the results of the genetic analyses and describe the specimens from Ariake Bay as *Acartia ohtsukai* sp. nov. together with *A. pacifica* s. str. from the Seto Inland Sea.

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Materials and methods

Sample collection

Acartia specimens were collected from the western Seto Inland Sea (33°58' N, 132°39' E) by vertical tows of 0.1-mm-mesh plankton net on 3 and 19 September 2002 and from the mouth part of the Rokkaku River, Ariake Bay (33°12' N, 130°13' E), by surface tows of 0.3-mm-mesh plankton net on 22 August 2002. Temperature and salinity in the surface water at the former sampling site were 26 °C and 33 psu, and those at the latter site were 29 °C and 5 psu, respectively. Immediately after sampling, specimens for genetic analyses were fixed in 95% ethyl alcohol; those for morphological examination were fixed in 1% formalin-seawater solution. The alcohol was changed after 24 h.

Morphological examination

Microscopic examinations and dissections were made in lactophenol using bright-field and differential interference microscopes. For detailed observation, specimens were stained with a 0.1% chlorazol-black E solution. Initial drawings were made with a camera lucida and final figures were prepared with computer software (Adobe Illustrator 10). Terminology follows Huys & Boxshall (1991). Specimens were deposited in the National Science Museum, Tokyo.

Genetic analysis

DNA sequences were determined for portions of two mitochondrial genes, cytochrome oxidase I (mtCOI) and 16S rRNA, for individuals used in the present study. Individual females were placed in microcentrifuge tubes with 35 µl dHOH and 3 drops of sterile mineral oil and microwaved for several minutes. The PCR reagents (5 µl 10X PCR buffer, 4 µl MgCl₂, 5 µl 2 mM dNTPs, 0.5 µl 10 µM primer solutions, 0.25 µl TAQ polymerase) were added to each reaction from a mixed pool of reagents (see Bucklin, 2000 for detailed methods). PCR primers for mtCOI were COI-1490-F (Folmer et al., 1994), 5'-GGTCAACAAATCATAAAGATATTGG-3', and COI-2364-R (Bucklin et al., unpublished data), 5'-GCATCTATACCTACAGTAAATATATG-3'; the PCR protocol was 94 °C (1 min); 45 °C (2 min);

72 °C (3 min); repeated for 40 cycles. For mt16S rRNA, the primers were: 16S-167 (Bucklin et al., 1998; Caudill & Bucklin, 2004), 5'-GAC-GAGAAGACCCTATGAAG-3', and 16SBR-H (Palumbi, 1996), 5'-CCGGTTTGAAGTCA-GATCATGT-3'; the PCR protocol was 94 °C (1 min); 37 °C (2 min); 72 °C (3 min); repeated for 40 cycles. PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN Sciences, MD). Sequencing was done using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences, Piscataway, NJ). Sequencing products were purified using the QIAGEN DyeEx 2.0 Spin Kit (QIAGEN Sciences, MD). DNA sequencing was done on a 377 Applied Biosystems Automated DNA Sequencer (Foster City, CA). All DNA sequences were edited manually.

Patterns of DNA sequence variation were described for both genes by evaluation of multiple alignments, distance matrices, and gene tree reconstructions. Multiple alignments of mtCOI and mt16S rRNA sequences were done using CLUSTAL X (Thompson et al., 1997). Pairwise distance measures and phylogenetic analyses were determined using the Molecular Evolutionary Genetics Analysis (MEGA), Ver. 2.1, software package (Kumar et al., 2001). DNA sequences for mtCOI and mt16S rRNA for *Acartia tsuensis* Ito (Bucklin et al., unpublished data) were used as outgroups for the phylogenetic analysis.

Results

Morphological description

Acartia ohtsukai sp. nov. (Figs. 1–4)

Synonymy:

Acartia pacifica, Brodsky, 1948: 73, plate 24, figures 1–6; Brodsky, 1950: 422, figure 299; Chen & Zhang, 1965: 112, plate 49, figures 9–12; Zheng et al., 1965: 149, figure 70.

Material examined:

Twenty-eight females and 27 males, collected from the Rokkaku River estuary, Ariake Bay, of which 8 females and 7 males were dissected and closely examined. This species and *Pseudodiaptomus inopinus* Burckhardt exclusively predominated in the in the sample. Holotype: adult female dissected and mounted on five glass slides using CMC-10,

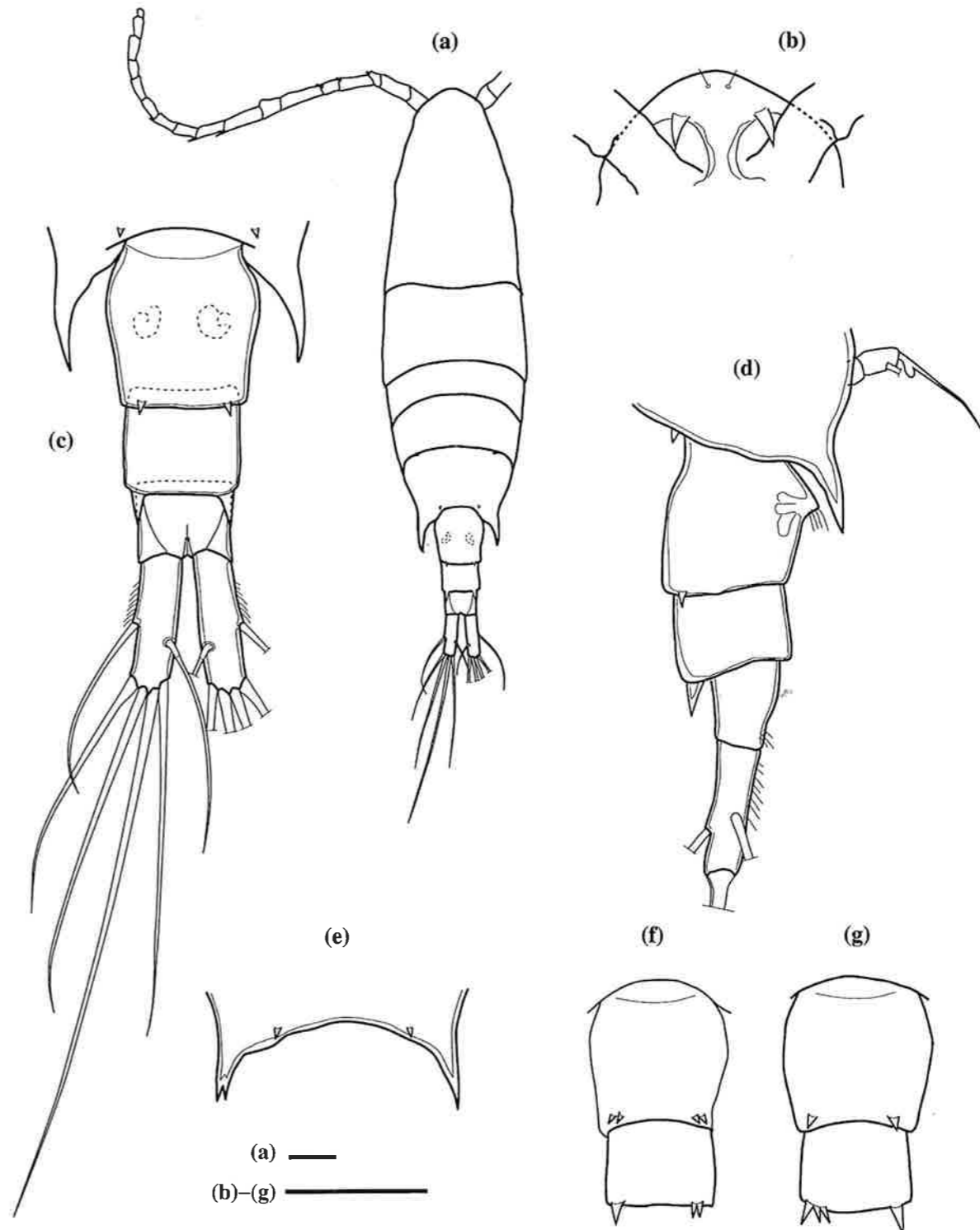


Figure 1. *Acartia ohtsukai* sp. nov. Female (a–d, holotype): (a) habitus, dorsal view; (b) forehead with rostrum filaments, ventral view; (c) fifth pediger and urosome, dorsal view; (d) fifth pediger with fifth leg and urosome, lateral view; (e) fifth pediger with bifurcated left lateral projection; (f) and (g) genital double-somite and second urosomite with dorsal spines, dorsal views. Scales = 0.1 mm.

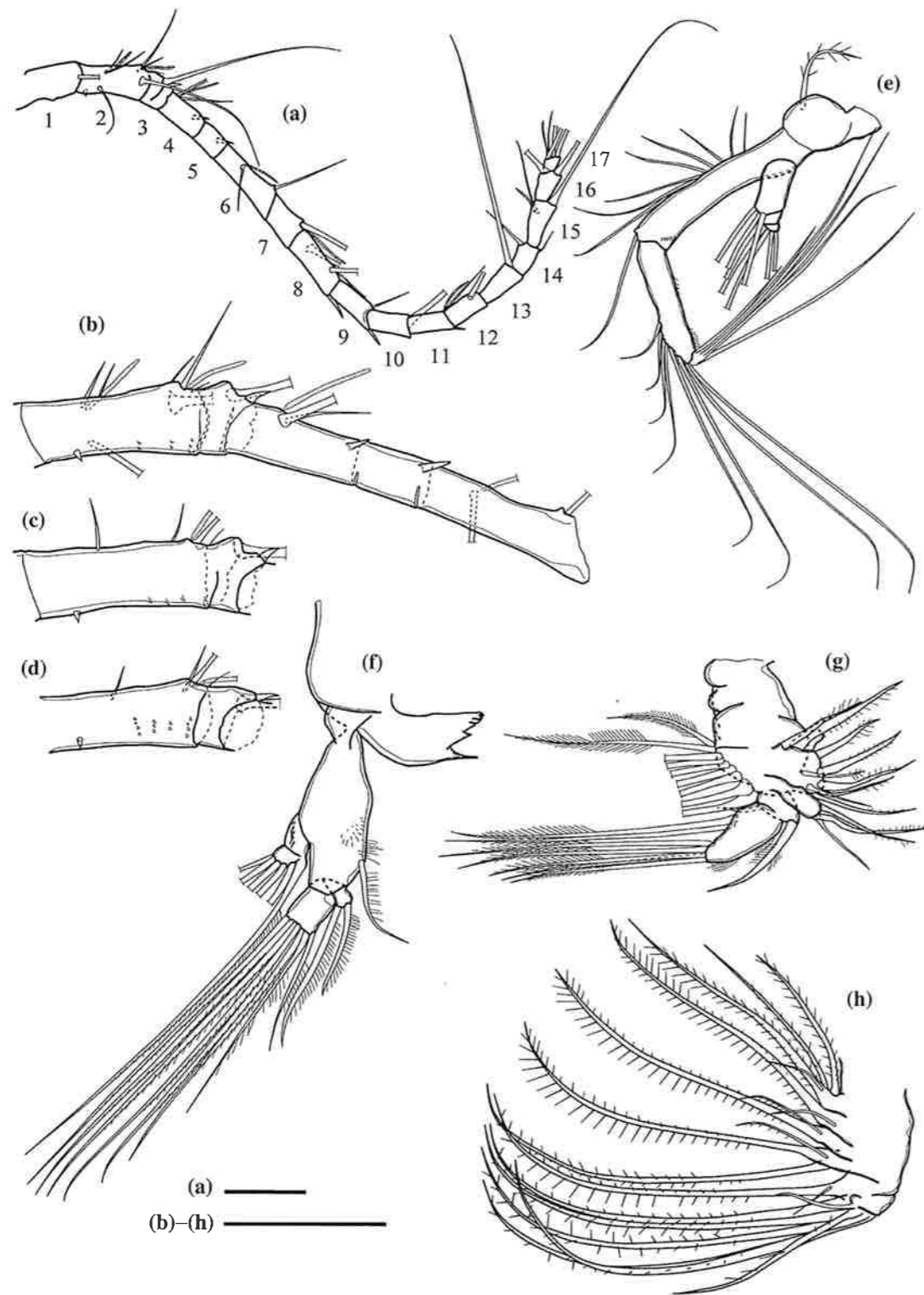


Figure 2. *Acartia ohtsukai* sp. nov. Female (a, b, e-h, holotype,): (a) left antennule with segment numbers, ventral view; (b) second to sixth segments of left antennule, dorsal view; (c) and (d) second and third segments of left antennule of other specimens, dorsal views; (e) left antenna; (f) left mandible; (g) left maxillule; (h) right maxilla. Scales = 0.1 mm.

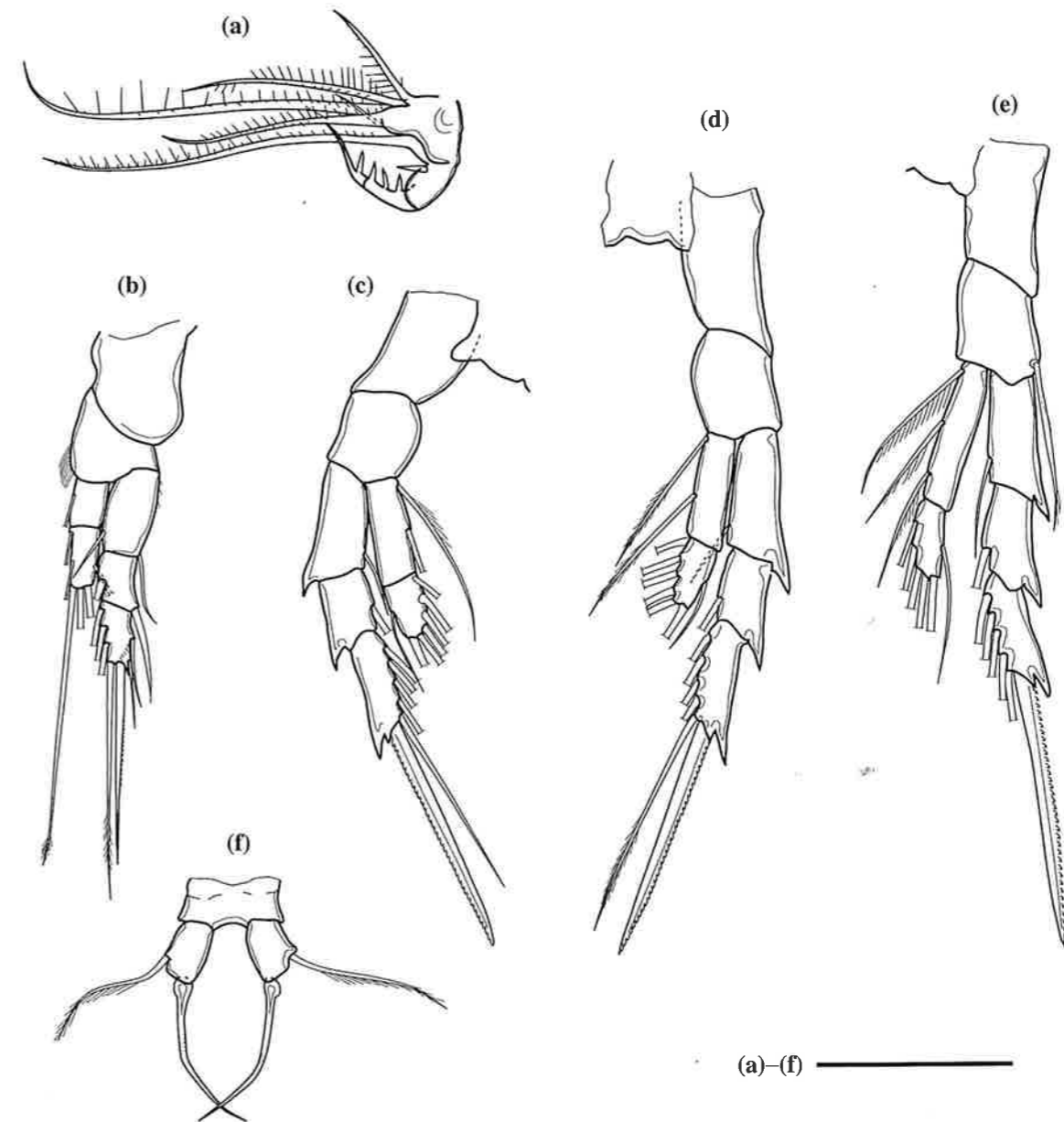


Figure 3. *Acartia ohtsukai* sp. nov. Female (holotype): (a) left maxilliped; (b) right first leg; (c) right second leg; (d) left third leg; (e) right fourth leg; (f) fifth legs, anterior view using a cover slip. Scale = 0.1 mm.

aqueous mounting medium (Masters Company, Inc., Wood Dale, IL), NSMT-Cr 16267. Paratypes: undissected 20 females and 20 males in two vials, NSMT-Cr 16268 and NSMT-Cr 16269.

Female:

Body (Fig. 1a) length 1.19–1.23 mm ($n = 5$, holotype 1.21 mm). Prosome with no hairs except for sensilla. Rostral filaments (Fig. 3b) thick and

short. Fifth pediger (Fig. 1c, d) with acute, slightly curved lateral projection and posterodorsal spine on each side; left process of one specimen bifurcated at tip (Fig. 1e). Genital double-somite as long as wide, bearing 2 dorsal spines, which nearly as long as spines on fifth pediger, along posterior margin, and ventral hairs around gonopores; one specimen bears 2 dorsal spines at each locus

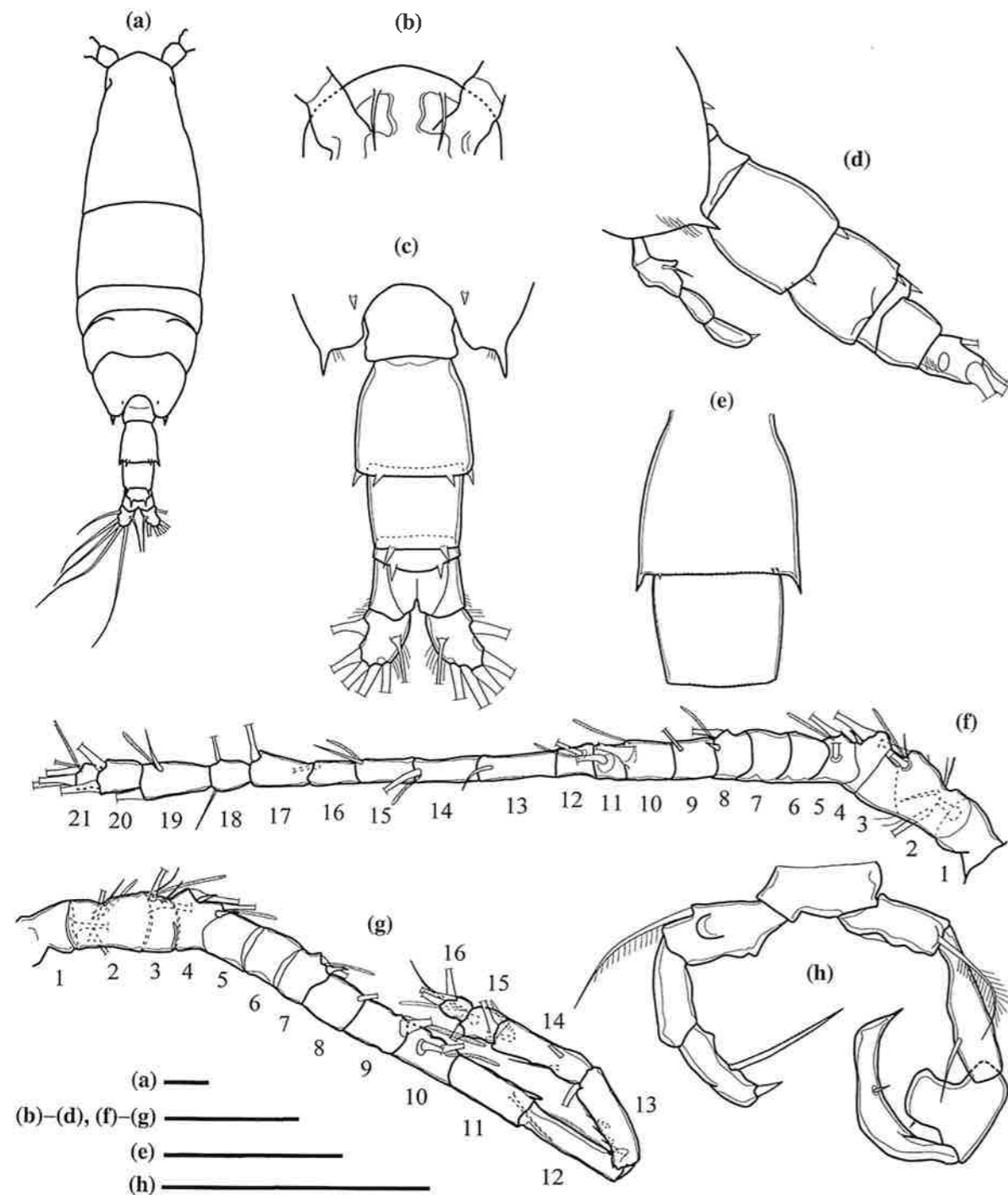


Figure 4. *Acartia ohtsukai* sp. nov. Male: (a) habitus, dorsal view; (b) forehead with rostrum filaments, ventral view; (c) fifth pediger and urosome, dorsal view; (d) fifth pediger with fifth leg and urosome, lateral view; (e) second and third urosomites, ventral view; (f) left antennule with segment numbers, dorsal view; (g) right antennule with segment numbers, dorsal view; (h) fifth leg, posterior view using a cover slip. Scales = 0.1 mm.

(Fig. 1f). Second urosomite with 2 dorsal spines, which twice or more as long as spines on genital double-somite; two specimens with 2 and 3 spines at one locus, respectively (Fig. 1f, g). Anal somite with hairs on posterior part of ventral surface. Caudal ramus 3 times longer than wide, with hairs proximally to lateral seta.

Antennule (Fig. 2a-d) incompletely 17-segmented; second to third and fourth to sixth segments partly fused on dorsal surface; segmentation and setation patterns as follows: (1) I=1, (2) II-VIII=7+2 aesthetascs(ae), (3) IX-X=2 (1 spiniform), (4) XI-XII=2+ae, (5) XIII=unarmed, (6) XIV-XV=2+ae, (7) XVI=1+ae, (8) XVII-XVIII=2+ae, (9) XIX=1, (10) XX=1, (11) XXI=1+ae, (12) XXII=1, (13) XXIII=1, (14) XXIV=2, (15) XXV=2+ae, (16) XXVI=2, (17) XXVII-XXVIII=4+ae. Second segment with short spine at one fourth proximal and 3-4 transverse rows of 1-4 spinules on distal half of dorsal surface; fourth and fifth segments each with subterminal spine dorsally; eighth, ninth and eleventh segments each with terminal spine, of which spine on eleventh segment smaller than others; ninth segment with ventral row of tiny teeth along distal margin.

Antenna (Fig. 2e) with 1 on coxa, 8 medially and 1 distally on allobasis, 8 on exopod, 8 on first free endopodal segment and 6 setae on second free endopodal segment; allobasis with oblique row of tiny spinules on distal part of anterior surface.

Mandible (Fig. 2f): basis with seta and group of fine spinules posteriorly; exopod with oblique row of spinules on first segment and 6 setae in total; endopod with 2 and 8 setae on first and second segments, respectively.

Maxillule (Fig. 2g): precoxal endite with 9 setae, of which anterior 2 thin; coxal endite with 3 setae, exite with 9 setae; basis with thick medial seta and thin, short lateral seta; 1-segmented exopod partly fused with basis and bearing 2 setae laterally and 5 setae terminally; endopod absent.

Maxilla (Fig. 2h) with 4 endites bearing thin, short seta on each and 3, 1, 1, 2 long setae, respectively; basis with long seta and short seta; endopod with 5 long setae, medium seta and short seta.

Maxilliped (Fig. 3a): syncoxal endite with 2 long, 2 medium and 2 short setae; basis with short spiniform seta; endopod with 3 spines on first

segment and spine on second segment; tip of which elongate and spiniform.

Segmentation of first to fourth legs typical for the genus (Fig. 3b-e). Seta and spine formula as follows:

	Coxa	Basis	Exopod segment			Endopod segment	
			1	2	3	1	2
Leg 1	0-0	0-0	1-1; 1-1; 2, 1, 4	0-1; 1, 3, 2			
Leg 2	0-0	0-0	0-1; 0-1; 0, 1, 5	0-2; 1, 2, 4			
Leg 3	0-0	0-0	0-1; 0-1; 0, 1, 5	0-2; 1, 2, 4			
Leg 4	0-0	1-0	0-1; 0-1; 0, 1, 5	0-3; 1, 2, 3			

Fifth leg (Figs. 1d, 3f): basis about 1.5 times longer than wide; lateral seta nearly as long as claw-like exopod; exopod with round, posteriorly produced lobe at base and fine teeth on both sides of distal half.

Male:

Body (Fig. 4a) length 1.03-1.05 mm ($n = 5$). Rostral filaments (Fig. 4b) thin. Fifth pediger (Fig. 4c, d) with pointed posterior process, which smaller than in female, posterodorsal spine and posterolateral hairs on each side. Second urosomite with 2 dorsal and 2 lateral spines, and ventral row of spinules along posterior margin; lateralmost one or two of ventral spinules conspicuous but those between them very fine and sometimes absent (Fig. 4e). Third and fourth urosomites each with 2 dorsal spines; spines on third urosomite slightly longer than or as long as spines on fourth urosomite. Anal somite bearing short hairs on distolateral surface. Caudal ramus about 1.5 times longer than wide, with hairs along lateral margin proximally to lateral seta and along distal half of medial margin.

Left antenna (Fig. 4f) incompletely 21-segmented; second to third segments fused dorsally; segmentation and setation patterns as follows: (1) I=1, (2) II-VIII=6+2 ae, (3) IX=1, (4) X=2 (1 spiniform), (5) XI=2+ae, (6) XII=unarmed, (7) XIII=unarmed, (8) XIV=2 (1 spiniform)+ae, (9) XV=1, (10) XVI=1+ae, (11) XVII=1, (12) XVIII=1+ae, (13) XIX=1, (14) XX=1+ae, (15) XXI=1+ae, (16) XXII=1, (17) XXIII=1, (18) XXIV=2, (19) XXV=2+ae, (20) XXVI=2, (21) XXVII-XXVIII=4+ae. Right antennule (Fig. 4g) 16-segmented, with geniculation between twelfth