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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.

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 $MgCl_2$, 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′-GACGAGAAGACCCTATGAAG-3′, and 16SBR-H (Palumbi, 1996), 5′-CCGGTTTGAACTCAGATCATGT-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,

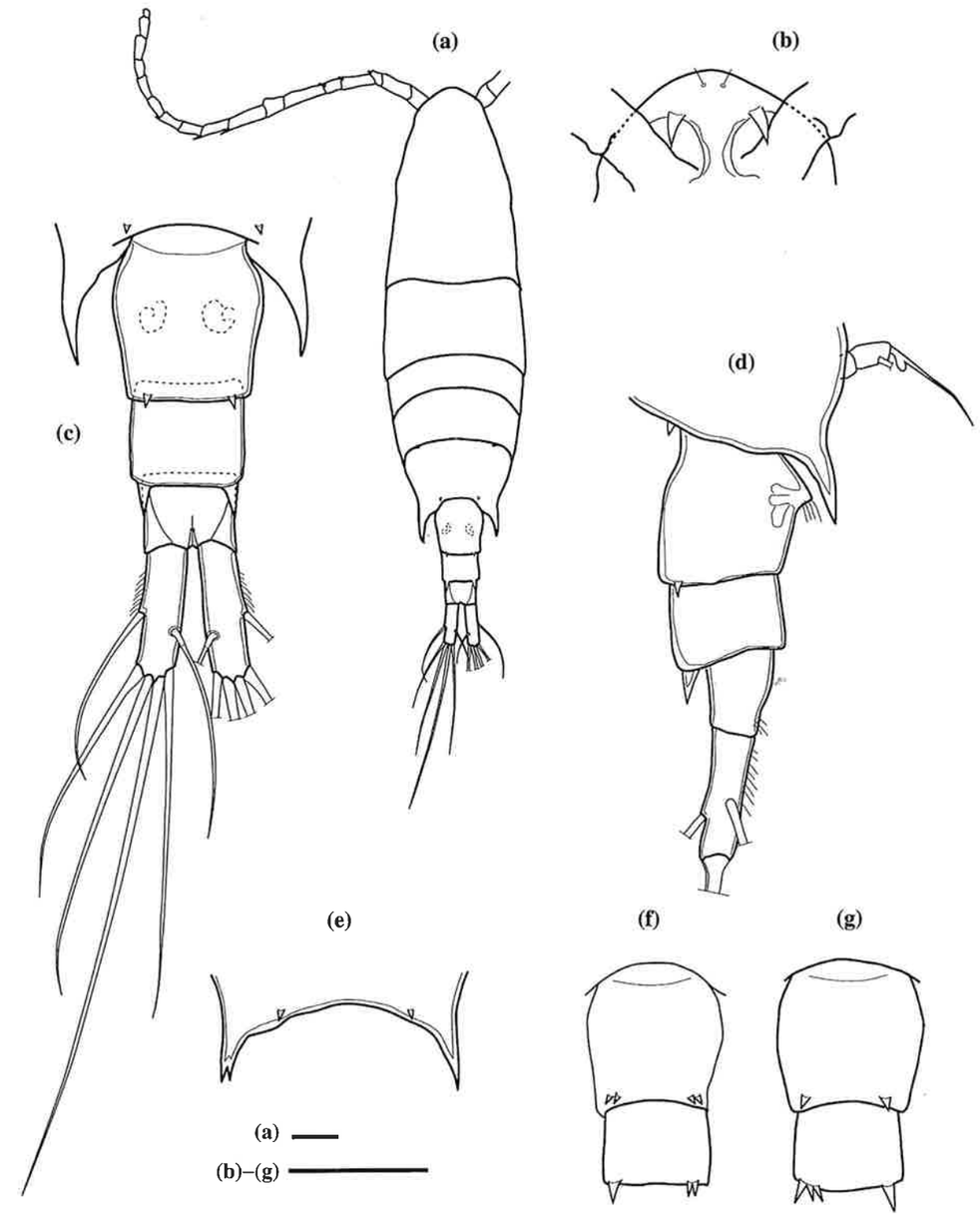

Figure 1. Acartia ohtsukai sp. nov. Female (a–d, holotype): (a) habitus, dorsal view; (b) forehead with rostrum filaments, ventral view; (c) fifth pediger and urosome, dorsal view; (d) fifth pediger with fifth leg and urosome, lateral view; (e) fifth pediger with bifurcated left lateral projection; (f)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

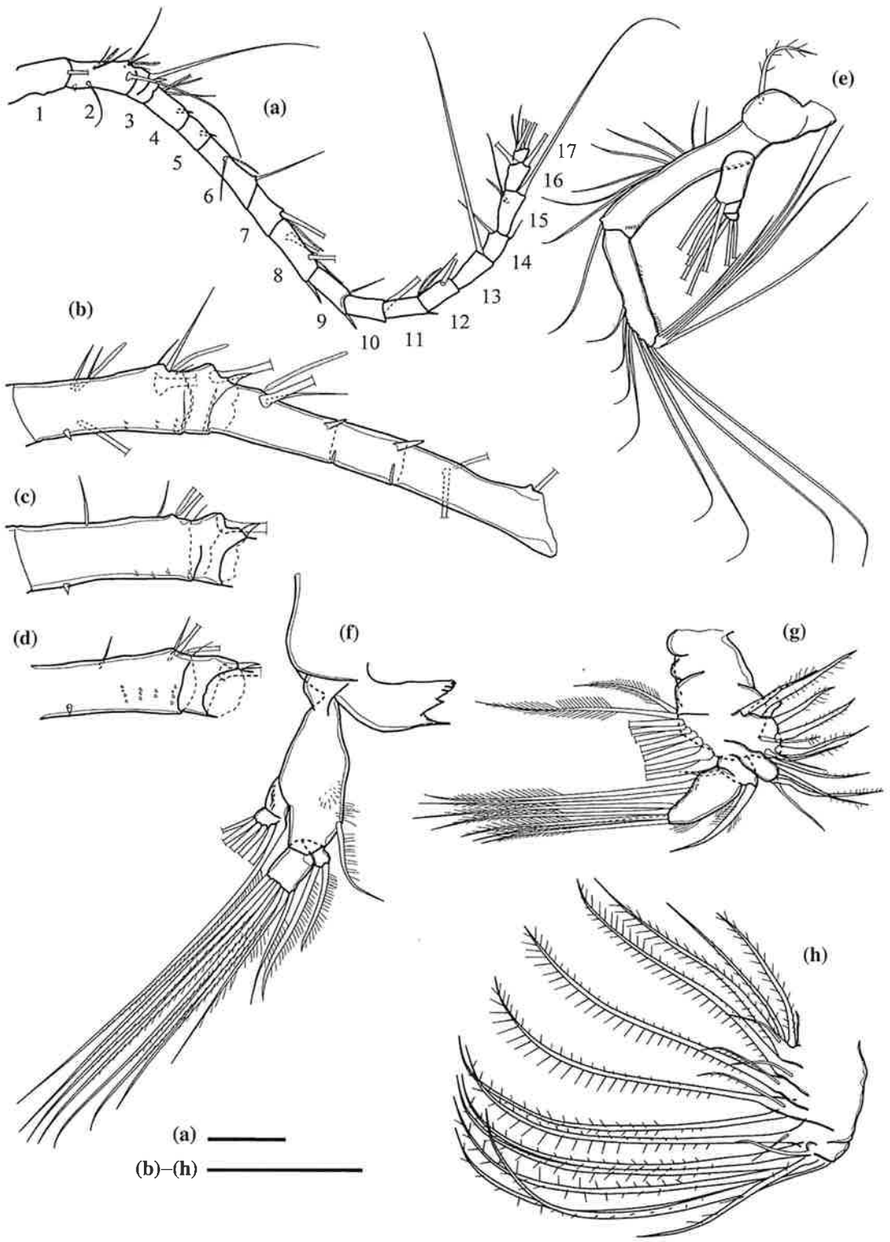

Figure 2. Acartia ohtsukai sp. nov. Female (a, b, e–h, holotype,): (a) left antennule with segment numbers, ventral view; (b) second to sixth segments of left antennule, dorsal view; (c) and (d) second and third segments of left antennule of other specimens, dorsal views; (e) left antenna; (f) left mandible;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

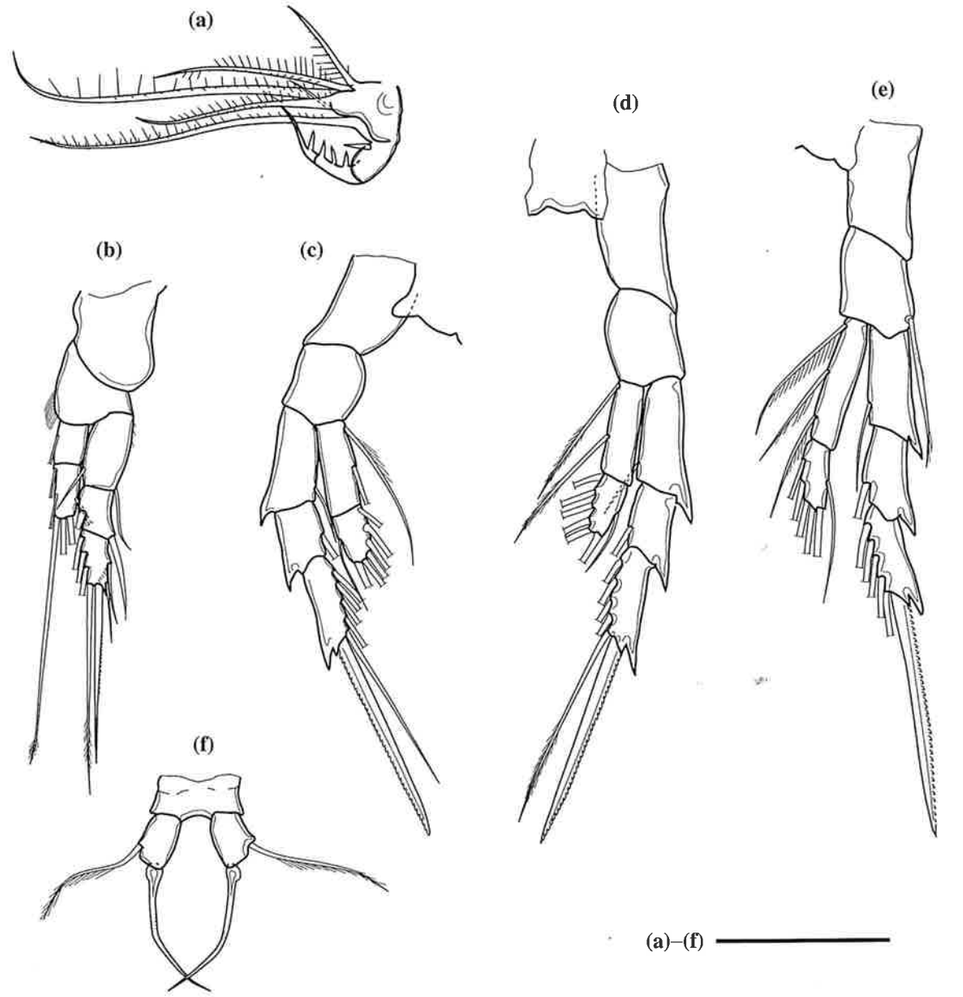

Figure 3. Acartia ohtsukai sp. nov. Female (holotype): (a) left maxilliped; (b) right first leg; (c) right second leg; (d) left third leg; (e) right fourth leg; (f) fifth legs, anterior view using a cover slip. Scale = 0.1 mm.

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

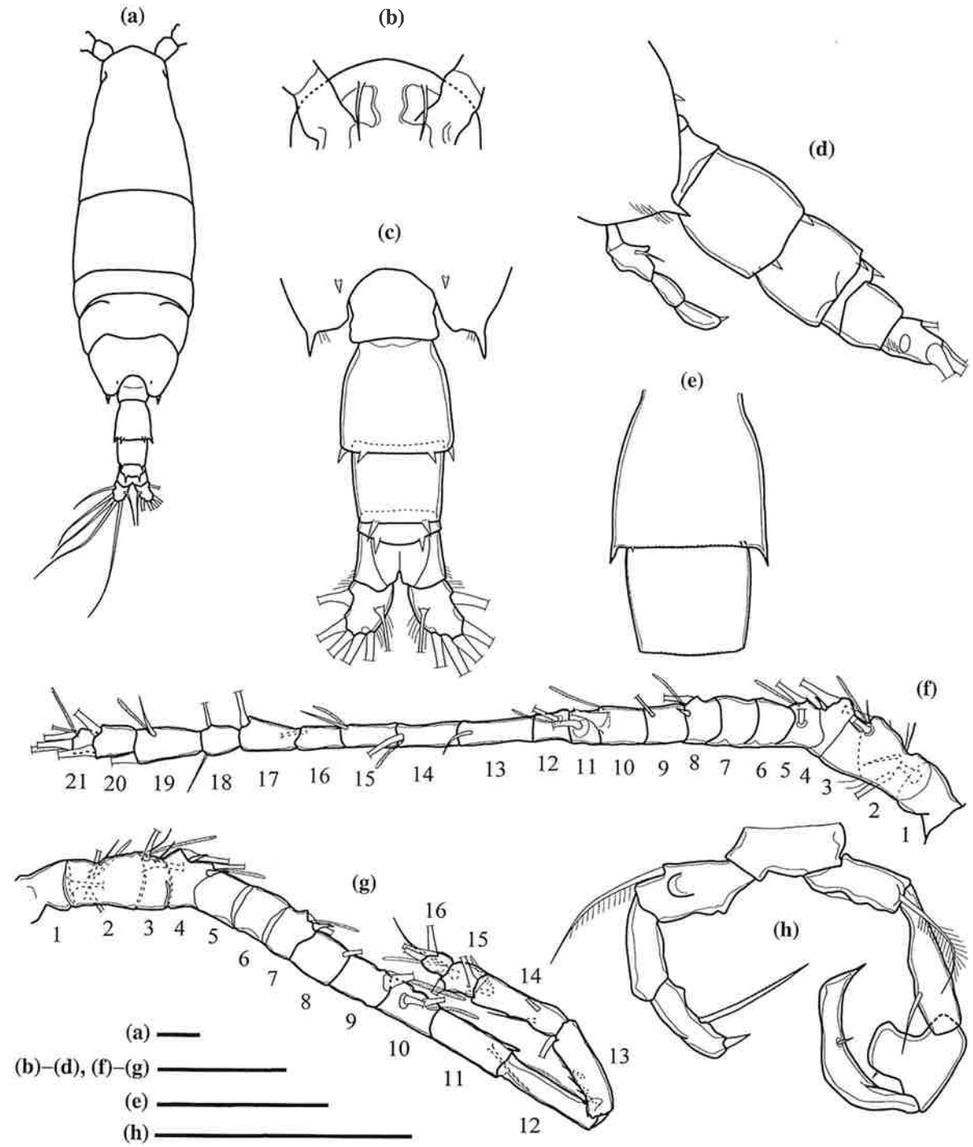

Figure 4. Acartia ohtsukai sp. nov. Male: (a) habitus, dorsal view; (b) forehead with rostrum filaments, ventral view; (c) fifth pediger and urosome, dorsal view; (d) fifth pediger with fifth leg and urosome, lateral view; (e) second and third urosomites, ventral view; (f) left antennule with segment 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	I–1; I-1; 2, I, 4	0–1; 1, 3, 2
Leg 2	0–0	0–0	0–1; 0–1; 0, I, 5	0–2; 1, 2, 4
Leg 3	0–0	0–0	0–1; 0–1; 0, I, 5	0–2; 1, 2, 4
Leg 4	0–0	1–0	0–1; 0–1; 0, I, 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

and thirteenth segments; second to fourth segments partly fused on dorsal surface; segmentation and setation patterns as follows: (1) I=1, (2) II–VI=4+ae, (3) VII–VIII=3+ae, (4) IX–XI=4 (2 spiniform)+ae, (5) XII=unarmed, (6) XIII=unarmed, (7) XIV=2 (1 spiniform)+ae, (8) XV=1, (9) XVI=1+ae, (10) XVII=1, (11) XVIII=1+ae, (12) XIX=1+process, (13) XX=1, (14) XXI–XXIII=4, (15) XXIV–XXV=4+ae, (16) XXVI=2, (17) XXVII–XXVIII=4+ae.

Left fifth leg (Fig. 4h): proportional lengths of basis and 2 exopodal segments 1:1:0.8; basis 2 times longer than wide, with round process on posterior surface; first exopodal segment as long as second exopodal segment and shorter than first exopodal segment of right leg; second exopodal segment about 3 times longer than wide, with slightly curved long spine at about midpoint of medial margin and a short terminal spine. Right fifth leg: proportional lengths of basis and first 2 exopodal segments 1:1.4:0.9; second exopodal segment as long as wide, with square medial projection; third exopodal segment with small terminal and medial spines.

Etymology:

The new species is dedicated to Dr. Susumu Ohtsuka (Hiroshima University) for his contributions to copepod taxonomy and for inviting the first author to participate in the ZooGene project.

Remarks:

The new species is distinguished from *Acartia pacifica* s. str. by a subterminal spine on the fifth segment of the antennule and long caudal rami in female, and shorter spines on the third urosomite, a square medial projection of the second exopodal segment of the right fifth leg and the shorter first exopodal segment of the left leg in male. Among the previous descriptions of the species named *A. pacifica*, the same characters are seen in figures described from estuaries along the continental coasts of Russia (Brodsky, 1948, 1950) and China (Chen & Zhang, 1965; Zheng et al., 1965), indicating these specimens are *A. ohtsukai*.

Acartia pacifica Steuer, 1915 (Fig. 5).

Synonymy:

Acartia pacifica Steuer, 1915: 379, figures 5–6; Steuer, 1923: 116, figures 134–137; Tanaka, 1965: 391, figure 247; Kim, 1987: 87, plate 18, figures g–j; Ueda, 1997: 672, plate 21, figure 15, plate 22, figure 15; Mulyadi, 2004: 146, figure 83.

Acartia pacifica forma *typica*, Greenwood, 1978: 15, figs 7a–c, f, i, j.

Acartia spinicauda Giestbrecht, Mori, 1937: 103, plate 50, figs 5–7.

?*Acartia pacifica*, Farran, 1936: 120, fig. 22.

Not *Acartia pacifica*, Wellershaus, 1969: 275, figs 82–85.

Material examined:

Twenty-five females and 25 males, collected from the Seto Inland Sea, of which five females and five males were dissected for close examination. Undissected 20 females and 20 males in two vials (NSMT-Cr 16270 and NSMT-Cr 16271) were deposited.

Female:

Body length 1.19–1.21 mm ($n=5$). Caudal rami (Fig. 5a, b) 2.5 times longer than wide. Antennule (Fig. 5c): second segment without spines and spinules on dorsal surface; fifth segment lacking subterminal dorsal spine; ninth segment without teeth along distal margin. Fifth leg (Fig. 5d): lateral seta of basis about 2 times longer than exopod; exopod with conspicuous teeth on distal half of segment. Other characters similar to *A. ohtsukai*.

Male:

Body length 1.03–1.16 mm ($n=5$). Fifth pediger (Fig. 5e, f) without posterolateral hairs on each side. Second urosomite (Fig. 5g) ventrally with 3 transverse rows of 2–4 long spinules on each side and without spinules along posterior margin. Posterior spines on urosomal somites stronger than those in *A. ohtsukai*; spines on third urosomite about 2 times longer than spines on fourth urosomite (Fig. 5f). Hairs on anal somite and caudal rami longer and more conspicuous than those in *A. ohtsukai*.

Left fifth leg (Fig. 5h): proportional lengths of basis and 2 exopodal segments 1:1.2:0.7; basis 1.5 times longer than wide; first exopodal segment longer than second exopodal segment and nearly as long as first exopodal segment of right leg; second exopodal segment with hairs distally to base of medial long spine. Right fifth leg: proportional lengths of basis and first 2 exopodal segments 1:1.3:0.9; second exopodal segment 1.4 times longer than wide, with round, triangular medial projection.

Remarks:

Mori's (1937) identification of *Acartia spinicauda* females from the Kii Channel, the eastern channel

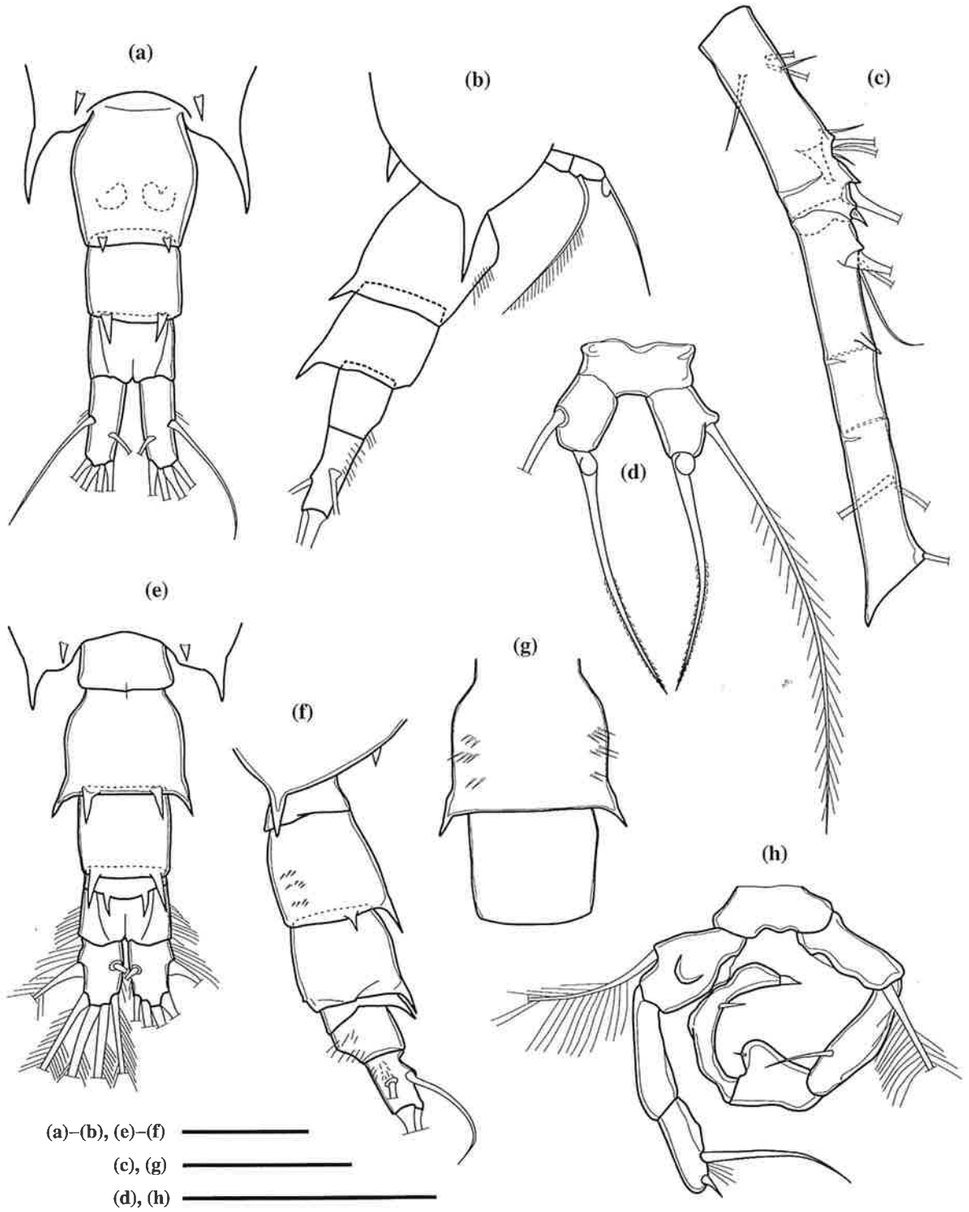

Figure 5. Acartia pacifica. Female: (a) fifth pediger and urosome, dorsal view; (b) fifth pediger with fifth leg and urosome, lateral view; (c) second to sixth segments of right antennule, dorsal view; (d) fifth legs, posterior view using a cover slip. Male: (e) fifth pediger and urosome, dorsal view; (f) fifth pediger and urosome, lateral view; (g) second and third urosomites, ventral view; (h) fifth leg, posterior view using a cover slip. Scales=0.1 mm.

of the Seto Inland Sea, is incorrect and these are probably *A. pacifica* as noted by Ueda (1997). In Mori's figures, the specific characters are seen in the second antennular segment without the spine characteristic of *A. spinicauda* and a round lobe at the base of the exopod of the fifth leg, which is pointed in *A. spinicauda* (cf. Giesbrecht, 1892). Farran's (1936) *A. pacifica* from the Great Barrier Reef may be a different species. The differences are found in the following urosomal spines in his illustrations: the spines of the female second urosomite is much longer (beyond the posterior margin of the anal somite) and those of the male third urosomite is much shorter (not beyond the posterior margin of the fourth urosomite). The *A. pacifica* female specimens described by Wellershaus (1969) from the Cochin Backwater, India, is apparently different from *A. pacifica* s. str. and *A. ohtsukai*, because of the short caudal rami (2 times longer than wide), a smaller projection on the lateral corner of the fifth pediger, the different pattern of spines on the antennular segment, and the short first exopodal segment of the left fifth leg.

Acartia mertoni Steuer, 1917 new rank

Synonymy:

Acartia pacifica var. *mertoni* Steuer, 1917: 255, figures 1–6; Steuer 1923: 117, figures 138–141.

Acartia pacifica forma *mertoni*, Greenwood, 1978: 15, figures 7d, e, g, h.

Remarks:

This species was originally described as a variety of *Acartia pacifica* from a brackish water of the Aru Islands, Indonesia, by Steuer (1917). Greenwood (1978) described it from Moreton Bay, Australia, together with coexisting the typical form of *A. pacifica*. According to their figures, this species is more closely allied to *A. ohtsukai* than to the typical form of *A. pacifca*. The similar morphologies between *A. mertoni* and *A. ohtsukai* are, for example, the shorter lateral seta of the female fifth leg than in *A. pacifica* and the square medial projection of the second exopodal segment of the male fifth leg in contrast to the triangular projection in *A. pacifica* (Table 1). However, their figures also clearly indicate that *A. mertoni* differs from *A. ohtsukai* in the following points: the female caudal rami are two times longer than wide (three times longer in *A. ohtsukai*); there are small teeth between posterodorsal spines of the female second urosomite and the male second to fourth urosomites (no teeth in *A. ohtsukai*); the male second urosomite bears ventral spinules (no spinules in *A. ohtsukai*); and the posterodorsal spines on the male third urosomites are much shorter than those on the fourth urosomite (slightly longer than or as long as in *A. ohtsukai*). Thus, *A. mertoni* is regarded as an independent species as is *A. ohtsukai*.

Molecular diversity

DNA sequences were determined for a 658 base-pair region of mtCOI for five individuals of *Acartia pacifica* collected from the Seto Inland Sea and five individuals of *A. ohtsukai* sp. nov. from Ariake Bay. Individuals from the same species differed in mtCOI sequence by 0–3%, while individuals of the different species differed by 23–24% (Table 2). The mtCOI gene tree demonstrated the clear separation between the species (Fig. 6).

DNA sequences were determined for a 264 base-pair region of mt16S rRNA for four individuals of *A. pacifica* from the Seto Inland Sea and four individuals of *A. ohtsukai* from Ariake Bay. Individuals from the same species differed in mt16S rRNA sequence by <1%, while individuals of the different species differed by 28%. The mt16S rRNA gene tree also clearly distinguished individuals of the two species.

MtCOI and 16S rRNA reference sequences (see Bucklin et al., 2003) or DNA barcodes (see Stoeckle, 2003) were selected to represent the new species from Ariake Bay (GenBank Accession No. DQ071176 for mtCOI and DQ071174 for 16S rRNA) and *A. pacifica* from the Seto Inland Sea (DQ071177 for mtCOI and DQ071175 for 16S rRNA).

Discussion

MtCOI has been used to examine population genetic diversity and structure of copepods (Burton & Lee, 1994; Edmands, 2001; Bucklin et al., 2003) and other crustaceans (Quan et al., 2001; Vainola et al., 2001). Based on a search of the NCBI GenBank molecular database, mtCOI sequence variation is becoming a standard tool for ecologists and evolutionary biologists interested in understanding intra- and inter-specific patterns of

Table 1. Comparative list of characters in the *Acartia pacifica* species group

	A. pacifica	*A. ohtsukai* n. sp.	*A. mertoni* new rank*
Female			
Length/width of caudal ramus	2.5	3	2
Teeth between posterodorsal spines of 2nd urosomite	Absent	Absent	Present
First antennular segment	Without spine and spinules	With spine proximally and spinule rows distally	Unknown
Subterminal spine on 5th antennular segment	Absent	Present	Unknown
Length ratio of lateral seta to exopod of 5th leg	ca. 2	ca. 1	ca. 1
Male			
Spinule rows on ventrolateral surface of 2nd urosomite	Present	Absent	Present
Length ratio of dorsal spines on 3rd urosomite to those on 4th urosomite	ca. 2	1 or slightly >1	<<1
Length ratio of 1st exopodal segment of left 5th leg to that of right leg	ca. 1	<1	<1
Medial projection of 2nd exopodal segment of right 5th leg	Triangular	Square	Square

*According to Steuer (1923) and Greenwood (1978).

variation in crustaceans and many other taxa. Pairwise differences between six other species of *Acartia* for the mtCOI DNA sequence ranged between 23 and 27% (Bucklin et al., unpublished data), similar to the differences between *A. pacifica* from the Seto Inland Sea and *A. ohtsukai* sp. nov. from Ariake Bay, which differed by 23–24%.

Mt16S rRNA has also been used to identify and discriminate closely-related copepod species (Bucklin et al., 1992, 1996), including *Acartia* species (Caudill & Bucklin, 2004). Interestingly, estuarine and neritic zooplankton species appear to exhibit rapid divergence among conspecific populations and cryptic species (Bucklin et al., 1998, unpublished data). Although mt16S rRNA is thought to evolve more slowly than mtCOI, recognized species of *Acartia* differed in mt16S rRNA sequence by 18–29% (Caudill & Bucklin, 2004). In comparison, *A. pacifica* from the Seto Inland Sea and *A. ohtsukai* from Ariake Bay differed by 28%. Thus, based on patterns of DNA sequence variation at two genes, mtCOI and 16S rRNA, within and between *A. pacifica* from Seto Inland Sea, *A. ohtsukai* from Ariake Bay, and six other recognized species of *Acartia* (Bucklin et al., unpublished data), *A. ohtsukai* is undoubtedly not a subspecies or phenotypic form of *A. pacifica*, but is an independent species.

The *Acartia* subgenus *Odontacartia* includes twelve species, *A. amboinensis* Carl, *A. australis* Farran, *A. bispinosa* Carl, *A. bowmani* Abraham, *A. centrura* Giesbrecht, *A. erythraea* Giesbrecht, *A. japonica* Mori, *A. lilljeborgii* Giesbrecht, *A. mertoni*, *A. ohtsukai* sp. nov., *A. pacifica*, and *A. spinicauda*. The present three species, *A. pacifica*, *A.mertoni* and *A. ohtsukai*, comprise 'the *pacifica*-group' by their close morphological similarity, e.g., a terminal spine on each the eight and ninth antennular segments, a round lobe at the base of the exopod of the female fifth leg, and slender

Table 2. Pairwise percent differences for mtCOI and mt16S rRNA sequences between individual females of *Acartia ohtsukai* sp. nov. from Ariake Bay and *A. pacifica* from the Seto Inland Sea

	o-1	o-2	o-3	o-4	o-5	p-1	p-2	p-3	p-4
MtCOI									
Acartia ohtsukai #1 (o-1)									
Acartia ohtsukai #2 (o-2)	0.019								
Acartia ohtsukai #3 (o-3)	0.003	0.022							
Acartia ohtsukai #4 (o-4)	0.019	0.000	0.022						
Acartia ohtsukai #5 (o-5)	0.000	0.019	0.003	0.018					
Acartia pacifica #1 (p-1)	**0.233**	**0.231**	**0.234**	**0.232**	**0.234**				
Acartia pacifica #2 (p-2)	**0.232**	**0.231**	**0.233**	**0.231**	**0.233**	0.003			
Acartia pacifica #3 (p-3)	**0.235**	**0.233**	**0.236**	**0.234**	**0.235**	0.011	0.011		
Acartia pacifica #4 (p-4)	**0.235**	**0.233**	**0.236**	**0.234**	**0.235**	0.012	0.012	0.005	
Acartia pacifica #5	**0.231**	**0.230**	**0.232**	**0.231**	**0.232**	0.002	0.002	0.009	0.011
	o-6	o-7	o-8	o-9	p-6	p-7	p-8		
Mt16S rRNA									
Acartia ohtsukai #6 (o-6)									
Acartia ohtsukai #7 (o-7)	0.021								
Acartia ohtsukai #8 (o-8)	0.000	0.021							
Acartia ohtsukai #9 (o-9)	0.000	0.021	0.000						
Acartia pacifica #6 (p-6)	**0.282**	**0.282**	**0.282**	**0.282**					
Acartia pacifica #7 (p-7)	**0.281**	**0.281**	**0.281**	**0.281**	0.004				
Acartia pacifica #8 (p-8)	**0.281**	**0.281**	**0.281**	**0.281**	0.000	0.004			
Acartia pacifica #9	**0.281**	**0.281**	**0.281**	**0.281**	0.000	0.004	0.000		

Values in bold face indicate pairwise comparisons between the different species.

second exopodal segment of the left male fifth leg with a long medial spine and a short apical spine. The morphologically closest relative of the *pacifica*-group is *A. spinicauda*, which has a similar pattern of spines on the female antennule (a long terminal spine on each the eighth and ninth segments) and the very similar male fifth legs according to figures described by Giesbrecht (1892) and Zheng et al. (1965). But it differs from the *pacifica*-group by the somewhat pointed lobe at the base of the exopodal segment of the female fifth leg, which is round-shaped in the *pacifica*-group. Wellershaus' (1969) *A. pacifica* female from the Cochin Backwater, which is another member of the group, has the same characters as those of *A. spinicauda*, i.e., both species have smaller dorsal spines on the urosomal somites and two spines along the anterior surface of the second antennular segment. This suggests that Wellershaus' (1969) *A. pacifica* is an intermediate form between *A. spinicauda* and Steuer's *A. pacifica*.

The species of the *pacifica*-group have been recorded from the Indo-West Pacific regions. *Acartia ohtsukai* is a brackish water form along the coast of the Northwest Pacific, judging from the localities so far recorded (Brodsky, 1948; Zheng et al., 1965), with high abundance in the Rokkaku River estuary in Ariake Bay. In contrast, *A. pacifica* is a marine species, which is distributed in high salinity coastal waters (Steuer, 1915; Tanaka, 1965; present study). Ariake Bay is known to have the peculiar fauna characterized by continental relicts, resulting from the isolation of Japanese island populations from populations in coastal regions of the neighboring Asian continent by marine transgressions during the geological history. Such relicts may presently survive in limited areas of the coastal waters of Japan. According to Sato & Takita (2000), fourteen species (six fishes, two calanoid copepods and six benthic invertebrates, including crabs and polychaetes) are referred to as continental relicts. They have been recorded from the innermost part of Ariake Bay but never from other localities in Japan. Both copepods, *Sinocalanus sinensis* (Poppe) and *Tortanus derjugini* Smirnov (Hiromi & Ueda, 1987; Ohtsuka et al., 1995), are distributed widely in estuarine waters along the coast of the Asian continent; *S. sinensis* from Fujian in China (Shen et al., 1979) to south Korea (Chang & Kim, 1986) and *T. derjugini* from Xiamen in China (Shen et al., 1979) to the Amur River estuary in Russia (Brodsky, 1948). *Acartia ohtsukai* is considered as the third member of the continental relict copepods in Ariake Bay because its distribution pattern is similar to those of *S. sinensis* and *T. derjugini*, in that it inhabits brackish waters, is restricted to Ariake Bay in Japan, and has been recorded together with *S. sinensis* and/or *T. derjugini* from brackish waters along the coast of the East Asian continent.

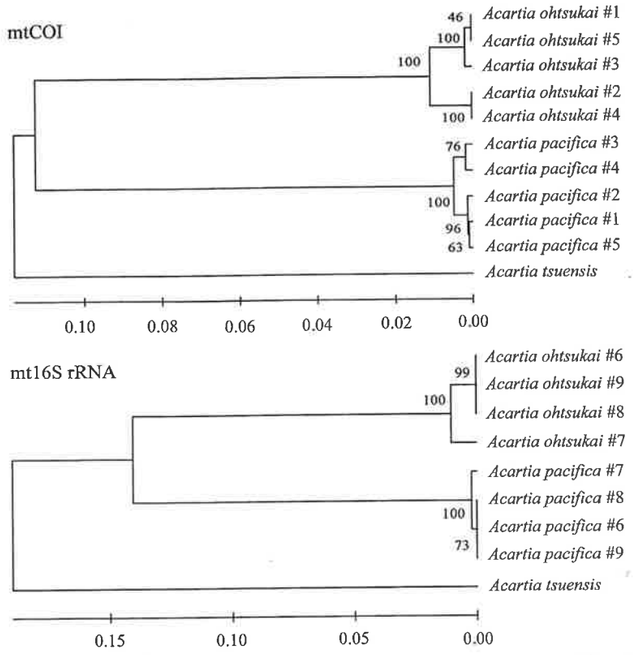

Figure 6. Gene trees for mtCOI (top) and mt16S rRNA (bottom) showing proportional differences between individual females of *Acartia pacifica* from Seto Inland Sea and *A. ohtsukai* sp. nov. from Ariake Bay. Numbers at branch points are bootstrap values (i.e., percentage of trees with that branch point among 1000 subreplicates). The gene sequence for *A. tsuensis* collected from a brackish pond in an island of the Seto Inland Sea on 22 August 2003 was used as an outgroup. The specimen number corresponds to that in Table 2.

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