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A new species closely related to *Acartia sinjiensis* (Copepoda: Calanoida), from river estuaries of northern Luzon, the Philippines

SAKIKO ORUI SAKAGUCHI[1,4] & HIROSHI UEDA[2,3]
[1]*Institute of Biogeosciences, Japan Agency for Marine-Earth Science and Technology, 2-15 Natsushima, Yokosuka, Kanagawa 237-0061, Japan.*
oruis@jamstec.go.jp; https://orcid.org/0000-0003-3699-5938
[2]*Usa Marine Biological Institute, Kochi University, 194 Inoshiri, Usa, Tosa, Kochi 781-1164, Japan.*
[3]*Present address: 13–22–703 Tanaka-cho 1-chome, Higashi-nada-ku, Kobe 658–0081, Japan.*
hueda@kochi-u.ac.jp; https://orcid.org/0000-0002-3304-9308
[4]*Corresponding author.*

Abstract

We describe a brackish-water calanoid copepod *Acartia* (*Acanthacartia*) *cagayanensis* **sp. nov.** collected from river estuaries in the northernmost Luzon, the Philippines. The new species has several characteristic features that are typical to the *A. plumosa* group (*A.* (*A.*) *plumosa* Scott T., 1894, *A.* (*A.*) *sinjiensis* Mori, 1940 and *A.* (*A.*) *tropica* Ueda & Hiromi, 1987); specifically, a short apical spine on the long terminal segment of male left leg 5, which is unique to the group. The morphological features of *A. cagayanensis* **sp. nov.** different from those of the *A. plumosa* group are the barrel-shaped genital double somite and the cylindrical basal part of the terminal segment of female leg 5. Among the species in the group, *A. cagayanensis* **sp. nov.** is closest to *A. sinjiensis* in terms of the spinule patterns on the female antennule, the posterior corner of the prosome, and the male second urosomite. The genetic analysis using DNA sequences of mitochondrial gene cytochrome oxidase subunit I (COI) revealed that *A. sinjiensis* from Japan and *A. cagayanensis* **sp. nov.** differed by 16.5–16.9%, in contrast to a small variation (0.0–0.5%) within each population. We confirmed that previous records of *A. sinjiensis* from the Philippines were not *A. cagayanensis* **sp. nov.**, and therefore, *A. cagayanensis* **sp. nov.** is the third species of the subgenus *Acanthacartia* Steuer, 1925, after *A. sinjiensis* and *A.* (*A.*) *tsuensis* Ito, 1956.

Key words: *Acanthacartia*, brackish-water copepods, *Acartia plumosa* species group, COI

Introduction

Generally, estuaries are inhabited by brackish-water copepods, which are predominantly limited to low-salinity waters. Brackish-water copepods are divergent within a genus, and most of them are limited to narrow geographical ranges compared with marine species (Ohtsuka *et al.* 1995; Ohtsuka & Ueda 1999). Sakaguchi *et al.* (2011) investigated planktonic copepod faunas in various river estuaries in western Japan and southern Korea, and revealed that the calanoid copepod genus *Acartia* Dana, 1846 was often the dominant mesozooplankton member. Furthermore, the dominant species of the genus were *A.* (*Acanthacartia*) *sinjiensis* Mori, 1940, *A.* (*A.*) *tsuensis* Ito, 1956, and *A.* (*Odontacartia*) *ohtsukai* Ueda & Bucklin, 2006. *Acartia ohtsukai* was previously identified as *A.* (*O.*) *pacifica* Steuer, 1915, but it was distinguished from the latter by morphological and molecular analyses (Ueda & Bucklin 2006). Recently, a similar case was reported for an *Acartia* species from the Philippines (Srinui *et al*. 2019); that is, *A.* (*O.*) *edentata* Srinui, Ohtsuka, Metillo & Nishibori, 2019 which was previously identified as *A. pacifica*. This study shows a similar case for another cryptic species of the genus from the Philippines.

One (SOS) of us had an opportunity to sample zooplankton at river mouths in the northernmost Luzon, the Philippines, in 2009. The samples contained an undescribed copepod closely related to *A. sinjiensis*. A genetic comparison with *A. sinjiensis* specimens in our collections from Japan confirmed that the specimens from Luzon are an independent species. Hence, herein, we describe them as a new species by distinguishing it from closely related species.

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Materials and methods

Copepods were collected from the Cagayan River estuary in the northernmost Luzon, the Philippines on February 2, 2009 and February 16, 2010, and from an estuary of the Pata River, approximately 58 km northeast of the Cagayan River, on February 15, 2010. Sampling was carried out by vertical hauls with a 0.2-mm mesh plankton net from the bottom of the Cagayan River and by oblique tows with the same net from the mid-depth in the Pata River using a bout. Immediately after sampling, the specimens were collected using a 0.2-mm mesh and fixed in 99.5% ethanol. The temperature and salinity of the surface and bottom waters in each sampling site were measured using a conductivity meter (DKK-TOA, CM-21P). Specimens of the new species were collected from 15 other sites (from 18.328861°N, 121.638444°E to 18.253917°N, 121.679500°E) in the Cagayan River, and three sites (from 18.621167°N, 121.155056°E to 18.613167°N, 121.159611°E) in the Pata River. The range of the surface and bottom water temperatures was 24.3–29.3°C and 24.2–28.5°C, respectively, and that of salinity was 0.1–8.3 and 3.2–28.8, respectively. The salinity in the sampling site in the Cagayan River where the species was the most abundant was 2.9 at the surface and 12.3 at the bottom.

For morphological examination, the specimens were stained with 0.1% chlorazol-black E solution and were dissected in lactophenol. Morphological examination, drawing, and measurements were performed under a differential interference microscope (Nikon Eclipse E600, Nikon, Japan) equipped with a drawing tube and an ocular micrometer. Illustrations for printing were prepared using computer illustration software (Adobe Illustrator®). The morphological terminologies followed those of Boxshall & Halsey (2004). The type specimens have been deposited in the National Museum of Nature and Science, Tokyo (NSMT). The morphology of *A. sinjiensis* collected from a milkfish farming pond in Panay, the Philippines, in late July of 1987, was used for comparison with the new species. The specimens were provided by Dr. Atsushi Ohno in 1991 to one of the authors (HU).

The genomic DNA was extracted from specimens following the methods described by Sakaguchi & Ueda (2010). The mitochondrial gene cytochrome oxidase subunit I (COI) sequences were analyzed for eight female specimens of the new species each from the Cagayan and Pata Rivers, and two female specimens of *A. sinjiensis* each from the Yamakuni River in Kyushu Island and the Ayaragi River in mainland Japan. The COI region was PCR-amplified using primers LCO1490 and HCO2198 (Folmer *et al.* 1994). The PCR was performed using TaKaRa Ex Taq (TaKaRa, Japan) under the following conditions: first 5 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 1 min, extension at 72°C for 1 min; second 35 cycles at 94°C for 30 s, 50°C for 1 min, and 72°C for 1 min. The PCR products were purified using the High Pure PCR Product Purification Kit (Roche, Germany) and directly sequenced with the 3130xl Genetic Analyzer DNA autosequencer (Applied Biosystems, USA) using the BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturers' instructions. The nucleotide sequences reported in the present study have been deposited in the DDBJ/EMBL/GenBank databases under accession nos. AB780968–AB780975 for *A. cagayanensis* **sp. nov.** and AB910756–AB910759 for *A. sinjiensis.*

The COI sequences were aligned using MEGA version X (Kumar *et al.* 2018) and ClustalX 2.1 (Larkin *et al.* 2007). The COI pairwise p-distances were calculated using MEGA X. The maximum likelihood (ML) phylogenetic trees and the corresponding bootstrap support values (1000 replicates) were calculated using MEGA X software with the resulting datasets. Initial trees for a heuristic search were constructed automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach. For these datasets, Bayesian analyses were performed using MrBayes v3.2.5 (Ronquist *et al.* 2012). Six parallel metropolis-coupled Markov chain Monte Carlo (MCMCMC) runs, each consisting of three heated chains and one cold chain with default chain temperatures, were run for 1,000,000 generations. Log-likelihood scores and trees with branch lengths were sampled every 1000 generations. The first 250,000 generations were excluded as burn-in, and the remaining trees were summarized to obtain Bayesian posterior probabilities. Convergence of parallel MCMCMC runs was judged by the average standard deviation of split frequencies. For ML and Bayesian analyses, the most appropriate models selected with MEGA X were applied. *Acartia tsuensis* (DDBJ/EMBL/GenBank accession no. AB910760) was used as the outgroup.

Results

Taxonomy

***Acartia* (*Acanthacartia*) *cagayanensis* sp. nov.**
(Figs. 1–3)

Material examined. Female holotype (NSMT-Cr 28389) and male allotype (NSMT-Cr 28390) collected from the mouth of the Cagayan River on February 2, 2009. Paratypes: 2 females and 2 males were collected from the mouth of the Cagayan River on February 2, 2009 (NSMT-Cr 28391– 28394) and 10 female specimens were collected from the river on February 16, 2010 (NSMT-Cr 28395). Furthermore, 46 females and 2 males were collected from the mouth of the Pata River on February 15, 2010.

Descriptions

Female. Body (Fig. 1A, B) length 0.83–0.95 mm (n = 5, holotype 0.95 mm); prosome length 0.22–0.26 mm (n = 5). Rostral filaments (Fig. 1C) almost straight in ventral view. Posterior corner of prosome (Fig. 1A, B, D) rounded, with row of posterodorsal spinules on each side; dorsalmost spinule larger than other spinules. Genital double somite (Fig. 1D, E) as long as wide, barrel-shaped especially in lateral view by having round ventral surface, with irregularly arranged many spinules on posterodorsal margin. Second urosomite with many spinules grouped in 4 loci along posterodorsal margin; spinules slightly larger than those on genital double somite. Anal somite naked on ventral surface. Caudal ramus length 1.7–1.9 times (n = 3, holotype 1.7) width, as long as second urosomite, with hairs proximal to lateral seta.

Antennule (Fig. 1F) 17-segmented, reaching posterior end of prosome, without row of spinules on any segments; setal formula (Roman numerals represent ancestral segments) 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 = 1, (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 = 5 + ae.

Antenna (Fig. 1G), coxa with medial seta; basis and first endopodal segment forming allobasis bearing 8 setae at mid part and 1 seta near distal corner on medial margin, and distolateral row of spinules; second endopodal segment with 8 setae medially and fine short hairs laterally; third endopodal segment with 6 setae; exopod 4-segmented, with 1, 2, 2, 3 setae.

Mandible (Fig. 1H), basis with distal seta and group of spinules on medial margin; exopod 5-segmaneted, with 1, 1, 1, 1, 2 setae; endopod 2-segmented, with 2, 9 setae.

Maxillule (Fig. 2A), precoxal endite bearing 9 spiniform setae, proximal one of them armed with strong spinules, and short spinules on lump; coxal endite with 3 setae, coxal epipodite with 9 setae; basis with 1 medial and 1 lateral setae; exopod partly fused with 5 distal and 2 lateral setae; endopod absent.

Maxilla (Fig. 2B), 4 syncoxal and 1 basal endites, with 3, 2, 2, 3, 2 setae; endopod 3-segmented, with 2, 1, 2 setae.

Maxilliped (Fig. 2C) 4-segmented; syncoxa with 5 setae, and row of spinules on anterior surface; basis with short seta; endopod 2-segmented, with 3, 2 naked spiniform setae fused to segment.

Legs 1–4 (Figs 3D–G) normal to genus. Seta and spine formula of legs 1–4 as follows:

	Coxa	Basis	Exopodal segment	Endopodal segment
Leg 1	0-0	0-0	1-1; 1-1; 2-1-4	0-1; 1-2-3
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

Leg 5 (Fig. 2H, I), basis approximately 1.7 times longer than wide; exopod (=terminal segment) consisting of basal part and blade-shaped long attenuation; basal part approximately 2 times longer than wide, cylindrical with lateral corner produced distally; blade-shaped attenuation inclined inward against axis of base, about 5 μm at widest, 2 times longer than basis, slightly longer than lateral seta, with fine teeth on distal 2/3 of both margins.

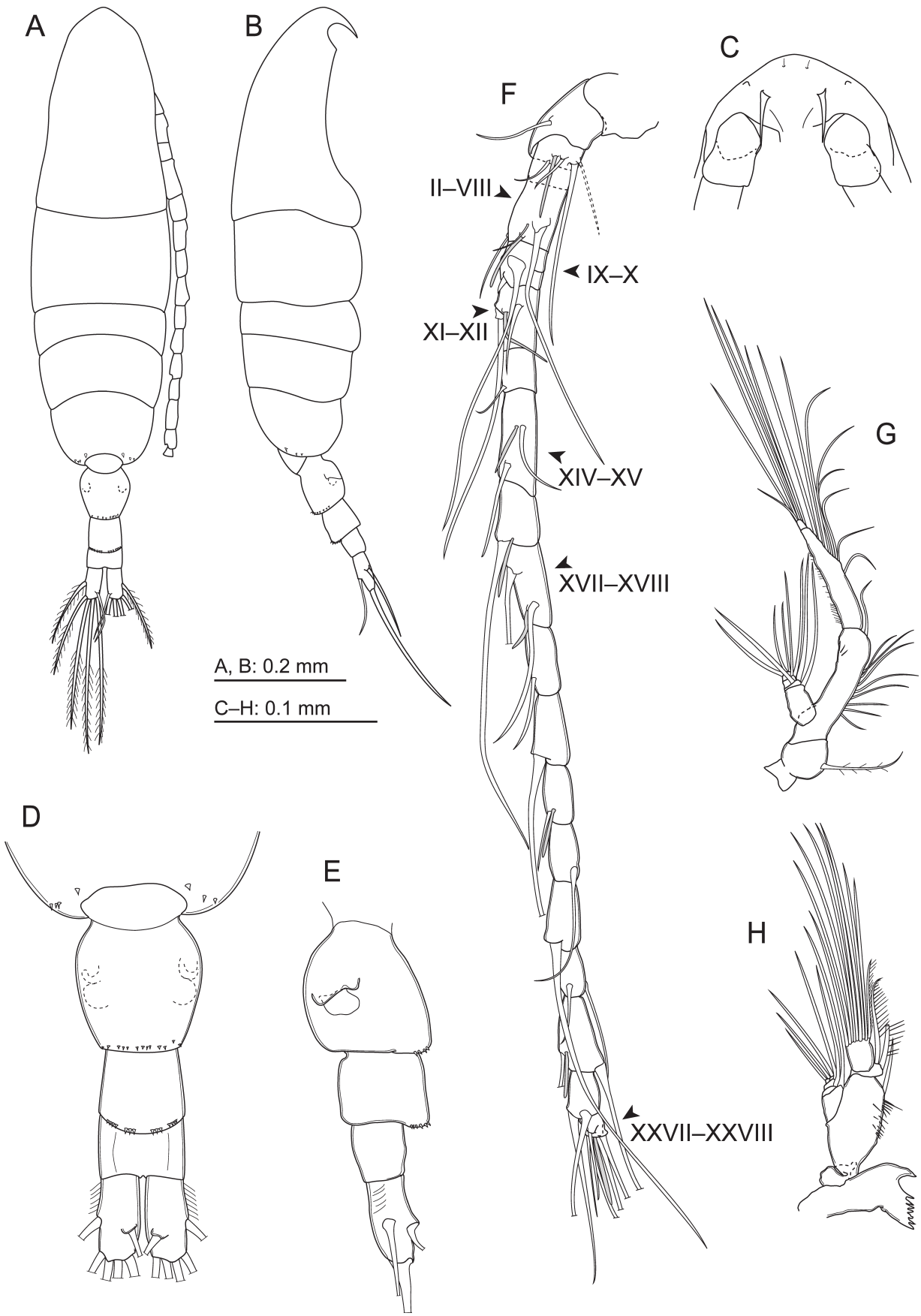

FIGURE 1. *Acartia cagayanensis* **sp. nov.** female (A–F, H, holotype; G, paratype from the Cagayan River). A, habitus, dorsal; B, habitus, right lateral; C, rostrum, ventral; D, fifth pediger and urosome, dorsal; E, urosome, left lateral; F, right antennule, ventral; G, right antenna; H, right mandible.

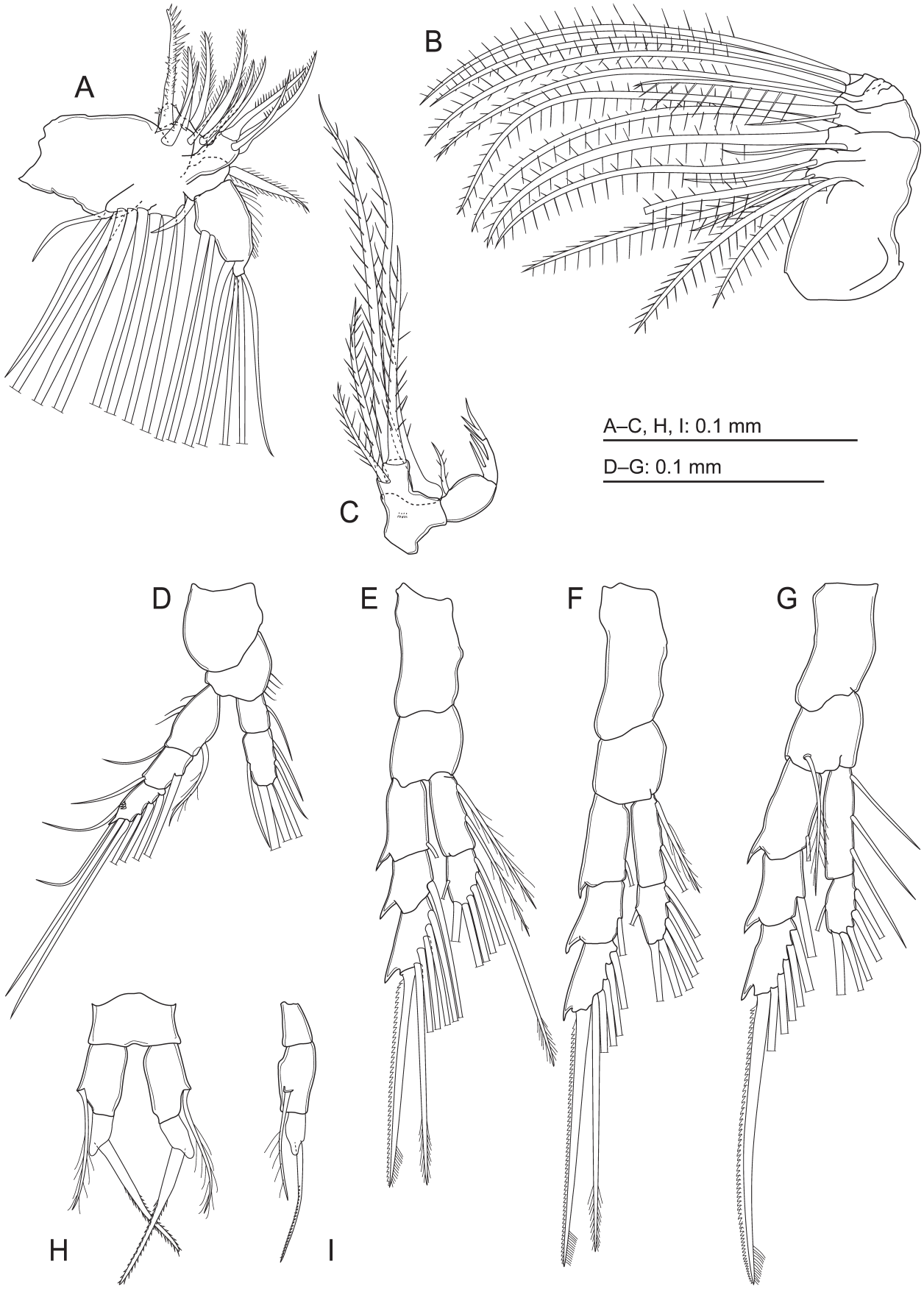

FIGURE 2. *Acartia cagayanensis* **sp. nov.** female (A–G, I, holotype; H, paratype from the Cagayan River). A, left maxillule; B, left maxilla; C, right maxilliped; D–H, legs 1–5, posterior; I, right leg 5, right lateral.

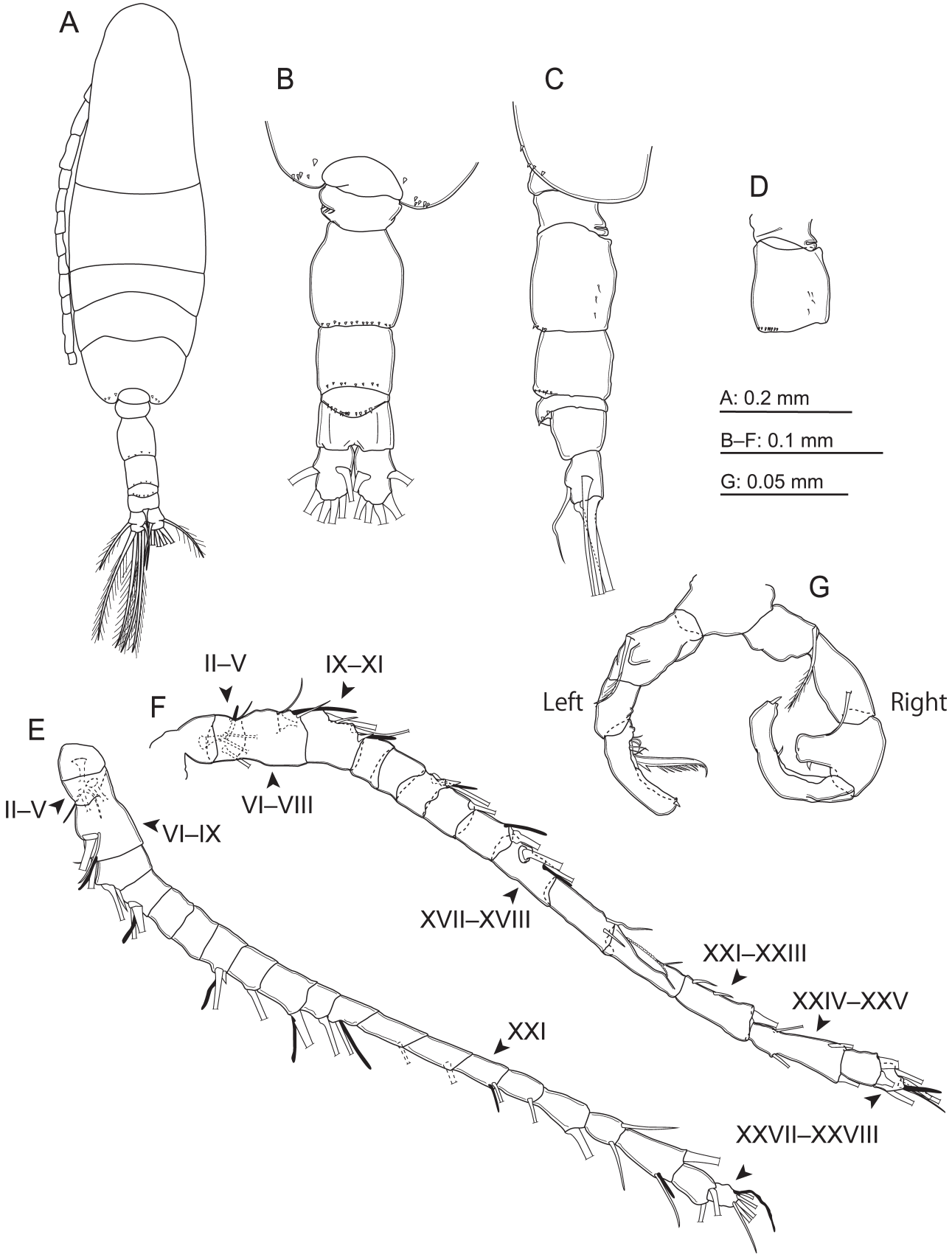

FIGURE 3. *Acartia cagayanensis* **sp. nov.** male (A–C, allotype; D–G, paratype from the Cagayan River). A, habitus, dorsal; B, fifth pediger and urosome, dorsal; C, fifth pediger and urosome, right lateral; D, first and second urosomites, right lateral; E, left antennule, dorsal; F, right antennule, dorsal; G, leg 5, posterior.

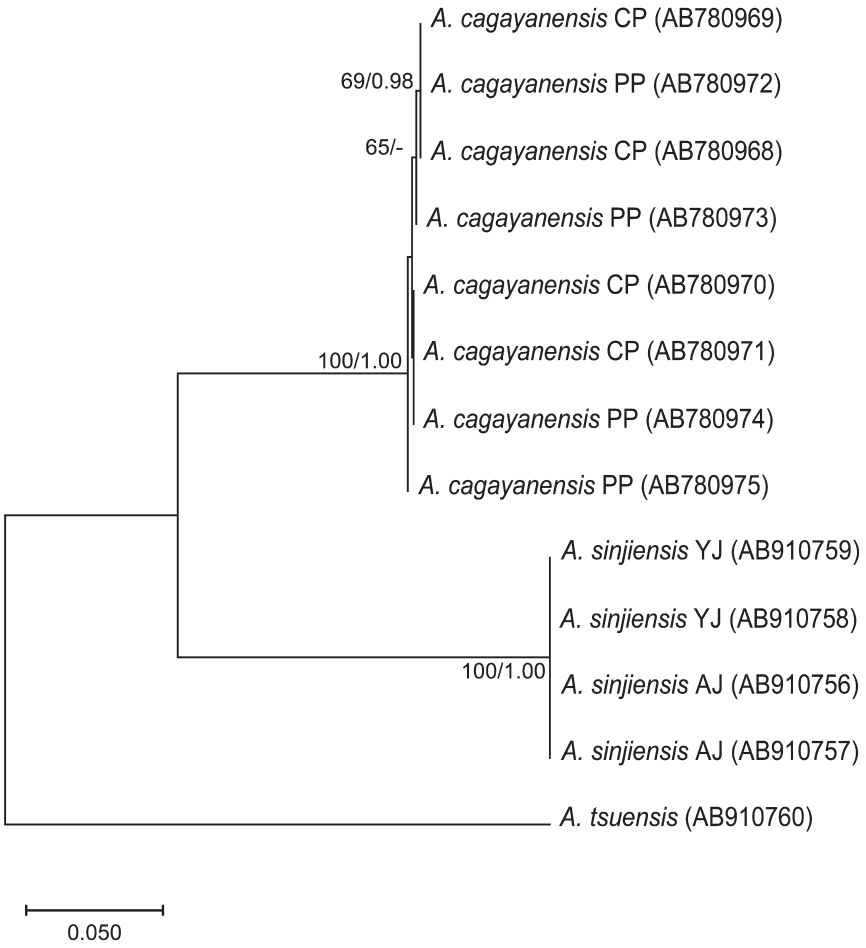

FIGURE 4. Maximum likelihood (ML) phylogenetic trees based on COI gene of *Acartia cagayanensis* **sp. nov.** from the Cagayan (CP) and Pata (PP) Rivers in the Philippines, *A. sinjiensis* from the Ayaragi River (AJ) in the mainland and the Yamakuni River (YJ) in Kyushu Island, Japan, and *A. tsuensis* (outgroup). ML bootstrap probabilities and Bayesian posterior probabilities for bipartitions with over 65% and 0.95 support, respectively, are shown. DDBJ/EMBL/GenBank accession nos. are provided in parentheses.

Male. Body (Fig. 3A) length 0.70–0.79 mm (n = 3, allotype 0.79 mm); prosome length 0.50–0.58 mm (n = 3); cephalosome slightly shorter than metasome. Armature on posterior corner of prosome as in female (Fig. 3A, B). Second urosomite (Fig. 3B–D) with row of many spinules on posterodorsal margin and longitudinally-located 3 or 4 hair-like spinules on each ventrolateral side; third and fourth urosomites each with row of spinules on posterodorsal margin; posterodorsal spinules on fourth urosomite slightly larger than those on second and third urosomites. Caudal ramus about 1.3 times longer than wide.

Left antennule (Fig. 3E) 21-segmented; 2nd and 3rd segments incompletely fused; setal formula as follows: (1) I = 1, (2) II–V= 3 + ae, (3) VI–IX = 4 + ae, (4) X = 2 (1 spine), (5) XI = 2 + ae, (6) XII = unarmed, (7) XIII = unarmed, (8) XIV = 2 (1 spine) + ae, (9) XV =1, (10) XVI = 1 + ae, (11) XVII = 1, (12) XVIII = 1 + ae, (13) XIX = 1, (14) XX = 1, (15) XXI = 1 + ae, (16) XXII = 1, (17) XXIII = 1, (18) XXIV = 2, (19) XXV = 2 + ae, (20) XXVI = 2, (21) XXVII–XXVIII = 5 + ae. Right antennule (Fig. 3F) 16-segmented with geniculation between 12th and 13th segments; 2nd to 4th segments incompletely fused; setal formula as follows: (1) I = 1, (2) II–V= 4 + ae, (3) VI–VIII = 3 + ae, (4) IX–XI = 4 (2 spines) + ae, (5) XII = unarmed, (6) XIII = unarmed, (7) XIV = 2 (1 spine) + ae, (8) XV = 1, (9) XVI = 1 + ae, (10) XVII–XVIII = 2 + ae, (11) XIX = 2 (1 spine), (12) XX = 1 + process, (13) XXI–XXIII = 3 (2 spines), (14) XXIV–XXV = 4, (15) XXVI = 2, (16) XXVII–XXVIII = 5 + ae.

TABLE 1. Morphological comparison of the three species belonging to *Acartia plumosa* group and the present new species. The morphological features of *A. plumosa*, *A. sinjiensis*, and *A. tropica* followed Ueda & Hiromi (1987). Abbreviations: GS, genital double somite; US2–4, urosomites 2–4; AS, anal somite.

	A. plumosa	*A. sinjiensis*	*A. tropica*	*A. cagayanensis* **sp. nov.**
Female				
Spinules on posterior corner of prosome	2 rows, spinules of outer row large	1 row of several small spines	1 row of several small spines	1 row of a few small spines
Relative size of dorsal spinules between GS and US2	GS ≒ US2	GS = US2	GS < US2	GS slightly < US2
Ventral setules on GS	3 rows	absent	several rows	absent
Ventral setules on AS	present	absent	Present	absent
Caudal ramus length/width	1.5-1.7	1.6-1.9	1.9-2.2	1.7–1.9
Antennular segment with spinule row	16th (penultimate) segment	absent	9th to 12th segments	absent
Basal part of leg 5 terminal segment	knob-like	knob-like	knob-like	cylindrical
Male				
Relative size of dorsal spinules among US2-4	US2>US3≒US4	US2>US3<US4	US2<US3=US4	US2=US3 slightly <US4
Ventral setules on AS	present	absent	Present	absent
Distal margin of medial process on 2 exopodal segment of right leg 5	bilobed	bilobed	Flat	flat
Distal half of terminal segment of left leg 5	tapering	tapering	not tapering	not tapering

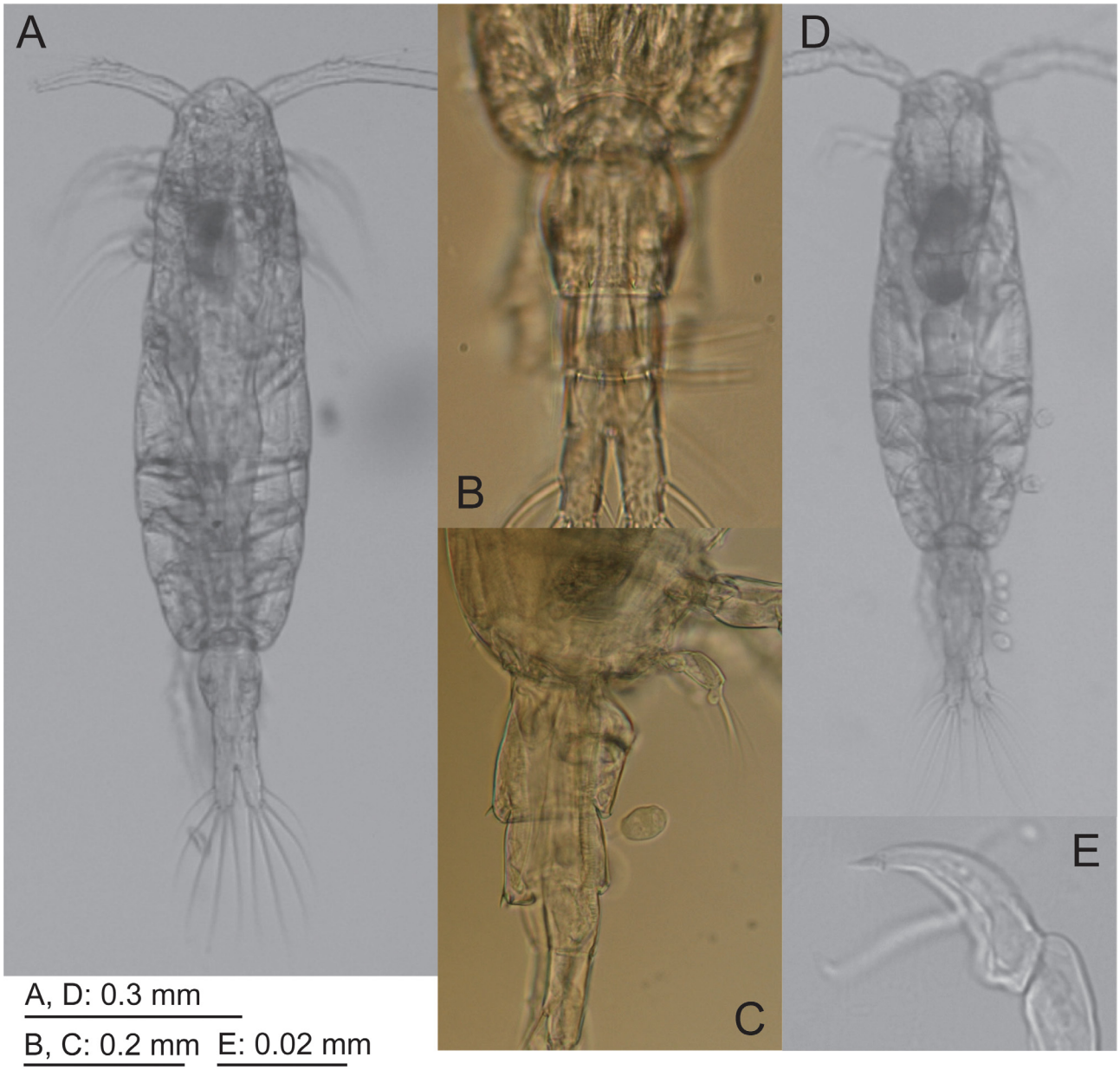

FIGURE 5. *Acartia sinjiensis* collected from a milkfish farming pond in Panay, the Philippines. A, female habitus; B, female urosomites, dorsal; C, female urosomites and leg 5, lateral; D, male habitus; E, terminal segment of the male left leg 5.

Left leg 5 (Fig. 3G), basis with thumb-like posterior process and 1 lateral seta; second exopodal segment (=terminal segment) slightly longer than basis, not tapering at distal half, medially with several hairs on proximal region, terminally-hooked long spine at 1/3 length, and short apical spine at tip; hooked medial spine 0.8–1.0 (allotype 1.0) times longer than segment. Right leg 5 basis with medially 2 depressions and laterally seta; first exopodal segment with posterior seta; second segment with medial process bearing small spine on distal side of segment; third segment with 1 medial and 1 apical spines.

*Etymology***.** The new species is named after the Cagayan River, where this it was collected.

Type locality. Female holotype, male allotype and paratypes were collected from the mouth of the Cagayan River (18.276917°N, 121.670167°E). The surface and bottom temperatures of the Cagayan River on February 2, 2009 and February 16, 2010 were 27.0–28.5°C and 26.1–27.8°C, respectively, and the salinities were 0.9–8.1 and 6.5–28.8, respectively.

TABLE 2. Pairwise percent differences among *Acartia cagayanensis* **sp. nov.** from the Cagayan (CP) and Pata (PP) Rivers in the Philippines, *A. sinjiensis* from the Ayaragi River (AJ) in the mainland and the Yamakuni River (YJ) in Kyushu Island, Japan, and *A. tsuensis* for the COI sequences. DDBJ/EMBL/GenBank accession nos. are provided in parentheses.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*A. cagayanensis* **sp. nov.** CP (AB780968)													
2	*A. cagayanensis* **sp. nov.** CP (AB780969)	0.000												
3	*A. cagayanensis* **sp. nov.** CP (AB780970)	0.004	0.004											
4	*A. cagayanensis* **sp. nov.** CP (AB780971)	0.004	0.004	0.000										
5	*A. cagayanensis* **sp. nov.** PP (AB780972)	0.000	0.000	0.004	0.004									
6	*A. cagayanensis* **sp. nov.** PP (AB780973)	0.002	0.002	0.002	0.002	0.002								
7	*A. cagayanensis* **sp. nov.** PP (AB780974)	0.004	0.004	0.000	0.000	0.004	0.002							
8	*A. cagayanensis* **sp. nov.** PP (AB780975)	0.005	0.005	0.002	0.002	0.005	0.004	0.002						
9	*A. sinjiensis* AJ (AB910756)	0.169	0.169	0.167	0.167	0.169	0.167	0.167	0.165					
10	*A. sinjiensis* AJ (AB910757)	0.169	0.169	0.167	0.167	0.169	0.167	0.167	0.165	0.000				
11	*A. sinjiensis* YJ (AB910758)	0.169	0.169	0.167	0.167	0.169	0.167	0.167	0.165	0.000	0.000			
12	*A. sinjiensis* YJ (AB910759)	0.169	0.169	0.167	0.167	0.169	0.167	0.167	0.165	0.000	0.000	0.000		
13	*A. tsuensis* (AB910760)	0.218	0.218	0.218	0.218	0.218	0.216	0.218	0.216	0.232	0.232	0.232	0.232	

*Remarks***.** *Acartia cagayanensis* **sp. nov.** belongs to the subgenus *Acanthacartia* Steuer, 1915 based on the following three morphological characters: presence of rostral filaments, round posterior corner of the prosome, and male right leg 5 without a distomedial lobe in the first exopodal segment (Steuer 1923; Bradford-Grieve 1999). The last feature is distinct to *Acanthacartia* belonging to the subgenus *Acartia*, which has a long distomedial lobe in the segment. *Acanthacartia* currently consists of 18 species (Walter & Boxshall 2019). Of these species, the closely related *A. plumosa* Scott T., 1894, *A. sinjiensis*, and *A. tropica* Ueda & Hiromi, 1987 form the *A. plumosa* group (Ueda & Hiromi 1987). The characteristic morphological features of the group described by Ueda & Hiromi (1987) are as follows: in the female, the terminal segment of leg 5 with a basal knob-like part, projecting noticeably backward; in the male, left leg 5 with a thumb-like process on the basis, the long terminal segment, which is as long as the basis, and a short apical spine. Among these features, the short apical spine on the long terminal segment of male left leg 5 is unique to the group. Leg 5 of *A. cagayanensis* **sp. nov.** has the same characteristic features as that of the *A. plumosa* group. However, they differ in the following female morphological features. The genital double somite is barrel-shaped in the new species, whereas rectangular shape in the dorsal view is common to the *A. plumosa* group (Fig. 2 in Ueda & Hiromi (1897)) (Fig. 5B). The basal part of the terminal segment of leg 5 is cylindrical in the new species, in contrast to knob-like in the *A. plumosa* group (Fig. 5C).

Acartia cagayanensis **sp. nov.** is distinguished from each species belonging to the *A. plumosa* group, except for *A. sinjiensis*, by the following characteristics: 1) the posterior corner of the female prosome has a single row of spinules, of which the dorsal-most one is larger than the others (*A. plumosa* has two rows of spinules), 2) the ventral setules on the genital double somite and anal somite in both sexes are absent, 3) the female antennule has no spinular row on any segment (the spinular row is present on the 16th segment in *A. plumosa* and on the 9–12 segments in *A. tropica*) (Table 1). These characteristics are common to *A. sinjiensis*.

Acartia cagayanensis **sp. nov.** differs from *A. sinjiensis* in the following morphological characteristics: 1) numerous spinules on the posterodorsal margins of the urosomites except for the anal somite (usually only 4–6 spinules in *A. sinjiensis*) and 2) the terminal segment of male left leg 5 not tapering distally (tapering in *A. sinjiensis* (Fig. 5E)).

*Distribution***.** The new species is known to exist only in the Cagayan River (type locality) and the Pata River. *Bestiolina* Andronov, 1991 sp. was dominant in all samples in which *Acartia cagayanensis* **sp. nov.** occurred, followed by *Parvocalanus crassirostris* (Dahl F., 1894) and *Pseudodiaptomus* Herrick, 1884 spp.

Genetic diversity. The DNA sequence of the 569-bp fragment of COI of the individuals was determined. In the pairwise genetic distance comparisons, the COI sequence differed by 16.5–16.9% between *Acartia cagayanensis* **sp. nov.** and *A. sinjiensis*, in contrast to the small variation (0.0–0.5%) within each population (Table 2). The genetic differences of these species from *A. tsuensis* used as the outgroup species was 21.6–23.2%. The phylogenetic tree demonstrated a clear separation between *A. cagayanensis* **sp. nov.** and *A. sinjiensis* (Fig. 4).

Discussion

The present study revealed a genetic difference of 16.5–16.9% in the COI sequence between *Acartia cagayanensis* **sp. nov**. and *A. sinjiensis*. This is similar to the 16–18% between *A. (Odontacartia) pacifica* and *A. (O.) edentata* (Srinui *et al*. 2019), and slightly more than 15.6% between *A. pacifica* and *A.* (*O.*) *spinicauda* Giesbrecht (Liu *et al*. 2008) and 13.7% between different clades of *A. (Acanthacartia) tonsa* Dana, 1849 (Chen & Hare 2008). Chen & Hare (2008) strongly suggested that the different clades of *A. tonsa* comprised reproductively isolated cryptic species. Speciation of cryptic copepods with a COI genetic difference of <20% has also been reported for the estuarine calanoid *Pseudodiaptomus yamato* Ueda & Sakaguchi, 2019 and *P. japonicus* Kikuchi K., 1928 (Ueda & Sakaguchi 2019), which were formerly known as *P. inopinus* Burckhardt, 1913 and differed by 12–15% in the COI region (Sakaguchi & Ueda 2018). Although the evolution rate depends on the taxa, these studies indicate that a genetic difference of <20% in the COI region is common for the speciation of estuarine copepods.

According to Razouls *et al.* (2005–2020), the species belonging to the subgenus *Acanthacartia* from the Philippines is only *A. tsuensis*. However, Metillo (2012) recorded *A. sinjiensis* from Mindanao, the southern Philippines; the photographs of *A. sinjiensis* (Fig. 22 in Metillo 2012) show the characteristic features of the species, that is, the terminal segment of female leg 5 with a knob-like basal part and blade-shaped attenuation, and the second exopodal

segment of male right leg 5 having the medial process with a bilobed distal margin (Table 1). A previous study on the population dynamics of *A. tsuensis* in a brackish-water fishpond in Guimbal, Panay Island, the middle Philippines provided another record of *A. sinjiensis* from the Philippines (Golez *et al.* 2002). In this pond, *A. sinjiensis* and *A. tsuensis* co-occurred at high abundances (>1,000 individuals per liter) from December to March (dry season) and from March to May (hot season), respectively (Golez *et al.* 2002). One (HU) of us has *A. sinjiensis* specimens collected from this pond, which were sent by Dr. Atsushi Ohno, a coauthor of the previous study, for species identification. Re-examination of the specimens confirmed that these are *A. sinjiensis*, based on the knob-like basal part of the terminal segment of female leg 5, the genital double somite with posterodorsal spinules as large as those on the next urosomite, and the anal somite without ventral setules (Fig. 5, Table 1). Thus, *A. cagayanensis* **sp. nov.** is the third *Acanthacartia* species in the Philippines, after *A. tsuensis* and *A. sinjiensis*, and the distribution of the new species is presumably limited to Luzon. Further studies on the geographical distribution of *A. cagayanensis* **sp. nov.** and *A. sinjiensis* in the Philippine Islands are necessary, because their distribution may provide novel insights on their speciation in relation to the geographical history of the islands.

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