Zootaxa 2623: 52–68 (2010)
www.mapress.com/zootaxa/

Article

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

A new species of *Pseudodiaptomus* (Copepoda: Calanoida) from Japan, with notes on the closely related *P. inopinus* Burckhardt, 1913 from Kyushu Island

SAKIKO ORUI SAKAGUCHI & HIROSHI UEDA[1]
Usa Marine Biological Institute, Kochi University, 194 Inoshiri, Usa, Tosa, Kochi 781-1164, Japan
[1]Corresponding author. E-mail: hueda@kochi-u.ac.jp

Abstract

A new species of the calanoid copepod, *Pseudodiaptomus nansei* **sp. nov.**, which has formerly been identified as *P. inopinus* Burckhardt, 1913, is described from estuaries of the Nansei Islands, southernmost Japan. This new species was compared with a population of the closely related *P. inopinus* from the neighboring Kyushu Island, western Japan, which is redescribed here. The new species is readily distinguishable from *P. inopinus* by the absence of dorsal spiniform processes on the fifth pediger in both sexes, short posterior projections of the genital operculum, and thin caudal setae of the female instead of swollen seta in *P. inopinus*. The mitochondrial gene cytochrome oxidase subunit I (mtCOI) sequences of *P. nansei* differed by 21–26% from the examined population of *P. inopinus*, in contrast to a 0–3% difference within the population of each species. The absence of descriptions identifiable to *P. nansei* in previous studies outside the Nansei Islands, coupled with no occurrence of *P. inopinus* there, suggests that *P. nansei* is endemic to Nansei Islands and geographically segregated from *P. inopinus*.

Key words: Nansei Islands, estuary, mtCOI, zoogeography

Introduction

The calanoid copepod genus *Pseudodiaptomus* Herrick, 1884 predominantly occurs in estuarine and coastal marine waters and presently comprises 77 species (Walter & Boxshall 2009). The temperate species *P. inopinus* Burckhardt, 1913 was originally described from a lake in China (Burckhardt 1913), and it is widely distributed in both fresh and brackish waters of East Asia, i.e. China (Shen & Tai 1962), Korea (Chang & Kim 1986; Lee *et al.* 2007; Chang 2009) and Japan (Kikuchi 1928; Mizuno & Miura 1984; Kawabata & Defaye 1994). One distinctive morphological characteristic of *P. inopinus* is the presence of very thick caudal setae on the female. However, Oka *et al.* (1991) recorded *P. inopinus* specimens having thin instead of thick setae from the Nansei Islands between Taiwan and Kyushu Island, western Japan, and suggested that thin caudal setae represent geographical variation within the species. In our investigation we also collected the same type of specimens from the Nansei Islands. Our morphological and molecular comparisons of these specimens with *P. inopinus* specimens with thick caudal setae collected from Kyushu Island revealed that specimens with thin setae from the Nansei Islands belong to a separate species. We herein describe the specimens collected from the Nansei Islands as *P. nansei* **sp. nov**. along with specimens identifiable as *P. inopinus* from Kyushu, and present the results of a genetic comparison between two.

Material and methods

Sample collection and examination. Zooplankton from brackish water was collected from 39 rivers and bays of seven islands of the Nansei Islands between 9 and 15 October 2008 and from 12 rivers of Kyushu between 18 and 21 August 2009 (Fig. 1). Collections were made by towing a plankton net with 0.2-mm mesh from the

 Accepted by T. Karanovic: 31 Aug. 2010; published: 23 Sep. 2010

shore or from a bridge. Immediately after sampling, specimens were sieved through a 0.2-mm mesh and preserved in 99.5% ethanol. Environmental data of surface and bottom water temperature and salinity were measured at the each sampling site using a conductivity meter (DKK-TOA CM-21P). Specimens were stained with a 0.1% chlorazol-black E solution and dissected in lactophenol. Morphological examinations, drawings, and measurements were made under a differential interference microscope (Nikon Eclipse E600) with a drawing tube and an ocular micrometer. Illustrations for printing were made with the aid of computer illustration software (Adobe Illustrator®). The type specimens were deposited in the National Museum of Nature and Science, Tokyo (formerly National Science Museum, Tokyo, NSMT) and the U.S. National Museum of Natural History in Washington (USNM). Morphological terminology follows Boxshall and Halsey (2004).

Molecular analysis. DNA sequences were determined for 631 base-pair regions of the mitochondrial gene cytochrome oxidase subunit I (mtCOI) for two male and two female specimens collected from the Hirakubo-gawa River, Ishigaki-jima Island, and two male and two female specimens collected from the Tsuri-kawa River, Kyushu. Total genomic DNA was extracted by placing individual copepods in microcentrifuge tubes with 8 mL of a buffer (10 mM Tris-HCl buffer pH 8.2), 1 mM EDTA, and 25 mM NaCl, then heated at 95°C for 5 min using a thermal cycler (Takara TP400 or TP240). After cooling the solutions in an ice bath, 2 µL of 2 mg/mL proteinase K was added to each solution, and the solutions were incubated at 37°C for 1 hr then warmed to 95°C for 5 min. The PCR reagents (50 µL) contained: distilled water, 2.5 µL of 10X PCR buffer, 2.0 µL of dNTP mixture (2.5 mM each), 1.0 µL of each primer (100 µM), 0.13 µl of 0.65 units Ex Taq (Takara, Japan) and 10 µL of template. PCR primers for mtCOI were LCO1490 and HCO2198 (Folmer *et al.* 1994). Reaction conditions were as follows: initial denaturation at 94°C for 1 min; 5 cycles at 94°C for 30 sec, 50°C for 1 min and 72°C for 1 min; 35 cycles of 94°C for 30 sec, 50°C for 1 min and 72°C for 1 min; final extension at 72°C for 3 min. PCR products were purified using the High Pure PCR Product Purification Kit (Roche, Germany) and sequenced directly using the BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing products were ethanol precipitated and run on a DNA autosequencer ABI PRISM3100-*Avant* Genetic Analyzer (Applied Biosystems, USA) according to the manufacturer's protocols.

The mtCOI sequences were aligned using Clustal X version 2.0 (Larkin *et al.* 2007). Pairwise distance measures and phylogenetic analyses were conducted in MEGA4 (Tamura *et al.* 2007). Ambiguous sites were eliminated from the dataset. There were a total of 631 positions in the final dataset. The Neighbor-Joining (Saitou & Nei 1987) tree with the Jukes-Cantor model was used for likelihood optimization. The bootstrap test (Felsenstein 1985) for NJ was carried out with 1000 replicates to evaluate statistical reliability. DNA sequences for *Pseudodiaptomus ishigakiensis* Nishida, 1985 collected from Kabira Bay on Ishigaki-jima Island on 10 October 2008 were acquired by the same procedure and they were used as outgroups for phylogenetic analysis.

Results

Pseudodiaptomus nansei **sp. nov.**
(Figs. 2, 3A, 4–6)

Synonym. *Pseudodiaptomus inopinus* Burckhardt, 1913, Oka *et al.* (1991), 85, fig. 3.

Type material. Female holotype (NSMT-Cr 21259), male allotype (NSMT-Cr 21260) dissected in lactophenol and mounted on 8 glass slides using CMC-10, aqueous mounting medium (Masters Company, Inc., Wood Dale, IL), and undissected 8 female and 10 male paratypes (NSMT-Cr 21261, 21262) in alcohol were deposited in the NSMT. Undissected 10 female and 10 male paratypes in alcohol were deposited in the USNM (USNM 1145707, 1145708). All type specimens (57 females and 48 males including the sequenced specimens) were collected from the mouth of the Hirakubo-gawa R. (24°35'39"N, 124°18'48"E, 10 October 2008, colls S.O. Sakaguchi and H. Ueda; #1 in Fig. 1) of Ishigaki-jima Island on 10 October 2008.

Other material examined. Five females and 4 males from the Miyara-gawa R. (24°21’31”N, 124°12’42”E, 10 October 2008; #2 in Fig. 1), southern Ishigaki-jima Island; 12 females and 4 males from the Yakashimoguchi-gawa R. (26°28’50”N, 127°50’48”E, 12 October 2008; #3) on the west coast, 1 male from the Tokuhina-gawa R. (26°27’16”N, 127°51’23”E, 12 October 2008; #4) and 8 males from the Ginozafukuchi-gawa R. (26°28’22”N, 127°57’05”E, 12 October 2008; #5) on the east of central Okinawa-jima Island; 1 female from the Chinaze-gawa R. (28°22’46”N, 129°26’47”E, 15 October 2008; #6) and 1 female from the Kawauchi-gawa R. (28°16’17”N, 129°18’28”E, 15 October 2008; #7) on the western coast, and 2 females and 8 males from the Yakugachi-gawa R. (28°15’17”N, 129°24’14”E, 15 October 2008; #8) and 5 females and 25 males from the Ura-gawa R. (28°24’36”N, 129°35’29”E, 15 October 2008; #9) on the eastern coast of Amami-ohshima Island. All the specimens were collected by S.O. Sakaguchi and H. Ueda.

Description. FEMALE (HOLOTYPE). Body (Fig. 2A, B) length 1.13 mm; prosome length 0.65 mm. Forehead rounded in dorsal and lateral views. Cephalosome and first pediger fused; fourth and fifth pedigers fused; dorsal and lateral surfaces of pedigers smooth, except 3–4 spinules on each posterolateral rounded corner of fifth pediger. Urosome symmetrical. Genital double-somite 1.3 times longer than wide, with several spinules on each anterolateral projection, long anterolateral seta and dorsolateral row of spinules at one-third anteriorly on each side; genital operculum (Figs. 2C, D, 3A) with broad hyaline frill laterally and rounded posterior process (indicated by arrowhead in Fig. 2C); second urosomite with dorsal row of spinules at on each side; genital double-somite and second and third urosomites with posterior row of spinules along dorsal margin. Caudal rami (Fig. 2E) symmetrical, 3.3 times longer than wide; 1 lateral and 4 terminal caudal setae thin and dorsal seta short and sinuate; lateral medial terminal caudal seta (longest caudal seta) twice longer than ramus.

Antennule (Fig. 2F) 22-segmented with incomplete suture between sixth to seventh segments; setal formula as follows: 1=1+ae (aesthetasc), 2 = 3 + ae, 3 = 2 + ae, 4 = 2 + ae, 5 = 3 + ae, 6 = 1 (spiniform), 7 = 2 + ae, 8 = 2 + ae, 9 = 2 + ae, 10 = 2(1 spiniform) + ae, 11 = 2 + ae, 12 = 2 + ae, 13 = 2 + ae, 14 = 2 + ae, 15 = 2, 16 = 2, 17 = 2 + ae, 18 = 1, 19 = 1, 20 = 2, 21 = 2, 22 = 6 + ae; 20th segment with seta bearing unique row of dense hairs (Fig. 2G).

Antenna (Fig. 4A) coxa and basis and first endopodal segment completely fused; coxa with seta; basis with 2 setae; first endopodal segment with 2 setae, second segment with 9 subterminal and 7 terminal setae; exopod 5-segmented, with setal formula 1, 5, 1, 2, 3 .

Mandible (Fig. 4B) basis with 4 setae; exopod 5-segmented, with seta on each first to fourth segments and 2setae on fifth segment; endopod 2-segmented, with 4 setae on first segment and 9 setae.

Maxillule (Fig. 4C) praecoxal arthrite with 9 spines and 6 setae; coxal endite with 4 setae, epipodite with 9 setae; basal endites with 3 and 5 setae, exite with seta; exopod with 9 seta; endopod 3-segmented, with setal formula 4, 4, 7

Maxilla (Fig. 4D) praecoxal with 4 setae on first endite and 3 setae on second endite; coxal endites with 3 and 3 setae; basal endite with 4 setae; endopod 4-segmented, with setal formula 2, 3, 2, 2.

Maxilliped (Fig. 4E) coxa with 2, 3, 4 setae on first to third endites; basis with 3 setae; endopod 6-segmented, with setal formula 2, 3, 2, 3, 3, 4; 2 setae on second segment and seta on third segment of endopod modified as shown in Fig. 4F and seta on fourth segment short and bearing teeth-like spinules.

Legs 1–4 (Fig. 5A–D, Table 1). Terminal spines on third exopodal segments of legs 1–3 with row of hairs along distal half of medial margin.

TABLE 1. Seta and spine formula of legs 1–4 of *Pseudodiaptomus nansei* **sp. nov.**

	Coxa	Basis	Exopodal segment	Endopodal segment
Leg 1	0-1	0-0	I-1; 0-1; II, I, 3	0-1; 0-1; 1, 2, 3
Leg 2	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 4
Leg 3	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 4
Leg 4	0-1	1-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 3

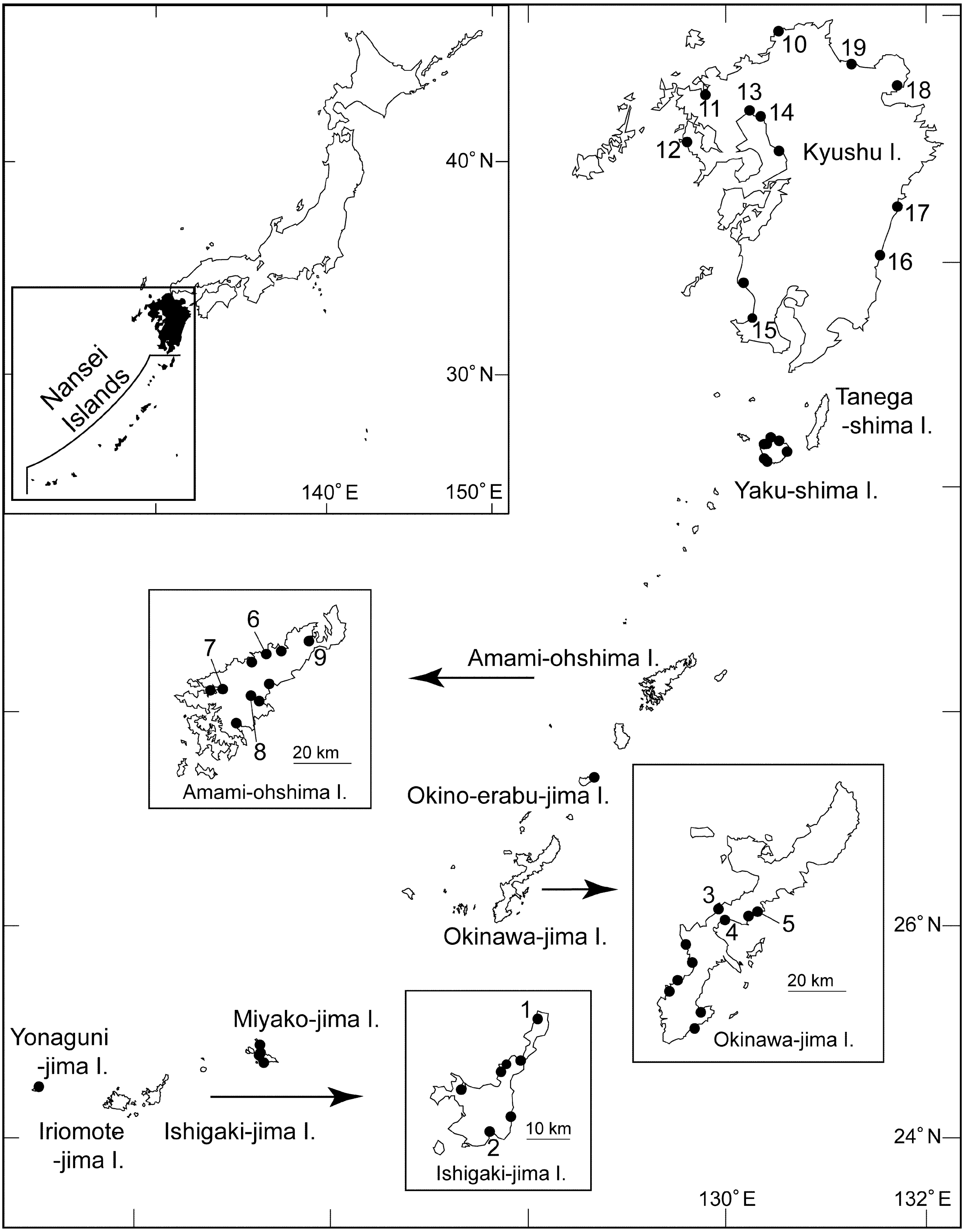

FIGURE 1. Map of the Nansei Islands and Kyushu Island. Locations of the rivers (R.) where sampling occurred are indicated by black spots, among which those with the numbers represent the rivers from which specimens of the new species or *P. inopinus* Burckhardt, 1913 were collected in the Nansei Islands (1, Hirakubo-gawa R.; 2, Miyara-gawa R.; 3, Yakashimoguchi-gawa R.; 4, Tokuhina-gawa R.; 5, Ginozafukuchi-gawa R.; 6, Chinaze-gawa R.; 7, Kawauchi-gawa R.; 8, Yakugachi-gawa R.; 9, Ura-gawa R.) and Kyushu Island (10, Tsuri-kawa R.; 11, Imari-gawa R.; 12, Yukiura-gawa R.; 13, Rokkaku-gawa R.; 14, Chikugo-gawa R.; 15, Manose-gawa R.; 16, Hitotsuse-gawa R.; 17, Shiomi-gawa R.; 18, Yasaka-gawa R.; 19, Yamakuni-gawa R.).

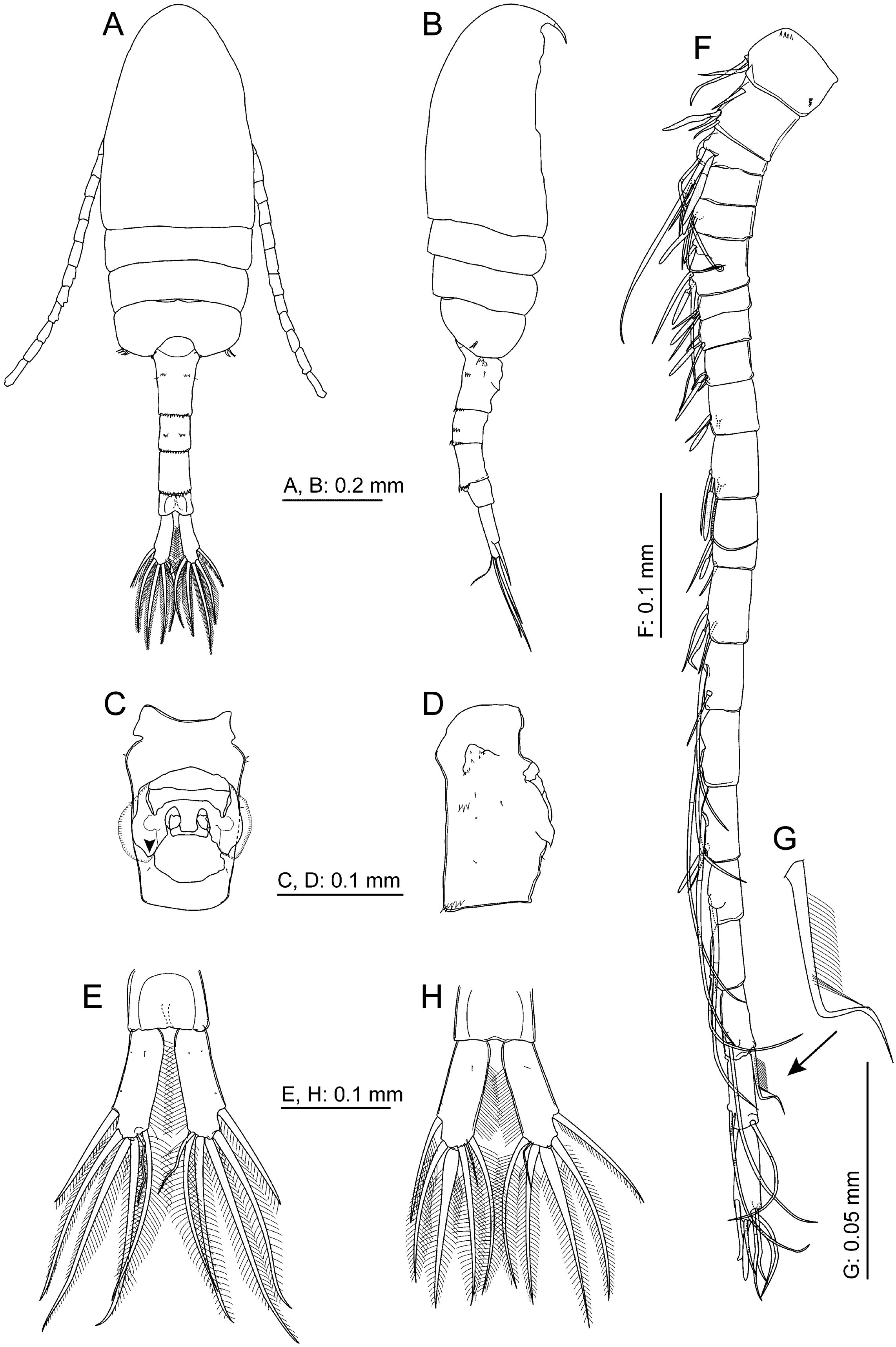

FIGURE 2. *Pseudodiaptomus nansei* **sp. nov.** Female (A–G, holotype; H, specimen from Yakugachi-gawa River, Amami-ohshima Island): A, habitus, dorsal; B, habitus, right lateral; C, genital double somite, ventral; D, genital double somite, right lateral; E, caudal rami, dorsal; F, antennule; G, seta on 20th segment of antennule; H, caudal rami with slightly thickened setae, dorsal.

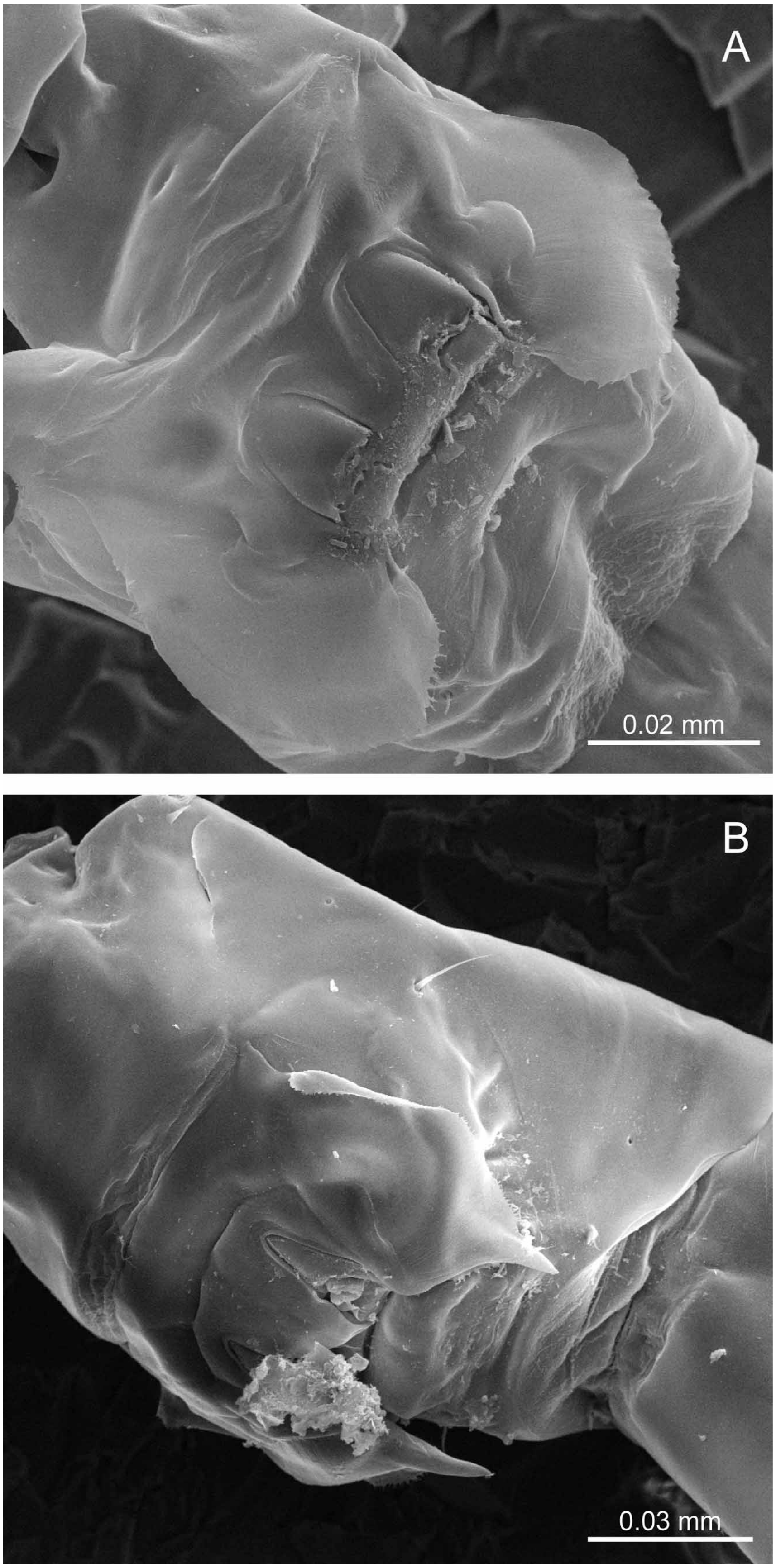

FIGURE 3. *Pseudodiaptomus nansei* **sp. nov.** and *P. inopinus* Burckhardt, 1913. SEM photographs of genital flaps: A, *P. nansei* **sp. nov.** from the Hirakubo-gawa River, ventral view; B, *P. inopinus* from the Tsuri-kawa River, with lateral hyline frill somewhat turned up, ventrolateral view.

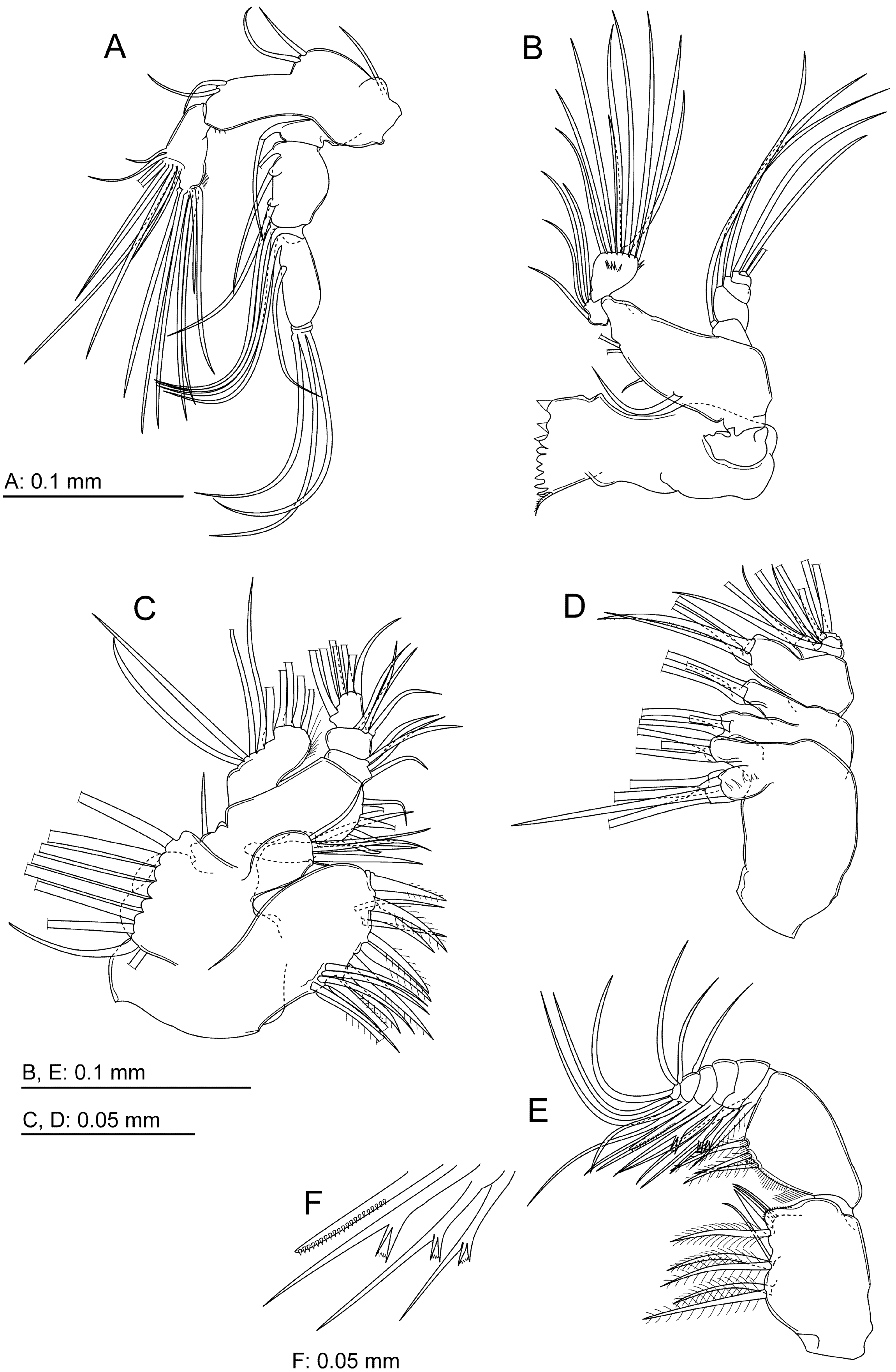

FIGURE 4. *Pseudodiaptomus nansei* **sp. nov.** Female (holotype): A, antenna; B, mandible; C, maxillule; D, maxilla; E, maxilliped; F, setae on third endopodal segment of maxilliped.

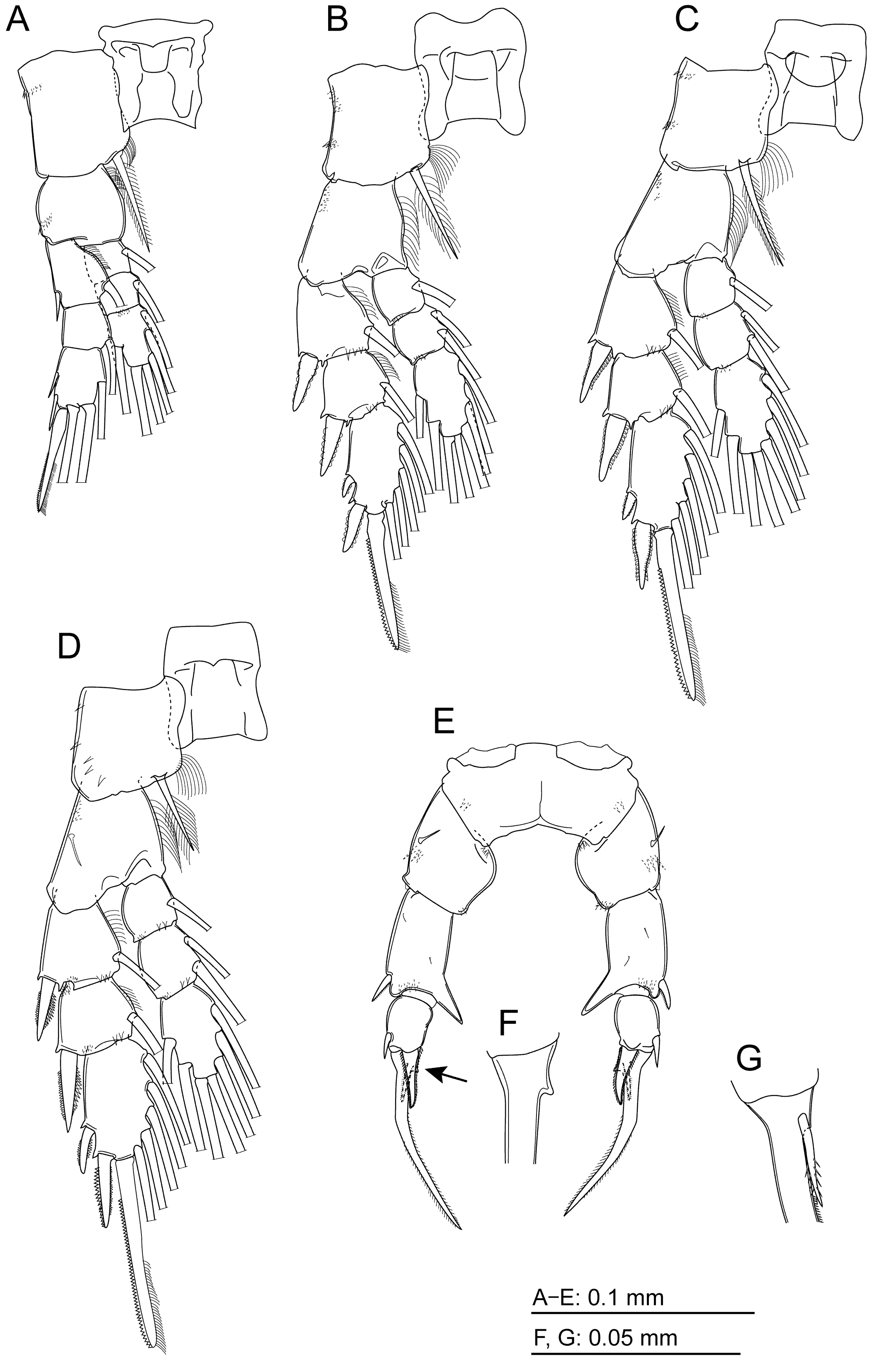

FIGURE 5. *Pseudodiaptomus nansei* **sp. nov.** Female (A–F, holotype; G, paratype): A–E, legs 1–5, posterior; F, base of third exopodal segment of leg 5, posterior; G, base of third exopodal segment of leg 5, anterior.

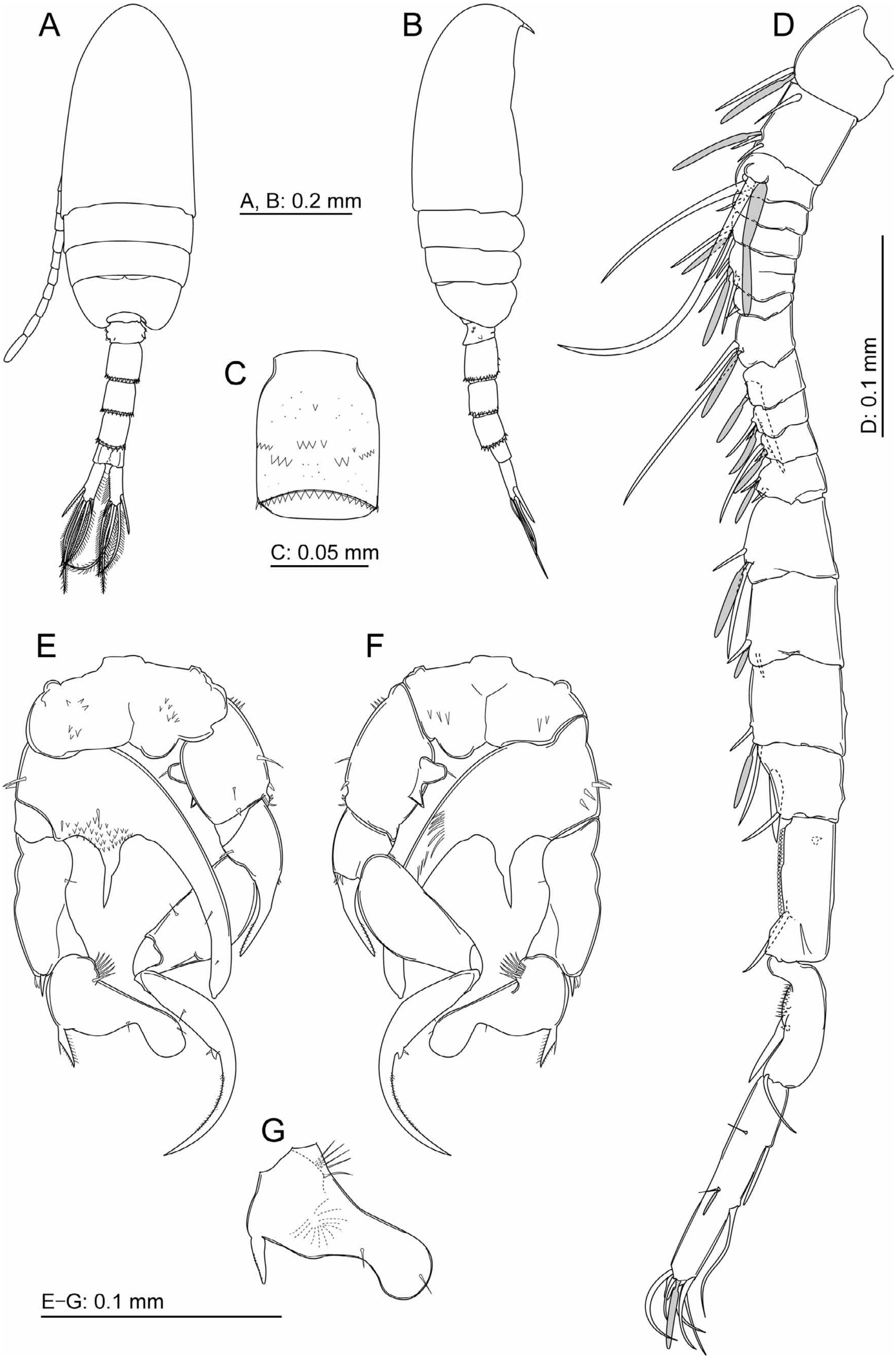

FIGURE 6. *Pseudodiaptomus nansei* **sp. nov.** Male (A–F, allotype; G, specimen from Ura-gawa River): A, habitus, dorsal; B, habitus, right lateral; C, second urosomite, ventral; D, right antennule; E, leg 5, posterior; F, leg 5, anterior; G, moderately paddle-shaped second exopodal segment of left leg 5.

Leg 5 (Fig. 5E) symmetrical; coxa with spinules anterolaterally; basis with proximal and distal spinules medially, posterior seta and anterior spinules laterally. Exopod 3-segmented; first segment with pointed distomedial process, distolateral spine, and anterodistal row of spinules; second segment with 1 large medial and 1 small lateral spines; third segment representing long terminal spine with notch (Fig. 5F) and short anterior spine at base (Fig. 5G).

MALE (ALLOTYPE). Body (Fig. 6A, B) length 0.90 mm, prosome length 0.54 mm. Cephalothorax as in female. Fifth pediger with no spinules on each posterolateral rounded corners. Genital somite with small posterolateral process on each side; second urosomite ventrally with transverse rows of spinules (Fig. 6C); second to fourth urosomites fringed with spinules on whole margins.

Right antennule (Fig. 6D) 20-segmented, with sixth to seventh segments incompletely fused; setal formula as follows: 1 = 1 + ae, 2 = 3 + ae, 3 = 2 + ae, 4 = 1, 5 = 2 + ae, 6 = 1, 7 = 2 + ae, 8 = 1 (spine), 9 = 2 + ae, 10 = 1 (spine) + ae, 11 = 2 (1 spine) + ae, 12 = 2 (1 spine) +ae, 13 = 2 (1 spine) + ae, 14 = 2 + ae, 15 = 2 + ae, 16 = 2 + ae, 17 = 2 (1 spine), 18 = 2 (1 pine), 19 = 3 (2 spine), 20 = 9 + 2ae; 18th segment with serrate ridge; 19th segment with hirsute proximal ridge; length ratios of 18–20th segments to 17th segment 1.9, 1.6, 3.2, respectively.

Leg 5 (Fig. 6E, F) coxa with spinule patch on both surfaces. Left basis and endopod completely fused, produced into 2 large medial processes, medial one large and laterally curved with anterior row of long spinules at mid length, and distal one smaller, and discontinuously tapering into thin spiniform process with many spinules on posterior surface of base, and with lateral seta and anterolateral row of spinules. Left exopod 2-segmented; first segment rectangular with spine and spinules at distolateral corner, second segment thumb-shaped with lateral notch one-third from tip, proximomedial row of spinules, proximolateral spine, 2 setae each at notch and tip. Right leg basis with 2 medial processes, proximal one rounded, tip bearing hair-like spinule, and distal one triangular, and with proximolateral spinules, lateral seta and distal spinules. Exopod 3-segmented; first segment with fused thick curved terminal spine extending at most to half length of second segment, with proximomedial spinule and anterolateral spinules; third segment shaped falcate long, proximally swollen along lateral margin with maximum width at proximal one-third, bearing medial seta and medial triangular process with seta, terminal third with medial spinules.

Other appendages as in female.

Variability. The body length ranged from 1.10–1.13 mm (n=5) in females and 0.86–0.91 mm (n=5) in males, and the prosome length 0.65–0.67 mm in females and 0.54–0.55 mm in males. One female specimen from the Yakugachi-gawa River had slightly swollen setae on the caudal rami (Fig. 2H). The male first urosomite lateral spinules and second urosomite ventral spinule rows varied and in some specimens the rows resembled those of *P. inopinus* Burckhardt, 1913 described below. Male left leg 5 posterior spinules patch on the fused basis-endopod segment was absent in specimens from the Amami-ohshima Island. Male left leg 5 the second exopodal segment of a specimen from the Ura-gawa River was the intermediate form between thumb- and paddle-types by having a weak lateral depression, and a spinule patch on the anterior surface (Fig. 6G), which was absent in the thumb-type specimens.

Etymology. The species name is a noun in apposition derived from the Nansei Islands, the distribution range of the species.

Remarks. This new species was collected from three islands of the Nansei Islands in southern Japan: Ishigaki-jima, Okinawa-jima and Amami-ohshima Islands (Fig. 1), but not found in the samples from Kyushu and the other islands of the Nansei Islands. Salinity and temperature at the sampling sites of the species ranged from 0.3–25.1 and from 21.6–29.7°C, respectively. This species was especially dominant at the site of the Hirakubo-gawa River, Ishigaki-jima Island, where the salinity ranged from 3.1–6.2. Oka *et al.*'s (1991, fig. 3) specimens described as *P. inopinus* from Iriomote-jima, Ishigaki-jima, Okinawa-jima, Amami-ohshima and Tanegashima Islands of the Nansei Islands are here attributed to *P. nansei*. The authors published illustrations showing the female caudal rami with thin terminal setae and the male right leg 5 with the third segment swollen at the proximal one-third. Oka *et al.* (1991) and Oka and Saisho (1994) also recorded the dominance of *P. nansei* in brackish waters of the Nakama-gawa River (salinity 1.5), Iriomote-jima Island, and the Sumiyo-gawa River (salinity 21.8±7.5), Amimi-oshima Island, respectively.

***Pseudodiaptomus inopinus* Burckhardt, 1913**
(Figs. 3B, 7, 8)

Synonyms. *Pseudodiaptomus inopinus* Burckhardt (1913), 379, pl. 11E, figs. 2–5, 7, 8, pl. 11F, figs. 1–4, 9, 10, pl. 11G, figs. 1–4, 6–8, pl. 12H, figs. 1–4, 7, 8, 10, 11; Smirnov (1929), 318, figs. 1–3; Tanaka (1966), 42, fig. 3; Mizuno and Miura (1984), 481, fig. 260; Chang and Kim (1986), 49, pl. 1, figs. 6–9; Cordell *et al.* (1992), 261; Soh *et al.* (2001), 203, fig. 3C; Cordell *et al.* (2007), 214, fig. 4; Eyun *et al.* (2007), 265; Lee *et al.* (2007), 140, figs. 6–7; Chang (2009), 110, figs. 24–25.
Pseudodiaptomus japonicus Kikuchi (1928), 68, pl. 18, fig. 9–12, pl. 19, figs. 13–18.
Schmackeria inopinus, Shen and Tai (1962), 101; Shen and Lee (1963), 578; Shen *et al.* (1979), 69, figs. 27, 28.

Material examined. Six females and 20 males from Tsuri-kawa R. (33°50’55”N, 130°30’08”E, 18 August 2009; #10 in Fig. 1), 1 female from Imari-gawa R. (33°16’22”N, 129°53’10”E, 19 August 2009; #11), 57 females and 47 males from Yukiura-gawa R. (33°56’34”N, 129°41’00”E, 19 August 2009; #12), 4 females and 4 males from Rokkaku-gawa R. (33°11’44”N, 130°12’27”E, 19 August 2009; #13), 39 females and 71 males from Chikugo-gawa R. (33°11’46”N, 130°21’36”E, 20 August 2009; #14), 36 females and 57 males from Manose-gawa R. (31°26’52”N, 130°18’31”E, 20 August 2009; #15), 4 females and 1 male from Hitotsuse-gawa R. (32°03’08”N, 131°28’18”E, 21 August 2009; #16), 11 females from Shiomi-gawa R. (32°24’57”N, 131°37’09”E, 21 August 2009; #17), 2 females and 1 male from Yasaka-gawa R. (33°24’23”N, 131°36’38”E, 21 August 2009; #18), and 2 females and 8 males from Yamakuni-gawa R. (33°36’09”N, 131°10’37”E, 21 August 2009; #19). All the specimens were collected by S.O. Sakaguchi and H. Ueda. One female and two male specimens from the Tsuri-kawa River were dissected for close examination of appendages. Ten female and 10 male specimens from the Tsuri-kawa River in alcohol were deposited in each the NSMT (NSMT-Cr 21263, 21264) and the USNM (USNM 1145709, 114510).

Redescription. FEMALE. Body (Fig. 7A, B) length 1.38–1.44 mm (n=5). Second to fourth prosomites laterally with row of fine spinules along posterior margins; fourth and fifth pediger fused with rounded corners, with spiniform process dorsally, small bump terminally (indicated by arrowhead *a* in Fig. 7A, C), several long spinules ventral to bump (arrowhead *b*), and row of spinules on posterolateral corner on each side (arrowhead *c*). Genital double-somite (Figs. 7D, E, 3B) 1.1 times longer than wide, with several spinules on each anterolateral projection, long anterolateral seta and dorsolateral row of spinules at one-third and two-third anteriorly on each side; posterior process of genital flap (indicated by arrowhead in Fig. 7D) pointed and longer than that of *P. nansei* **sp. nov.** Caudal left rami (Fig. 7F) slightly larger, lateral and terminal caudal setae thicker and shorter than those of *P. nansei*, the medial terminal seta especially swollen and as long as ramus, except one specimen from the Yukiura-gawa River with thin, long setae on both rami (Fig. 7G). Leg 5 (Fig. 7H) coxa with spinules on posterior and anterior surfaces. First exopodal segment with round distomedial process; terminal spine of third segment without notch at base and bearing short anterior spine with teeth medially (Fig. 7I). Other morphological characters as in *P. nansei*.

MALE. Body (Fig. 8A, B) length 1.10–1.15 mm (n=5). Second to fourth prosomites each with group of minute spinules near anteroventral corner, fifth pediger with spiniform process dorsally, and posterolateral spinules on each corner. Second urosomite with patch of minute spinules anterolaterally and ventral transverse rows of spinules (Fig. 8C). Right antennule (Fig. 8D) segmentation pattern and setal formula as in *P. nansei*; serration of 18th with longer teeth than those of *P. nansei*; length ratios of 18–20th segments to 17th segment 1.5, 1.4, 2.6, respectively. Left leg 5 (Fig. 8E) without spinules at base of distal smaller process of basoendopod; second exopodal segment of 5 specimens paddle-shaped among 20 specimens examined (Fig. 8F). Right leg 5 first exopodal segment with proximomedial spinule and distolateral spine extending more than mid length of second segment with small lateral spine; third segment not swollen proximally. Other morphological characters as in *P. nansei*.

Remarks. Diagnostic characters of our specimens closely agree with Burckhardt’s (1913) original description from Taifu Lake of the Yangtze River delta. However notable differences were observed in the following two respects. First, the female leg 1 is much shorter than that illustrated by Burckhardt (1913, plate 11G, fig. 6); e.g. the second exopodal segment is as long as wide in our specimens as in the new species, but two times longer than wide in the original description. The proportions of the leg 1 segments illustrated by

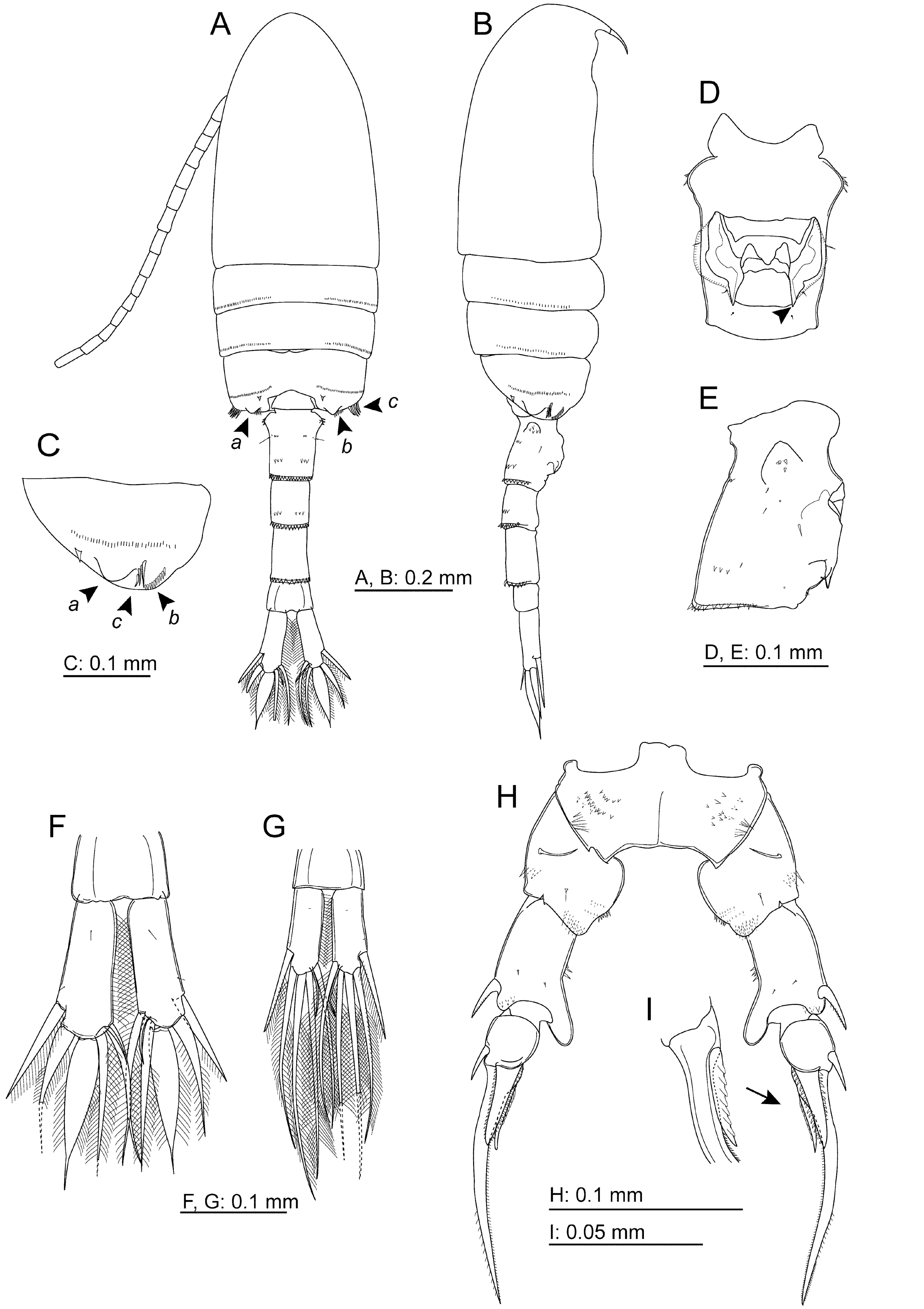

FIGURE 7. *Pseudodiaptomus inopinus* Burckhardt, 1913. Female (A–F, H, I, the same specimen from the Tsuri-kawa River; G, from Yukiura-gawa River): A, habitus, dorsal; B, habitus, right lateral; C, fifth pediger, right lateral; D, genital double somite, ventral; E, genital double somite, right lateral; F, caudal rami, dorsal; G, caudal rami with thin setae, dorsal; H, leg 5, posterior; I, base of third exopodal segment of leg 5, anterior.

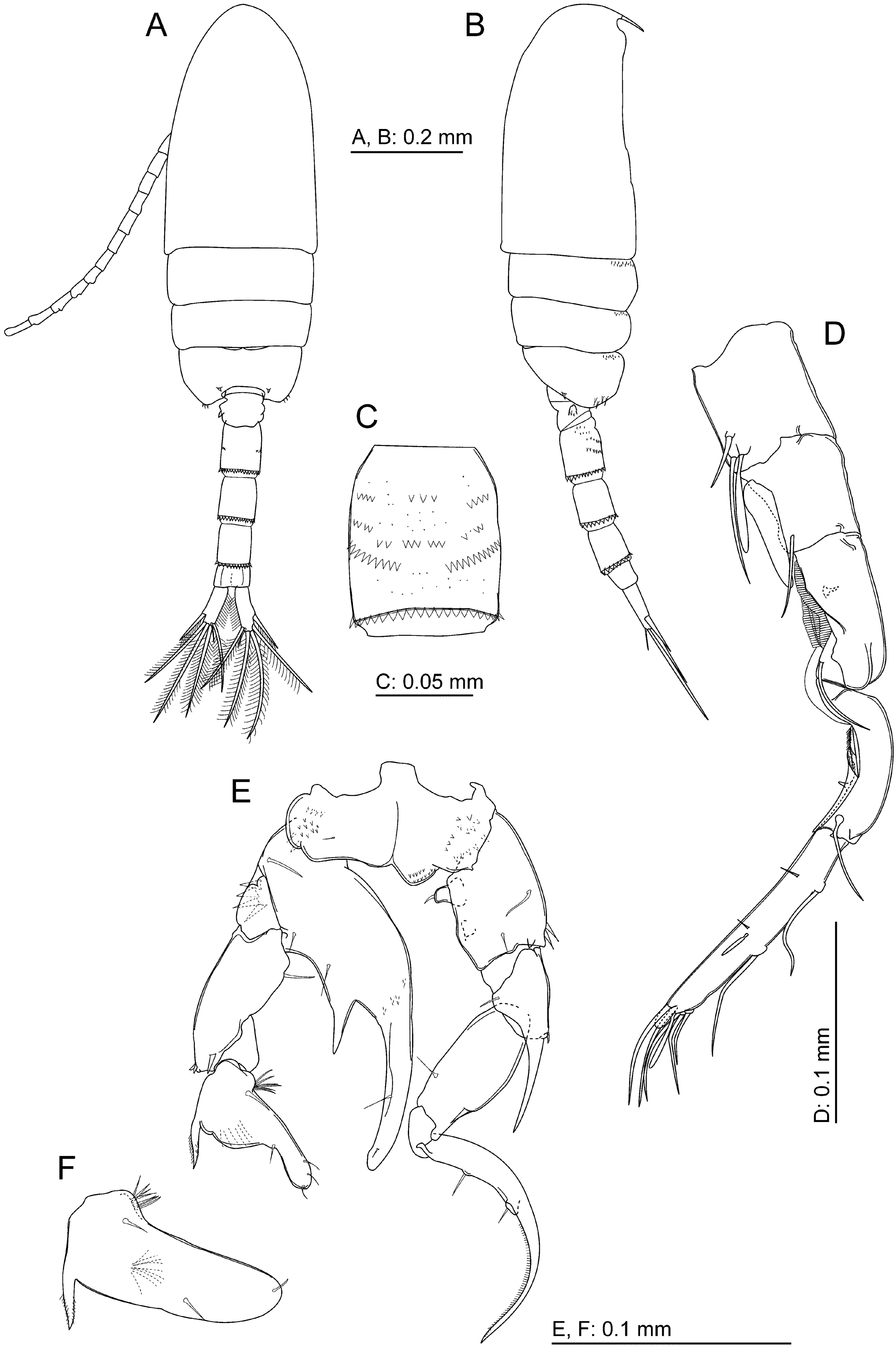

FIGURE 8. *Pseudodiaptomus inopinus* Burckhardt, 1913. Male (A–E, the same specimen from Tsuri-kawa River; F, from Tsuri-kawa River) A, habitus, dorsal; B, habitus, right lateral; C, second urosomite, ventral; D, 16–17th segments of right antennule; E, leg 5, posterior; F, paddle-shaped second exopod of left leg 5, posterior.

Lee *et al.* (2007) and Chang (2009), who provided illustrations of *P. inopinus* from Korea, are similar to those of our specimens. Second, a clear row of spinules is present medially to the conspicuous dorsal spinule of the female fifth pediger in Burckhardt's illustration (plate 11E, fig. 5), whereas in our specimens a spinule row is present ventrally to the bump. Lee *et al.* (2007) and Chang (2009) described another type of ornamentation in which no spinule rows were present except for those on the distolateral corner of the somite.

Considering these morphological differences among specimens from different localities, they may be a species complex. However, we call them *P. inopinus* for convenience in this study. Revising this potential complex worldwide is clearly beyond the scope this paper. This species occurred in most river estuaries in Kyushu but was never found in collections from the Nansei Islands.

Molecular diversity

The mtCOI sequences differed by 21–26% between *Pseudodiaptomus nansei* **sp. nov.** from the Hirakubo-gawa River on Ishigaki-jima Island and *P. inopinus* Burckhardt, 1913 from the Tsuri-kawa River from Kyushu, in contrast to the very small variation (0–3%) within each population (Table 2). The genetic differences between these morphologically allied species were almost comparable to those from *P. ishigakiensis* Nishida, 1985 (GenBank Accession No. AB576158) used as the outgroup species (24–32%). The mtCOI gene tree demonstrated a clear separation between *P. nansei* and *P. inopinus* (Fig. 9). MtCOI reference sequences were selected to represent the new species from the Hirakubo-gawa River (GenBank Accession No. AB576157) and *P. inopinus* from the Tsuri-kawa River (AB576159).

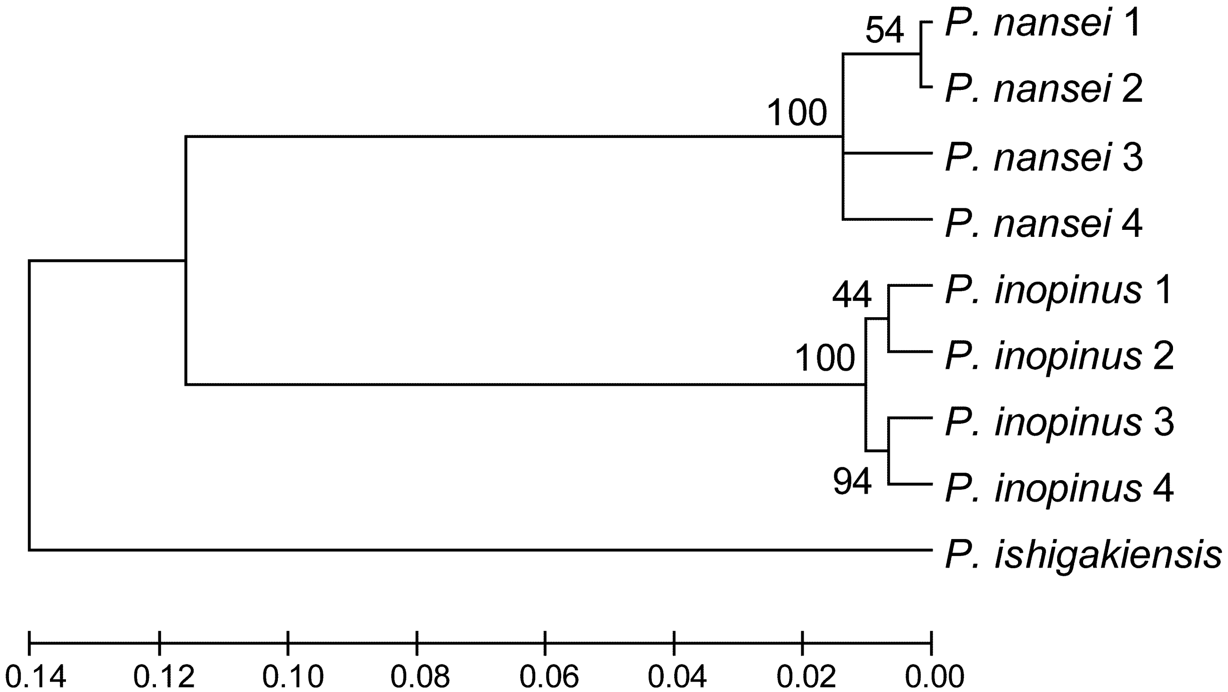

FIGURE 9. Cladogram of mtCOI showing proportional differences between individuals of *Pseudodiaptomus nansei* **sp. nov.** from Hirakubo-gawa River and *P. inopinus* Burckhardt, 1913 from Tsuri-kawa River. Numbers at branch points are bootstrap values. The cladogram is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the tree. *Pseudodiaptomus ishigakiensis* Nishida, 1985 collected from Kabira Bay, Ishigaki Island, was used as the outgroup. Specimen numbers correspond to those in Table 2.

TABLE 2. Pairwise percent differences of mtCOI sequences between individuals of *Pseudodiaptomus nansei* **sp. nov.** from the Hirakubo-gawa River and *P. inopinus* Burckhardt, 1913 from Tsuri-kawa River. Bold face values indicate pairwise comparisons between the different species.

	na-1	na-2	na-3	na-4	in-1	in-2	in-3	in-4
P. nansei 1								
P. nansei 2	0.00							
P. nansei 3	0.02	0.02						
P. nansei 4	0.03	0.03	0.03					
P. inopinus 1	**0.21**	**0.22**	**0.24**	**0.25**				
P. inopinus 2	**0.21**	**0.22**	**0.24**	**0.25**	0.02			
P. inopinus 3	**0.23**	**0.23**	**0.25**	**0.26**	0.01	0.03		
P. inopinus 4	**0.21**	**0.22**	**0.24**	**0.25**	0.02	0.01	0.01	
P. ishigakiensis	**0.24**	**0.24**	**0.25**	**0.27**	**0.31**	**0.31**	**0.32**	**0.31**

Discussion

The genetic difference of 21–26% in the mtCOI sequence between *P. nansei* **sp. nov.** and *P. inopinus* Burckhardt, 1913 from Kyushu Island is that at the species level (Bucklin *et al.* 2003; Eyun *et al.* 2007), indicating that they are independent species. The sequence of a *P. inopinus* specimen from Korea (GenBank Accession No. AF536520) differs from the new species by 23–25% and *P. inopinus* from Kyushu Island by 26–27%, also indicating that the Korean *P. inopinus* is a different species from our specimens.

According to the key to species groups of *Pseudodiaptomus* Herrick, 1884 (Walter *et al.* 2006), *P. nansei* belongs to the *forbesi*-subgroup of the Lobus group. The male left leg 5 of this subgroup has a non-bifurcated, large endopod fused to the basis. The new species is most closely related to *P. inopinus* among them, but readily distinguishable from *P. inopinus* by the following morphologies (corresponding morphologies of *P. inopinus* are present in brackets): 1) absence of dorsal spiniform processes on the fifth pediger in both sexes [present], 2) short posterior processes of the genital operculum [long posterior processes], 3) thin lateral medial terminal caudal seta of the female [swollen seta], and 4) pointed distomedial process of the first exopodal segment of the female leg 5 [round process], and 5) the proximally swollen third exopodal segment of the male left leg 5 [not swollen]. Among these morphologies, the most easily observable is the thickness of caudal setae in females. However, it is not critical in distinguishing the two species because specimens from the present study rarely displayed a different or intermediate type of the setae in both *P. nansei* and *P. inopinus*. Lee *et al.* (2007) found that about 10% of Korean specimens of *P. inopinus* did not have swollen caudal setae, and Shen and Lee (1963) noted that few specimens displayed female caudal setae from Guangdong, China, and they were similar in thickness. Exceptional variation of swollen caudal setae was also observed for *P. poplesia* Shen, 1955 from Korean brackish waters (Soh *et al.* 2001).

There are three subspecies of *P. inopinus*, viz. *P. i. gordiodes* Brehm, 1952, *P. i. succupodus* Shen and Tai, 1962, and the nominotypical *P. i. inopinus* (Walter & Boxshall 2009). The male left leg 5 of *P. i. gordiodes* is unique in having a bifurcated tip of the endopod and a deep lateral notch of the second exopodal segment (Brehm 1952), which are very obviously different from those of the new species. *Pseudodiaptomus i. succupodus* was created by Shen and Tai (1962) for male specimens having a paddle-shaped second exopod of the left leg 5, but later Shen and Song (1979) regarded the characteristics as a "form" of the species. The paddle-shaped second exopod of the male left leg 5 has been commonly observed for *P. inopinus* (Cordell *et al*. 2007; present study). The other members of the *forbesi*-subgroup are all distinguishable from the new species by the presence of a dorsal spiniform processes on the fifth pediger and/or a very different male leg 5 (see figures in Walter & Boxshall 2009). Although *P. curvilobatus* (Dang, 1967) is not present in the list of the species group by Walter *et al.* (2006), it apparently belongs to the *forbesi*-subgroup by having the male right leg 5 characteristic to the subgroup; figures are shown by Walter and Boxshall (2009). This species also has a dorsal spiniform process on the fifth pediger in both sexes and is distinct from the new species.

The present study and Oka *et al.* (1991) revealed that *P. nansei* is distributed throughout the full range of the Nansei Islands. On the other hand, *P. inopinus* was completely absent in our collections from the islands. Oka *et al.* (1991), who examined copepods from five islands between Iriomote-jima and Tanegashima Islands (Fig. 1), noted that all caudal setae of the specimens collected in their survey were thin. This indicates that *P. inopinus* does not occur in the Nansei Islands. In contrast, *P. nansei* was never found in the samples collected from Kyushu Island, suggesting that they are geographically segregated with a boundary between Kyushu and Tanegashima Islands, the northernmost island of the Nansei Islands.

Specimens described as *P. inopinus* have so far been recorded across a wide geographical range from both temperate and subtropical coasts of East Asia, i.e. the Chinese coast from Guangdong to Hebei (Shen & Tai 1962; Shen & Lee 1963; Shen & Song 1979), almost all around the South Korean coast (Chang & Kim 1986; Soh *et al.* 2001; Chang 2009), the Suifun River estuary near Vladivostok, Russia (Smirnov 1929), and the Japanese coast from Okinawa to Hokkaido (Kikuchi 1928; Tanaka 1966; Mizuno & Miura 1984; Oka *et al.* 1991). The species has been introduced to the Pacific coast of USA (Cordell *et al.* 1992, 2007). In these studies, the description identifiable to *P. nansei* is only from the Nansei Islands of Japan, by Oka *et al.* (1991), and the population with females having thin caudal setae is only dominant in the *P. nansei* population; this characteristic has never been reported from adjacent continental waters. The absence of descriptions corresponding to *P. nansei* in adjacent continental waters suggests that *P. nansei* is endemic to the Nansei Islands.

Acknowledgments

We thank to Drs Shigeki Fujiwara (Kochi University), Aika Yamaguchi (University of British Columbia) and Shinsuke Saito (Sessile Research Corp. Co. Ltd.) for their help in molecular analyses, and Takuya Matsuzaki (Kochi University) for taking SEM photographs. Our thanks are also due to Dr Todd W. Miller (Ehime University) for reviewing the text.

References

Boxshall, G.A. & Halsey, S.H. (2004) *An introduction to copepod diversity.* The Ray Society, London, 966 pp.

Brehm, V. (1952) Ein *Pseudodiaptomus* aus Südostasien. *Anzeiger der Österreichischen Akademie der Wissenschaften, Mathematisch- Naturwissenschaftlichen Klasse*, 89, 122–124.

Bucklin, A., Frost, B.W., Bradford-Grieve, J., Allen, L.D. & Copley, N.J. (2003) Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Marine Biology*, 142, 333–343.

Burckhardt, G. (1913) Zooplankton aus ost- und süd-asiatischen Binnengewässern. *Zoologische Jahrbucher, Abteilung für Systematik, Ökologie und Geographie der Tiere*, 34, 341–471.

Chang, C.Y. (2009) *Illustrated encyclopedia of fauna & flora of Korea. Vol. 42*, *Inland-water Copepoda.* Jeonghaeng-sa, Ministry of Education, Seoul, Soith Korea, 687 pp. [In Korean with English descriptions]

Chang, C.Y. & Kim, H.S. (1986) The freshwater Calanoida (Crustcea: Copepoda) of Korea. *Korean Journal of Systematic Zoology*, 2, 49–60.

Cordell, J.R., Morgan, C.A. & Simenstad, C.A. (1992) Occurrence of the Asian calanoid copepod *Pseudodiaptomus inopinus* in the zooplankton if the Columbia River estuary. *Journal of Crustacean Biology*, 12, 260–269.

Cordell, J.R., Rasmussen, M. & Bollens, S.M. (2007) Biology of the introduced copepod *Pseudodiaptomus inopinus* in a northeast Pacific estuary. *Marine Ecology Progress Series*, 333, 213–227.

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

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.

Folmer, O., M. Black, 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.

Kawabata, K. & Defaye, D. (1994) Description of planktonic copepods from Lake Kahoku-gata, Japan. *Japanese*

Journal of Limnology, 55, 143–158.
Kikuchi, K. (1928) Freshwater Calanoida of middle and south-western Japan. *Memoirs of the College of Science Kyoto Imperial University, Series B*, 4, 65–79, pls. 18–22.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. & Higgins, D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948.
Lee, J.M., Yoon, H.J. & Chang, C.Y. (2007) A faunistic study on the brackish-water calanoid copepods from South Korea. *Korean Journal of Systematic Zoology*, 23, 135–154.
Mizuno, T. & Miura, Y. (1984) *Inland-water Copepoda in Japan. In Chinese/Japanese Freshwater Copepoda*. ed. by Shen, C. & T. Mizuno, Tatara-shobo, Yonago, pp. 471–620. [In Japanese]
Oka, S., Saisho, T. & Hirota, R. (1991) *Pseudodiaptomus* (Crustacea, Copepoda) in the brackish waters of mangrove regions in the nansei Islands, Southwestern Japan. *Bulletin of the Biogeography Society of Japan*, 46, 83–87.
Oka, S. & Saisho, T. (1994) Occurrence of zooplankton in Sumiyo Bay, Amamioshima Island. Composition and abundance in the estuary and surf zone in winter season. *Memoirs of Faculty of Fisheries, Kagoshima University*, 43, 51–59. [In Japanese with English abstract]
Saitou, N. & Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology Evolution*, 4, 406–425.
Shen, C.J. & Tai, A.Y. (1962) The Copepoda of the Wu-Li Lake, Wu-Sih, Kiangsu Province. I. Calanoida. *Acta Zoologica Sinica*. 14, 99–118. [In Chinese with English summary]
Shen C.J. & Lee, F.S. (1963) The estuarine Copepoda of Chiekong and Zikong Rivers, Kwangtung Province, China. *Acta Zoologica Sinica*. 15, 571–596. [In Chinese with English summary]
Shen, C.J. & Song, D.X. (1979) Calanoida, Sars, 1903, *In*: Research Group of Carcinology, Institute of Zoology, Academia Sinica (Eds.), *Fauna Sinica, Crustacea, Freshwater Copepoda*. Science Press, Beijing, pp. 53–163. [in Chinese]
Smirnov, S.S. (1929) Beiträge zur Copopodenfauna Ostasiens. *Zoologischer Anzeiger*, 81, 317–329.
Soh, H.Y., Suh, H.L., Yu, O.H. & Ohtsuka, S. (2001) The first record of two demersal calanois copepods, *Pseudodiaptomus poplesia* and *P. nihonkaiensis* in Korea, with remarks on morphology of the genital area. *Hydrobiologia*, 448, 203–215.
Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology Evolution*, 24, 1596–1599.
Tanaka, O. (1966) Neritic Copepoda Calanoida from the north-west coast of Kyushu. *Proceedings of the Symposium Crustacea*, 1, 38–50.
Walter, T.C. & Boxshall, G. (Eds.) (2009) World of Copepoda. Available from http://www.marinespecies.org/copepoda/ (accessed 13 July 2010)
Walter, T.C., Ohtsuka, S. & Castillo, L.V. (2006) A new species of *Pseudodiaptomus* (Crustacea: Calanoida: Pseudodiaptomidae) from the Philippines, with a key to pseudodiaptomids from the Philippines and comments on the status of the genus *Schmackeria*. *Proceedings of the Biological Society of Washington*, 119, 202–221.