Zoosyst. Evol. 99 (2) 2023, 545–556 | DOI 10.3897/zse.99.108032

A new freshwater species of *Gnorimosphaeroma* (Crustacea, Isopoda, Sphaeromatidae) from Chichi-jima Island, Ogasawara Islands, Japan

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Academic editor: Luiz F. Andrade ♦ **Received** 15 June 2023 ♦ **Accepted** 16 October 2023 ♦ **Published** 21 November 2023

Abstract

This study describes *Gnorimosphaeroma rivulare* **sp. nov.** from a stream on Chichi-jima Island, Ogasawara Islands, Japan. This is the second freshwater species of *Gnorimosphaeroma* and the third Sphaeromatidae from oceanic islands. *Gnorimosphaeroma rivulare* **sp. nov.** is morphologically similar to *G. boninense* Nunomura, 2006, *G. naktongense* Kwon & Kim, 1987 and *G. saijoense* Nunomura, 2013. However, *G. rivulare* **sp. nov.** differs from these species in various morphological features, such as the shape of pleotelson and pereopod 2, relative length of antennule peduncular articles and pleopod 3 rami, number of setae on maxillula and maxilliped, and setation on pereopod 3. Phylogenetic analyses revealed that *G. akanense* is sister to *G. saijoense*, and together they are sister to *G. hokurikuense*. This three taxa clade is sister to *G. rivulare* **sp. nov.** with *G. iriei* basal to them all. Our analysis concludes that *G. boninense* from Haha-jima Island, Ogasawara Islands is only distantly related to *G. rivulare* and may represent an independent colonization event.

Key Words

freshwater, inland water, isopod, molecular phylogeny, oceanic island, stream, taxonomy

Introduction

Generally, oceanic islands do not occur on continental shelves. These are islands that have never been connected to a continental landmass. Inland water-dwelling organisms cannot reach oceanic islands without crossing the ocean or speciating from organisms that find themselves in a new habitat to which they have adapted, e.g., marine and then isolated in freshwater. Therefore, the occurrence of freshwater fish and invertebrates on oceanic islands is generally limited (Lévêque et al. 2008; Strong et al. 2008; Väinölä et al. 2008; Wilson 2008).

The Ogasawara Islands, oceanic islands, are a group of approximately 30 islands located in the Pacific Ocean approximately 1000 km southeast of the Japanese archipelago. The Ogasawara Islands have small and well-developed rivers inhabited by freshwater crustaceans, molluscs, and caddisflies. These taxa have limited diversity on oceanic islands (Satake and Cai 2005; Nunomura and Satake 2006; Miura et al. 2008; Tomikawa et al. 2022; Ito et al. 2023).

More than 10,600 species of Isopoda have been described worldwide, occurring in diverse aquatic and terrestrial environments (Boyko et al. 2023). Approximately 950

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species of isopods have been recorded from inland waters; however, the number of species occurring in inland waters of oceanic islands is far fewer, with only a few species recorded from Pacific Ocean islands (Jaume and Queinnec 2007; Wilson 2008). The aquatic sphaeromatid genus *Gnorimosphaeroma* Menzies, 1954 has 26 described species (Boyko et al. 2023). They are restricted to the Japanese Islands, Korean Peninsula, Mainland China, Alaska, and the eastern Pacific coast of North America (Tattersall 1921; Jang and Kwon 1993; Nunomura 2013; Wetzer et al. 2021). Most Sphaeromatidae are marine (Sket and Bruce 2004; Bruce 2005; Jaume and Queinnec 2007). The genus *Gnorimosphaeroma* is unusual because it includes not only marine species, but also has seven brackish and eight freshwater described species (Fig. 1, Table 1).

Satake and Ueno (2013) reported an unidentified species of *Gnorimosphaeroma* from a stream on Chichi-jima Island, Ogasawara Islands. A recent collection of *Gnorimosphaeroma* was made on Chichi-jima Island during the author's KT and NN research expedition to the Ogasawara Islands. A colleague provided an additional collection of seven specimens. In this study, we describe this previously undescribed species of *Gnorimosphaeroma*. To clarify the phylogenetic position of this *Gnorimosphaeroma* species, we performed a molecular phylogenetic analyses based on nuclear 18S rRNA and mitochondrial 16S rRNA genes.

Materials and methods

Samples

Specimens of an unidentified species were collected from under the boulders in the upper stream of Nagatani River, Chichi-jima Island, Ogasawara Islands, Japan (Fig. 2). Our molecular phylogenetic analysis includes nine described species of *Gnorimosphaeroma*, one unidentified species from Chiba, Japan, one unidentified species from San Francisco Bay, and the new undescribed freshwater species described in this paper (Table 2). The specimens were collected using a fine-mesh hand-net and samples were subsequently fixed in 70% or 99% ethanol in-situ. Specimens fixed in 70% ethanol were transferred to 99% ethanol in the laboratory.

Morphological observation

All appendages of *G. rivulare* sp. nov. were dissected in 80% ethanol and mounted in gum-chloral medium. The slides were examined using a stereomicroscope (Olympus SZX7, Japan) and a light microscope (Nikon Eclipse Ni, Japan), and the body and appendages were illustrated using a camera lucida. One male (paratype, NSMT-Cr 31507) was dehydrated through a graded ethanol series, and dried using hexamethyldisilazane (HMDS) (Nation 1983). They were then sputter-coated with gold and observed using scanning electron microscopy (SEM, JSM-6510LV). Body length was measured as a straight-line distance from the rostral point to the posterior margin of pleotelson within the nearest 0.1 mm. Type specimens were deposited at the National Museum of Nature and Science, Tsukuba, Japan (NSMT).

Molecular phylogenetic analyses

Genomic DNA was extracted from the appendage muscles of the specimen following procedures detailed by Tomikawa et al. (2014). The primer set 16Sar and 16Sbr (Palumbi et al. 1991) was used to target the mitochondrial 16S rRNA (16S), whereas the 18SF [5′-AAGATTAAGCCATGCATGTC-3′] and 18SR [5′- GCTGGAATTACCGCGGCTGC-3′] primer pair were designed to target the nuclear 18S rRNA (18S). PCR and DNA sequencing were performed using the method detailed by Tomikawa (2015). The newly obtained DNA sequences were deposited in the International Nucleotide Sequence Databases (INSD) through the DNA Data Bank of Japan (DDBJ) (Table 2).

The phylogenetic analyses were conducted based on 16S and 18S sequence data generated for this project and also includes previously published sequences. *Ancinus* sp. (Ancinidae) and two *Chitonosphaera* species (Sphaeromatidae), *C. lata* (Nishimura, 1968) and *C. salebrosa* (Nishimura, 1969) were used as the outgroup (Wetzer et al. 2013, 2018). The sequences were aligned using the Muscle algorithm implemented in MEGA XI (Tamura et al. 2021). The aligned lengths of the 16S and 18S were found to be 518 and 559 bp, respectively. Moreover, the concatenated sequences yielded 1077 bp of alignment positions.

Phylogenetic relationships were reconstructed via maximum likelihood (ML) and Bayesian inference (BI) and partitioned by 16S and 18S datasets. ML analyses were conducted using IQ-TREE web server (ver. 1.6.12, see http://www.iqtree.org/; Trifinopoulos et al. 2016) with 1000 ultrafast bootstrap replicates (Hoang et al. 2018). The best evolutionary models were selected based on the corrected Bayesian information criterion (BIC) using ModelFinder (Kalyaanamoorthy et al. 2017): for 16S, TIM2+F+G4; for 18S, TNe+G4. BI and Bayesian posterior probabilities (PPs) were estimated using MrBayes v. 3.2.5 (Ronquist et al. 2012). The best-fit partition scheme and models for each partition were selected with the BIC using PartitionFinder with the 'greedy' algorithm, and for 16S, GTR+G; for 28S, JC+I. Two independent runs of four Markov chains were conducted for 10 million generations, and the tree was sampled every 100 generations. The parameter estimates and convergence were checked using Tracer v. 1.7.1 (Rambaut et al. 2018), and the first 50,001 trees were considered burn-in and discarded.

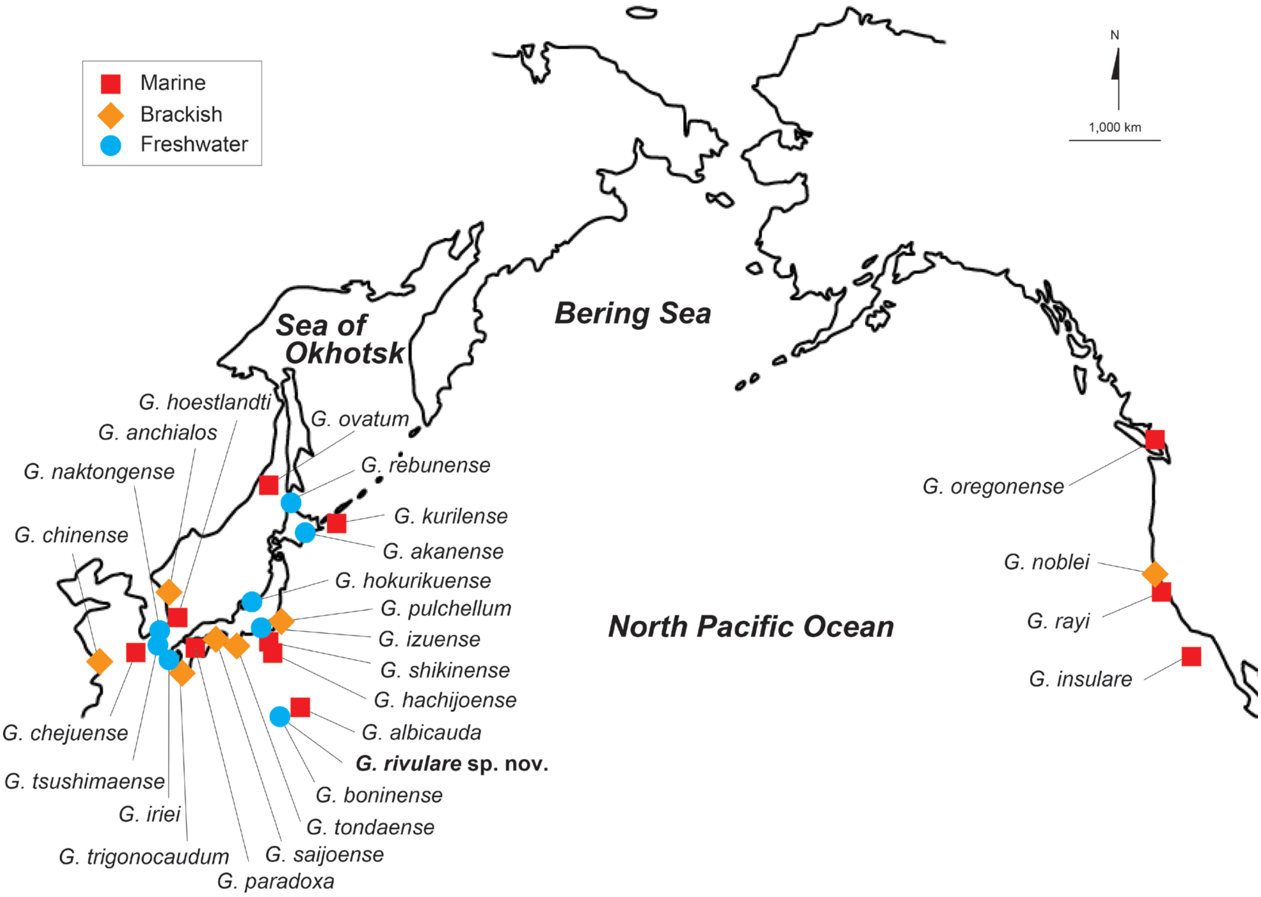

Figure 1. Map showing the type localities of *Gnorimosphaeroma* species.

Table 1. The type localities and habitats of *Gnorimosphaeroma* species.

Species	Type locality	Habitat
G. akanense Nunomura, 1998	Akan River, Hokkaido	freshwater
G. albicauda Nunomura, 2005	Hatsuneura, Chichi-jima Island, Ogasawara Islands	marine
G. anchialos Jang & Kwon, 1993	Lake Songjiho	brackish
G. boninense Nunomura & Satake, 2006	Haha-jima Island, Ogasawara Islands	freshwater
G. chejuense Kim & Kwon, 1988	Cheju Island	marine
G. chinense (Tattersall, 1921)	Whangpoo River, Shanghai	brackish
G. hachijoense Nunomura, 1999	Hachijo Island, Tokyo	marine
G. hoestlandti Kim & Kwon, 1985	Mukho	marine
G. hokurikuense Nunomura, 1998	Yomokuro-ike Pond, Toyama	freshwater
G. insulare (Van Name, 1940)	San Nicolas Island, California	marine
G. iriei Nunomura, 1998	Lake Ezu, Kumamoto	freshwater
G. izuense Nunomura, 2007	Izu, Shizuoka	freshwater
G. kurilense Kussakin, 1974	off Shikotan Island, Kuril Islands	marine
G. naktongense Kwon & Kim, 1987	Nakdong River, Busan	freshwater
G. noblei Menzies, 1954	Tomales Bay, California	brackish
G. oregonense (Dana, 1853)	Vancouver, British Columbia	marine
G. ovatum (Gurjanova, 1933)	Sea of Japan (details unknown)	marine
G. paradoxa (Nunomura, 1988)	Uwajima, Ehime	marine
G. pulchellum Nunomura, 1998	Obitsu River, Chiba	brackish
G. rayi Hoestlandt, 1969	Tomales Bay, California	marine
G. rebunense Nunomura, 1998	Rebun Island, Hokkaido	freshwater
***G. rivulare* sp. nov.**	Nagatani River, Chichi-jima Island, Ogasawara Islands	freshwater
G. saijoense Nunomura, 2013	Shiotori River, Ehime	brackish
G. shikinense Nunomura, 1999	Shikine Island, Tokyo	marine
G. tondaense Nunomura, 1999	Tonda River, Wakayama	brackish
G. trigonocaudum Nunomura, 2011	Hijikuro River, Nagasaki	brackish
G. tsutshimaense Nunomura, 1998	Tsushima Island	freshwater

Table 2. Samples used for the phylogenetic analyses. Sequences marked with an asterisk (*) were newly obtained in the present study. ND, no sequence available.

Species	Voucher or isolate #	Locality	INSD # 16S	INSD # 18S	Note
Sphaeromatidae					
Gnorimosphaeroma akanense	G1913	Lake Akan, Hokkaido, Japan	LC765314*	LC765326*	Topotype
Gnorimosphaeroma boninense	G1814	Chibusa Dam, Haha-jima I., Ogasawara Is., Japan	LC765315*	LC765327*	Topotype
Gnorimosphaeroma hokurikuense	G1943	Ota, Toyama, Japan	LC765316*	LC765328*	Topotype
Gnorimosphaeroma iriei	G1894	Lake Ezu, Kumamoto, Japan	LC765317*	LC765329*	Topotype
Gnorimosphaeroma noblei	RW02.021.1541	Tomales Bay, California, USA	KU248168	JF699554	Topotype
Gnorimosphaeroma oregonense	RW10.003.3131	Vancouver, British Columbia, Canada	MH427781	ND	Topotype
Gnorimosphaeroma rayi	RW09.002.2567	Tomales Bay, California, USA	MH427784	ND	Topotype
Gnorimosphaeroma saijoense	G1902	Kamo R., Ehime, Japan	LC765319*	LC765331*	Topotype
***Gnorimosphaeroma rivulare* sp. nov.**	G1820	Nagatani River, Chichi-jima I., Ogasawara Is., Japan	LC765320*	LC765332*	Paratype
***Gnorimosphaeroma rivulare* sp. nov.**	NSMT-Cr 31490; G1826	Nagatani River, Chichi-jima I., Ogasawara Is., Japan	LC765321*	LC765333*	Paratype
***Gnorimosphaeroma rivulare* sp. nov.**	NSMT-Cr 31486; G1892	Nagatani River, Chichi-jima I., Ogasawara Is., Japan	LC765322*	LC765334*	Paratype
Gnorimosphaeroma sp.	G1954	Obitsu R., Chiba, Japan	LC765318*	LC765330*	
Gnorimosphaeroma sp.	RW02.060.2550	San Francisco Bay, California, USA	MH427743	ND	
Gnorimosphaeroma tondaense	G1972	Takase River, Wakayama, Japan	LC765323*	LC765335*	Topotype
Outgroup					
Ancinidae					
Ancinus sp.	RW05.010.1475	Naos Island, Republic of Panama	KU248307	JF699514	
Sphaeromatidae					
Chitonosphaera lata	G1935	Takase R., Wakayama, Japan	LC765324*	LC765336*	
Chitonosphaera salebrosa	G1937	Edura,Wakayama, Japan	LC765325*	LC765337*	

Results

Taxonomy

Family Sphaeromatidae Latreille, 1825
Genus *Gnorimosphaeroma* Menzies, 1954

Gnorimosphaeroma rivulare Tomikawa, Yoshii & Nunomura, sp. nov.

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Figs 2–7

New Japanese name: Chichijima-kotsubumushi

Type materials. ***Holotype*:** male 4.9 mm (NSMT-Cr 31485), upper stream of Nagatani River (27°04.051'N, 142°12.938'E), alt. 160 m, Chichi-jima Island, Ogasawara Islands, Tokyo, Japan, collected by Tetsuro Sasaki on 2 February 2021. ***Paratypes*:** 3 males, 5.3 mm (NSMT-Cr 31486; G1892), 4.7 mm (NSMT-Cr 31487; G1893), 5.2 mm (NSMT-Cr 31507) 1 female 3.2 mm (NSMT-Cr 31488), data same as for holotype; 2 males, 4.1 mm (NSMT-Cr 31489), 4.0 mm (NSMT-Cr 31490; G1826), locality same as for holotype, collected by Noboru Nunomura on 16 December 2019.

Type locality. Japan, Tokyo: Ogasawara Islands, Chichi-jima Island, upper stream of Nagatani River (Figs 1, 2A).

Diagnosis. Pleonites incompletely fused; anterior suture line longer than posterior one. Pleotelson posterior margin rounded. Maxillula medial lobe with 4 plumose setae and a short single seta; lateral lobe with 10 robust setae. Maxilla medial lobe with 17 setae; middle lobe with 12 setae; lateral lobe with 13 setae. Pereopod 1 basis with a single seta on posterodistal corner; merus with

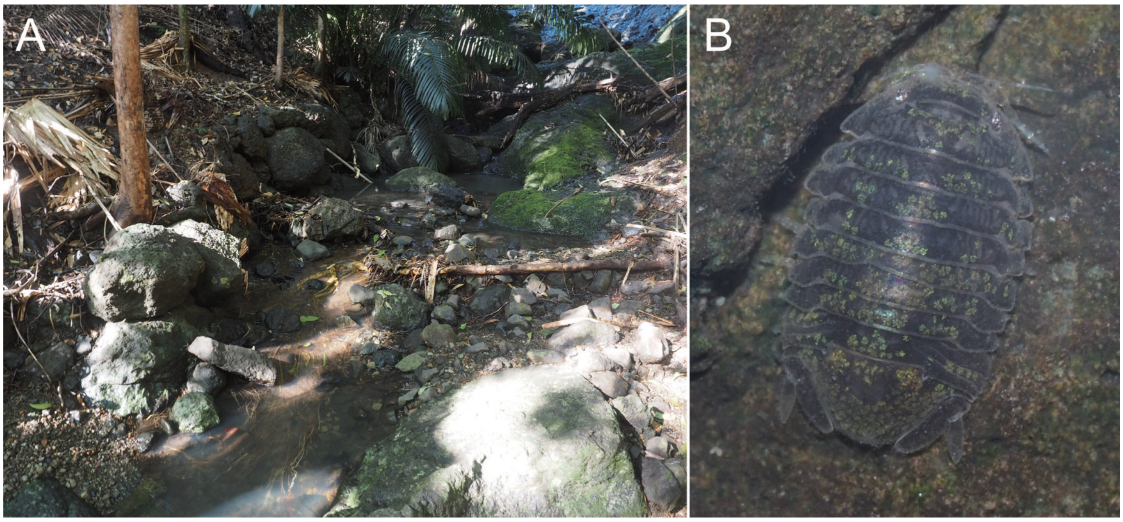

Figure 2. Habitat and live specimen of *Gnorimosphaeroma rivulare* sp. nov. **A.** Habitat; **B.** Living state of the holotype, male 4.9 mm (NSMT-Cr 31485).

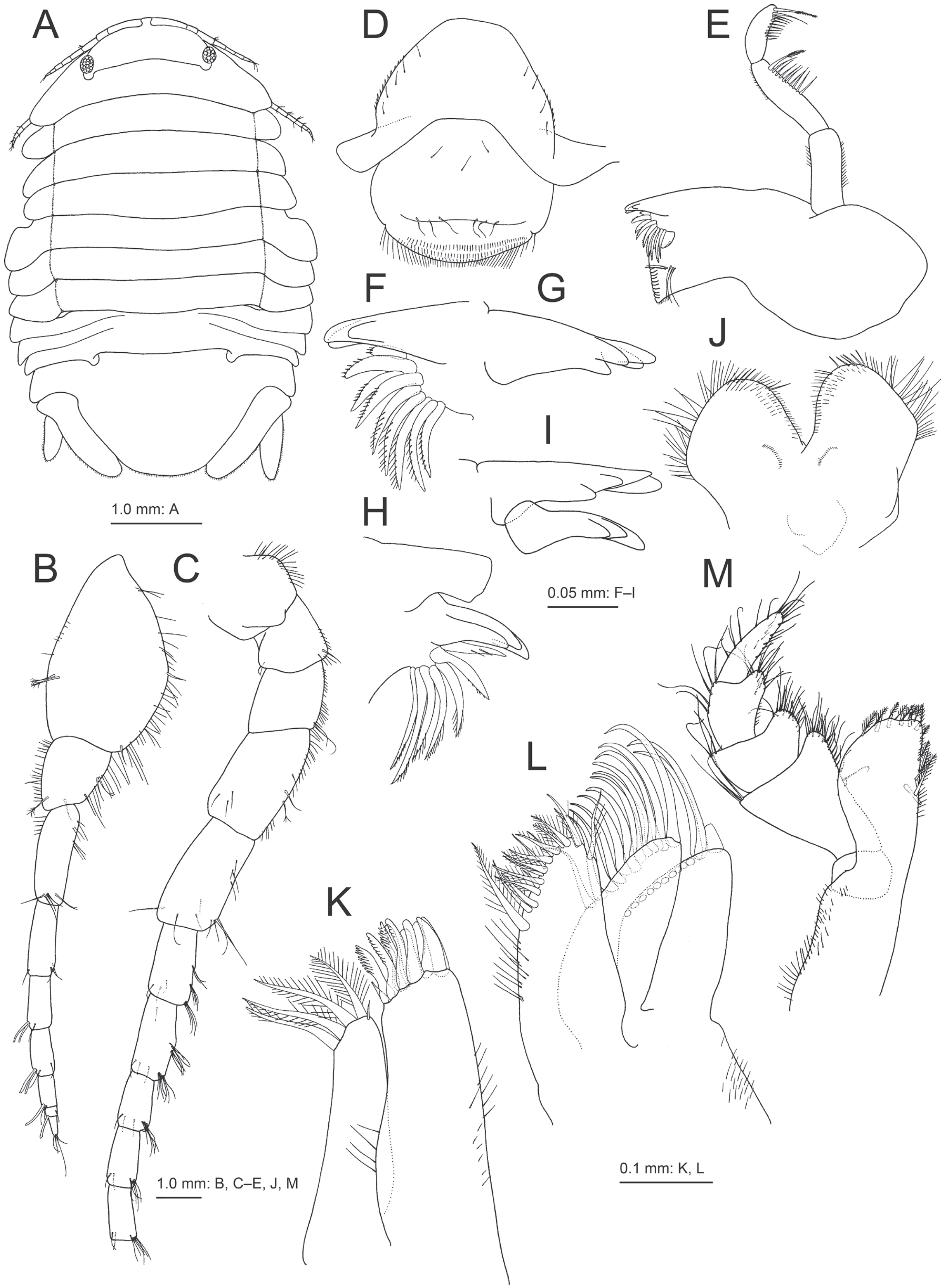

Figure 3. *Gnorimosphaeroma rivulare* sp. nov. **A–F, H, J–M.** Holotype, male 4.9 mm (NSMT-Cr 31485); **G, I.** Paratype, male 5.3 mm (NSMT-Cr 31486). **A.** Habitus, dorsal view; **B.** Antennula, medial view; **C.** Antenna, medial view; **D.** Labrum, dorsal view; **E.** Right mandible, medial view; **F.** Incisor and setal row of right mandible, medial view; **G.** Incisor of right mandible, lateral view; **H.** Incisor, lacinia mobilis and setal row of left mandible, medial view; **I.** Incisor and lacinia mobilis of left mandible, medial view; **J.** Paragnaths, posterior view; **K.** Maxillula, anterior view; **L.** Maxilla, anterior view; **M.** Maxilliped, anterior view.

4 setae on anterodistal corner. Pereopod 2 propodus subrectangular, not swollen. Pereopod 3 merus, carpus, and propodus sparsely setulose. Uropod exopod length 0.7 times as long as endopod.

Description. Male [NSMT-Cr 31485, holotype, 4.9 mm]. Body (Figs 2B, 3A) ovate, vaulted, ca. 1.5 times longer than wide, dorsal surface unornamented, without marginal setae; eyes ovate, simple. Coxal plates (Figs 3A, 7A) without visible distinct articulation to pereonites, partly overlapped, distal coxal margins rounded without setae; coxa 3 tapered anteriorly and posteriorly such that the animal can conglobulate. Pleon (Figs 3A, 7B) incompletely fused; pleonite 1 almost invisible, hidden by pereonite 7; anterior suture line between pleonites 2 and 3 longer than posterior one between pleonites 3 and 4; pleonite 5 fused with pleonite 4, barely recognizable. Pleotelson (Figs 3A, 7B) slightly narrower than pleon, length 0.5 times longer than wide, posterior margin entire, rounded, with minute setae.

Antennule (Fig. 3B) exceeding posterior margin of head; length ratio of peduncular articles 1–3 as 1.0: 0.4: 0.5; peduncular article 1 ovate, length 1.6 times as long as wide, anterior margin with a few setae, posterior margin lined with setae; peduncular article 2 subquadrate, length 1.2 times as long as wide, anterior and posterior margins lined with setae; peduncular article 3 rectangular, slender, length 3.0 times as long as wide, with setae on anterior and posterior margins; flagellum 6-articulate, articles 2 and 3 each with 2 aesthetaescs and articles 4 and 5 each with an aesthetasc. Antenna (Fig. 3C) reaching distal margin of pereonite 2; length ratio of peduncular articles 1–5 as 1.0: 0.9: 0.9: 1.5: 1.5; peduncular articles 1–3 subquadrate with fine setae on anterior margin; peduncular articles 4 and 5 subrectangular, each length 1.9 times longer than wide, anterior margin of article 4 with fine setae, article 5 with a few setae; flagellum 12-articulate (only the first five articles drawn in Fig. 3C).

Labrum (Fig. 3D) anterior margin rounded with fine setae. Mandibles (Fig. 3E–I) left and right incisors with 4 and 3 cusps, respectively; lacinia mobilis of left mandible with 3 cusps; setal row of left and right mandibles comprised 7 and 9 serrate setae; palp 3-articulate, length ratio of articles 1–3 as 1.0: 1.2: 0.8, article 1 with fine setae, article 2 with 10 setae on lateral margin and fine setae on medial margin, article 3 with 11 setae on lateral margin. Paragnaths (Fig. 3J) with rounded shoulders, bearing setae. Maxillula (Fig. 3K) medial lobe narrow, slightly shorter than lateral lobe, with 4 plumose setae and a short simple seta; lateral lobe with 10 robust setae, 4 of which serrate. Maxilla (Fig. 3L) medial lobe with 17 setae, some of which plumose; middle lobe with 12 setae; lateral lobe with 13 setae. Maxilliped (Fig. 3M) endite reaching distal margin of palp article 2, distal margin with 8 plumose setae and 5 simple setae, medial margin with a coupling hook and 4 plumose setae; palp 5-articulate, articles 2–4 with 3, 3 and 6 setae on lateral margin, respectively, produced mediodistally with setae, article 5 narrow with marginal setae.

Pereopod 1 (Fig. 4A, B) basis with a ventrodistal simple seta, ventral margin with fine setae; merus lobate dorsodistally, dorsodistal corner with 4 setae, ventral margin with robust setae and fine setae; carpus short with 2 robust setae and fine setae on ventral margin; propodus oval, swollen, length 2.5 times as long as wide, with 2 robust setae on ventral margin and 5 setulate setae close to ventral margin; dactylus length 0.6 times as long as propodus. Pereopod 2 (Fig. 4C, D) basis with a ventrodistal simple seta; merus weakly lobate dorsodistally, with 5 setae on dorsodistal corner, ventral margin with fine setae; carpus with 6 robust and 3 slender setae on distal surface, ventral margin with fine setae; propodus subrectangular, not swollen, length 2.8 times as long as wide, with 3 robust setae on ventral margin; dactylus length 0.5 times as long as propodus. Pereopod 3 (Fig. 4E, F) basis with a ventrodistal simple seta; merus lobate dorsodistally, dorsodistal corner with 6 setae, ventral margin with sparse fine setae; carpus with 5 robust and some slender setae on distal margin, ventral margin with sparse fine setae; propodus length 2.8 times as long as wide, with 2 robust setae and a slender single seta on ventral margin; dactylus length 0.5 times as long as propodus. Pereopod 4 (Fig. 4G) basis with a simple seta on ventrodistal corner and some broom setae on dorsal margin; ischium with fine setae on ventral margin; merus lobate dorsodistally with robust setae, dorsal and ventral margins with fine setae; carpus with sparse fine setae on ventral margin; propodus with 3 robust setae on ventral margin, dorsal and ventral margins with sparse fine setae. Pereopod 5 (Fig. 4H) basis with a simple seta on dorsodistal corner; merus and carpus with robust setae on distal margins; propodus with 3 robust setae on dorsal margin. Pereopods 6 and 7 (Fig. 5A, B) basis with a simple seta on dorsodistal corner; merus and carpus with robust setae on distal margins; propodus with 2 robust setae on dorsal margin.

Penial process (Fig. 5C) simple, length 3.5–4.2 times as long as basal width, close set.

Pleopod 1 (Fig. 5D) peduncle length 0.5 times width with 4 setae on mediodistal corner; exopod oval, length 1.9 times width, 1.2 times length of endopod, with plumose setae marginally; endopod subtriangular, length 1.4 times width, with distal plumose setae. Pleopod 2 (Fig. 5E) peduncle length 0.4 times width, mediodistal and laterodistal corners with 2 and 1 setae, respectively; exopod oval, length 1.8 times width, with marginal plumose setae; endopod subtriangular, length 1.6 times width, with plumose setae on distal margin; appendix masculina slender, length 6.0 times width, 1.2 times as long as endopod. Pleopod 3 (Fig. 5F) peduncle length 0.4 times width, mediodistal and laterodistal corners with 2 and 1 setae, respectively; exopod oval, length 1.9 times width, with transverse suture, bearing plumose setae marginally; endopod slightly shorter than exopod with plumose setae on distal margin. Pleopod 4 (Fig. 5G) peduncle small, length 0.5 times width, laterodistal corner with a single seta; exopod length 1.8 times width with transverse suture, bearing 4 plumose setae and simple setae marginally; endopod oval, length 1.4 times width,

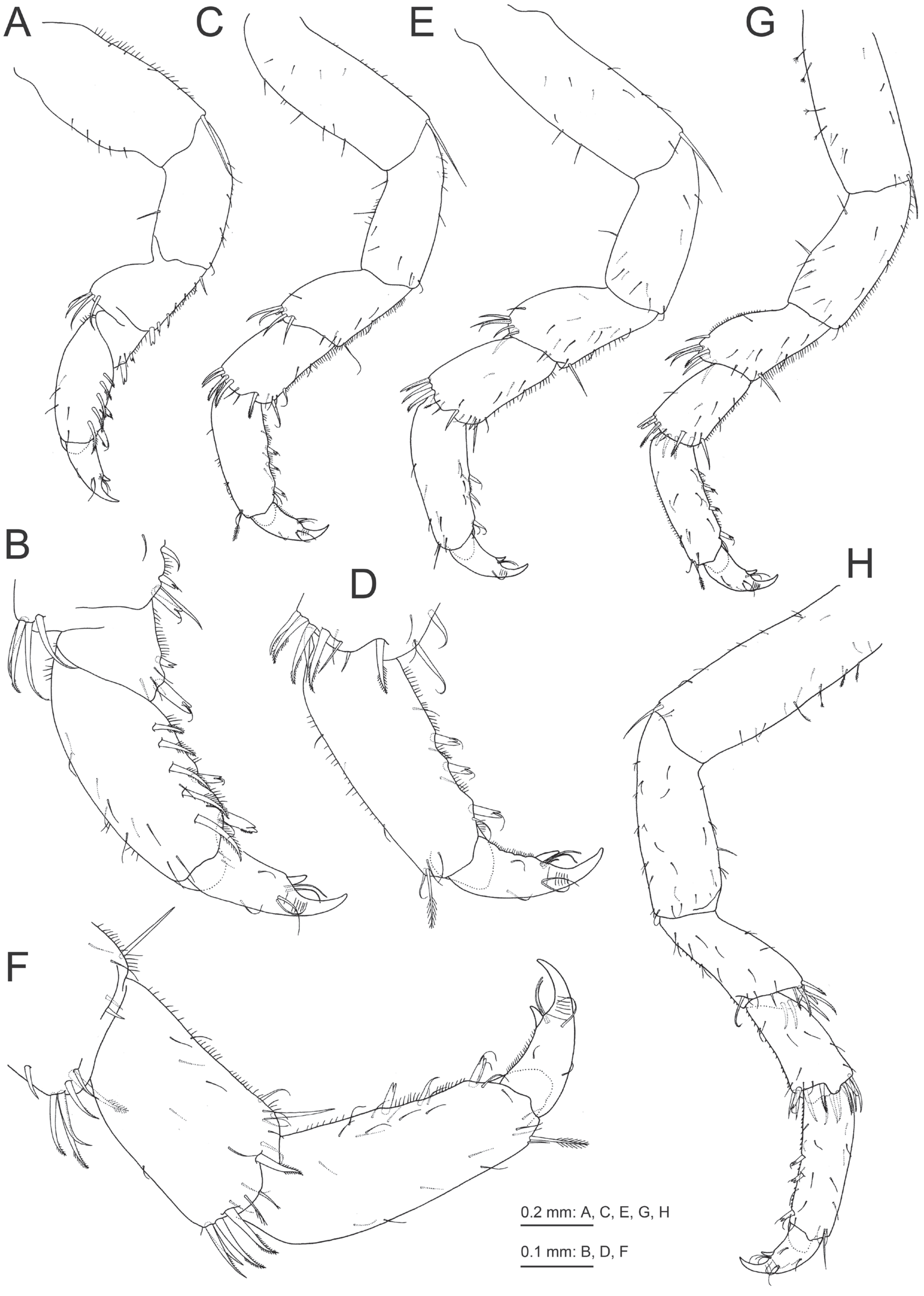

Figure 4. *Gnorimosphaeroma rivulare* sp. nov., holotype, male 4.9 mm (NSMT-Cr 31485). **A.** Right pereopod 1, medial view; **B.** Merus, carpus, propodus and dactylus of right pereopod 1, medial view; **C.** Right pereopod 2, medial view; **D.** Carpus, propodus and dactylus of right pereopod 2, medial view; **E.** Right pereopod 3, medial view; **F.** Merus, carpus, propodus and dactylus of right pereopod 3, medial view; **G.** Right pereopod 4, medial view; **H.** Right pereopod 5, medial view.

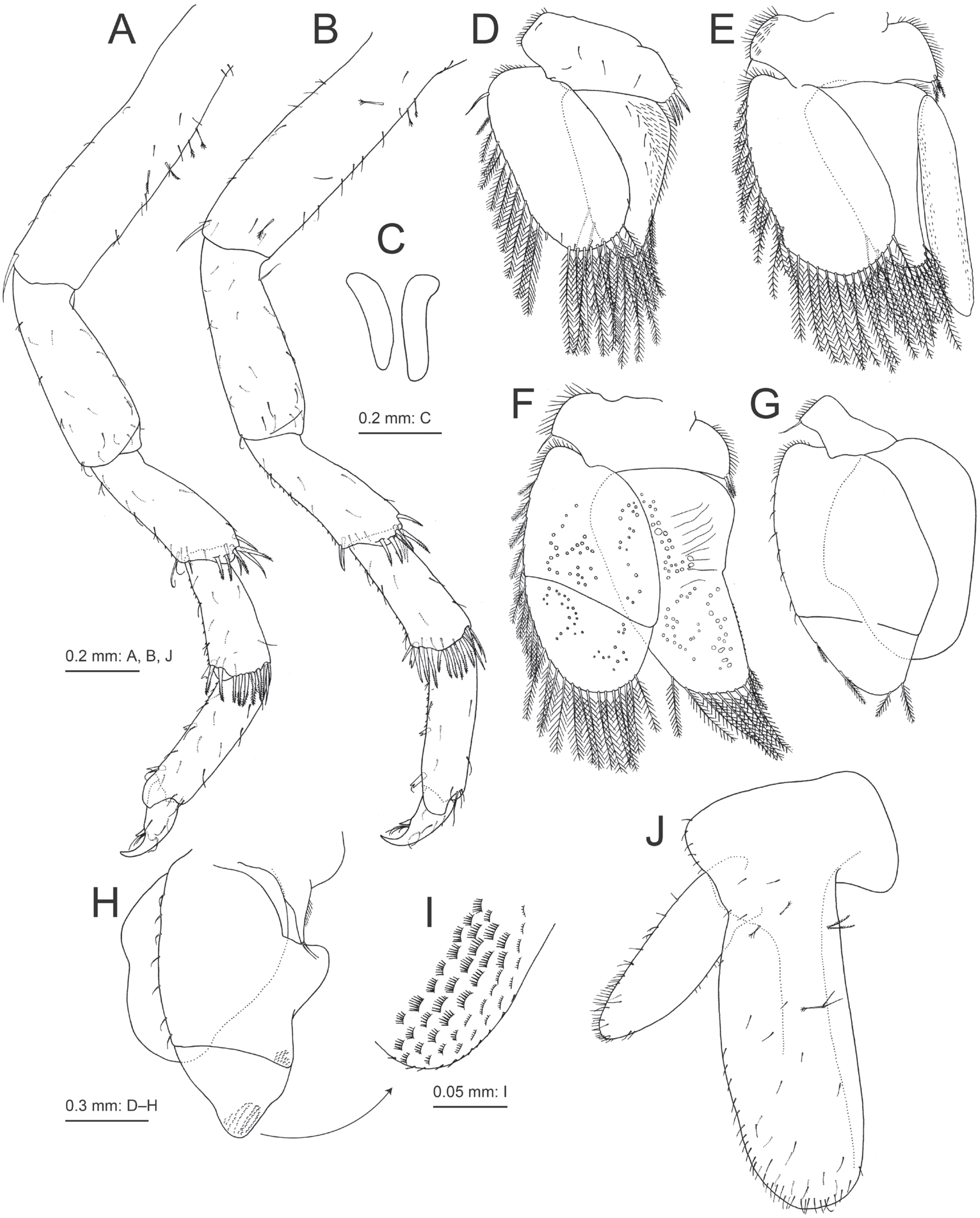

Figure 5. *Gnorimosphaeroma rivulare* sp. nov., holotype, male 4.9 mm (NSMT-Cr 31485). **A.** Right pereopod 6, medial view; **B.** Right pereopod 7, medial view; **C.** Penes, anterior view; **D.** Right pleopod 1, anterior view; **E.** Right pleopod 2, anterior view; **F.** Right pleopod 3, anterior view; **G.** Right pleopod 4, anterior view; **H.** Left pleopod 5, posterior view; **I.** Detail of distal endopod of pleopod 5, posterior view; **J.** Left uropod, dorsal view.

marginally bare. Pleopod 5 (Fig. 5H, I) peduncle with 2 setae on mediodistal corner; exopod length 1.6 times width transverse suture, bearing lateral and distal scale patches, lateral margin with simple setae; endopod oval, length 0.7 times as long as exopod, marginally bare.

Uropod (Figs 3A, 5J, 7B) not quite extending to posterior margin of pleotelson; peduncle rectangular, length 0.6 times width; exopod length 3.5 times width, 0.7 times as long as endopod, with fine sparse setae; endopod length 2.5 times width with fine setae.

Female [NSMT-Cr 31488, paratype, 3.2 mm]. Body shape similar to that of male. Body length 1.7 times width.

Pereopod 1 (Fig. 6A) propodus subrectangular, length 2.6 times as long as wide. Pereopod 2 (Fig. 6B) propodus subrectangular, length 3.1 times as long as wide, with 2 robust setae on posterior margin.

Pleopod 1 (Fig. 6C) peduncle length 0.5 times width with 3 setae on mediodistal corner; exopod oval, length 1.6 times width, 1.2 times length of endopod; endopod subtriangular, length 1.5 times width. Pleopod 2 (Fig. 6D) peduncle length 0.4 times width, mediodistal and laterodistal corners with 2 and 1 setae, respectively; exopod oval, length 1.8 times width; endopod subtriangular, length 1.6 times width.

Uropod (Fig. 6E) peduncle rectangular, length 0.6 times width; exopod length 3.0 times width, 0.7 times as long as endopod; endopod length 2.6 times width.

Variation. Mandible left incisor of paratype male (NSMT-Cr 31486) with 4 cusps.

Etymology. The specific name *rivulare* is derived from a Latin adjective *rivularis*, which means brook living, referring to the habitat of the new species.

Distribution and habitat. This species is known only from the type locality. The specimens were collected from beneath the cobbles in an upper stream of Nagatani River.

Remarks. *Gnorimosphaeroma rivulare* sp. nov. is morphologically similar to *G. boninense* Nunomura, 2006, *G. naktongense* Kwon & Kim, 1987, and *G. saijoense* Nunomura, 2013 in having anterior suture line longer than posterior one, maxilla middle and lateral lobes each with more than 10 setae, pereopod 1 basis with a simple seta on ventrodistal corner, pereopod 1 merus with 4 setae on dorsodistal corner, and uropodal exopod twice as long as endopod. However, *G. rivulare* sp. nov. differs from these three species by the following features (features of the species that are being compared are in parentheses): from *G. boninense*, pleotelson posterior margin rounded (almost straight), pereopod 3 carpus and propodus sparsely setulose (densely setulose), pereopod 2 propodus subrectangular (subtriangular), and pleopod 3 exopod almost same size as endopod (smaller than endopod); from *G. naktongense*, antennule peduncular article 3 not elongate, length 1.3 times as long as article 2 (elongate, 1.7 times as long as article 2), pereopod 2 propodus ventral margin straight (weakly expanded), and pleopod 5 endopod length 0.7 (0.9) times as long as exopod; from *G. saijoense*, maxillula medial lobe with 4 (5) plumose setae, maxilliped palp articles 2 and 3 each with 3 (1) setae on laterodistal corner, pereopod

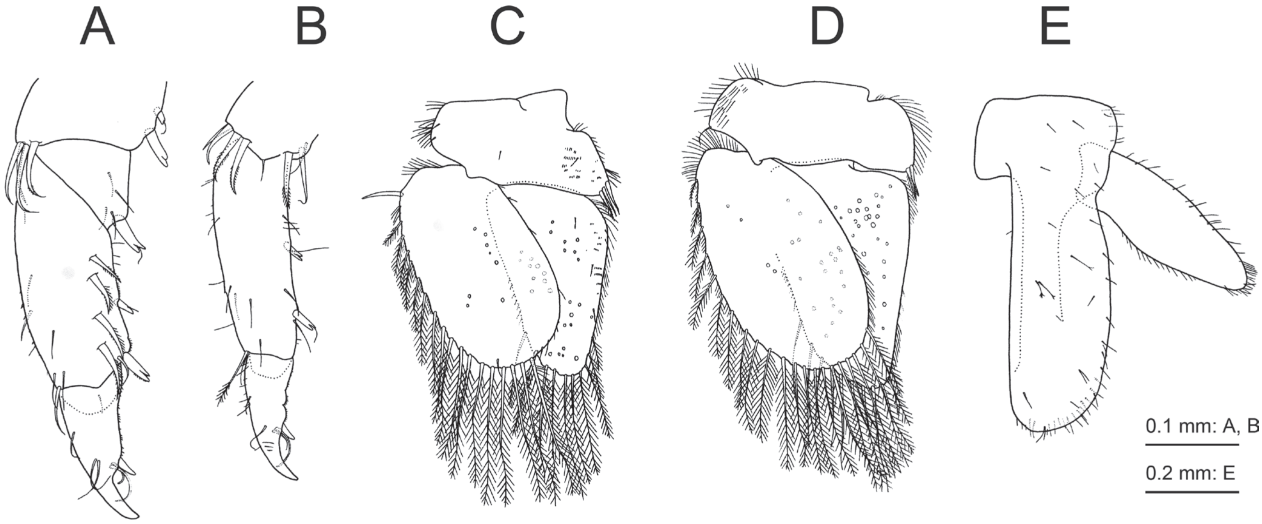

Figure 6. *Gnorimosphaeroma rivulare* sp. nov., paratype, female 3.2 mm (NSMT-Cr 31488). **A, B.** Merus, carpus, propodus and dactylus of right pereopods 1 and 2, medial views; **C, D.** Right pleopods 1 and 2, anterior views; **E.** Right uropod, dorsal view.

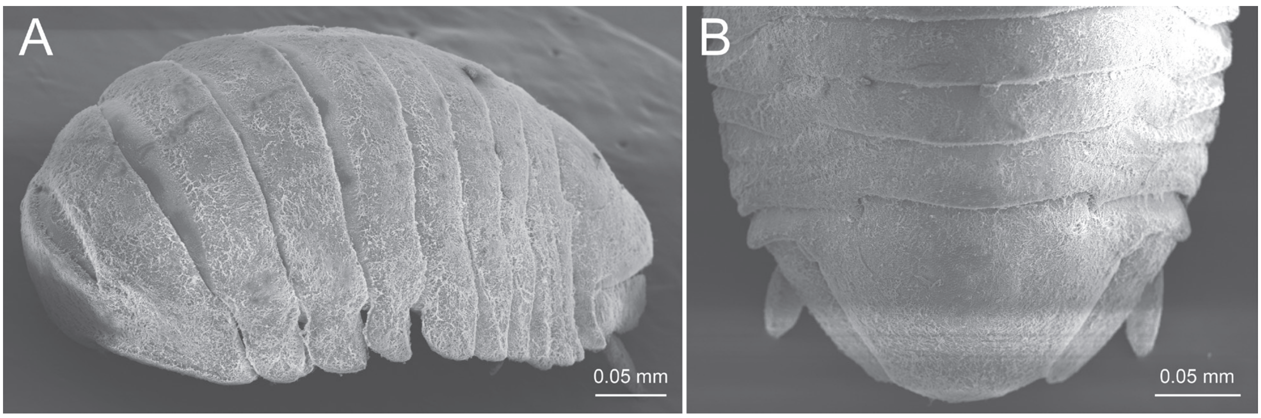

Figure 7. SEM photographs of *G. rivulare* sp. nov., paratype, male 5.2 mm (NSMT-Cr 31507). **A.** Habitus, lateral view; **B.** Pleon and pleotelson, dorsal view.

2 propodus ventral margin straight (weakly expanded), pereopod 3 merus, carpus, and propodus sparsely setulose (densely setulose).

Molecular phylogeny

Bayesian and ML phylogenies were similar. Only the ML is shown (Fig. 8). *Gnorimosphaeroma rivulare* sp. nov. forms a monophyletic clade with *G. akanense* Nunomura, 1998, *G. hokurikuense* Nunomura, 1998, *G. iriei* Nunomura, 1998, and *G. saijoense* (Fig. 8). Genetic differentiation of *G. akanense*, *G. hokurikuense*, and *G. saijoense* was small, with a *p*-distance of 0.4–1.7% for 16S rRNA sequences. Marine *G. rayi* Hoestlandt, 1969 and brackish water *G. tondaense* Nunomura, 1999 are sister group. The 16S rRNA sequences of unidentified species from Chiba, Japan and California were identical.

Discussion

Our molecular phylogenetic analyses revealed that marine, brackish, and freshwater taxa appear mixed throughout the tree. *Gnorimosphaeroma boninense* from a small stream on Haha-jima Island, Ogasawara Islands and *G. rivulare* sp. nov. are not sister taxa; however, the latter forms a monophyletic clade with *G. akanense* from Akan River, *G. hokurikuense* from freshwater streams in Honshu, *G. iriei* from springs in Kyushu, and *G. saijoense* from brackish waters at the mouths of rivers in Shikoku. This suggests that *G. boninense* and *G. rivulare* sp. nov. may have colonised the Ogasawara Islands independently. However, without including all *Gnorimosphaeroma* species in the analyses and also carefully reviewing the ecologies of each species it is not possible to definitively determine invasion and evolutionary history. Multiple invasions of freshwater invertebrates into the Ogasawara Islands have been reported for the amphipod Crustacea (Tomikawa et al. 2022). Tomikawa et al. (2022) showed that two species of melitid amphipods, *Melita nunomurai* Tomikawa & Sasaki, 2022 and *M. ogasawaraensis* Tomikawa & Sasaki, 2022, which occur in freshwater of Ogasawara Islands, are closely related to the species occurring on Honshu Island and the Nansei Islands, respectively. Possibly, they may originate from different regions. Although the origin of freshwater *Gnorimosphaeroma* on the Ogasawara Islands cannot been clarified in this study, we suggest that further elucidation of the evolutional history of freshwater isopods on oceanic islands will be possible with enhanced taxon sampling and broad genetic sampling in the future.

The freshwater invertebrate fauna of Chichi-jima Island, Ogasawara Islands, has been relatively well investigated (Satake and Ueno 2013; Satake et al. 2019). However, *G. rivulare* sp. nov. has only been found at one site in the upper reaches of Nagatani River on Chichi-jima Island (Satake and Ueno 2013). In addition to the low water volume in this species' habitat, the water flow is being dammed up by the roots of the non-native plant Bishop wood, *Bischofia javanica*. Freshwater *Gnorimosphaeroma* have been found in springs and mountain streams (e.g. Nunomura 1998) and is considered to prefer oxygenated

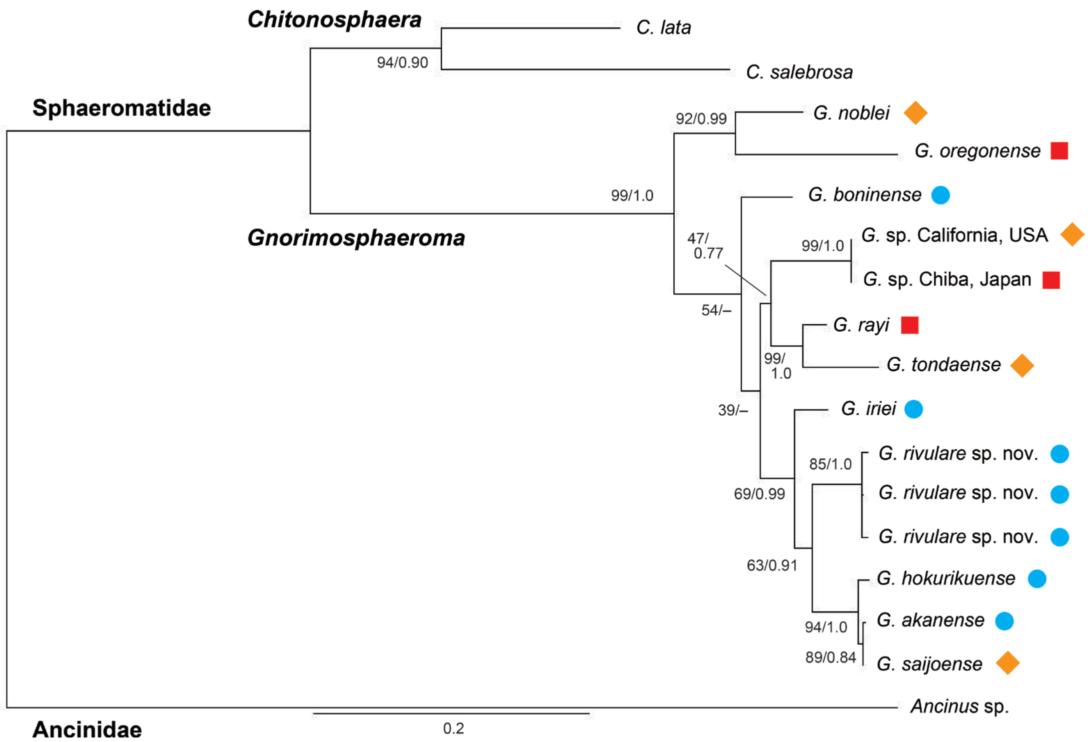

Figure 8. Maximum likelihood tree for 1077 bp of 16S rRNA and 18S rRNA markers. Numbers on nodes represent ultra-bootstrap values for maximum likelihood and Bayesian posterior probabilities. Symbols beside the species name indicate the habitats shown in Fig. 1.

lotic waters. A stagnant bottom environment may not be favourable for *G. rivulare* sp. nov., and could severely limit its future persistence. For this newly described species survive, conservation of this restricted habitat is an urgent issue that needs to be addressed.

Author contributions

KT, JY, AN, TS, NK, and NN collected the samples used in this study. KT, JY, AN, and CL performed molecular phylogenetic analyses. KT, JY, and NN were responsible for describing and naming the new species. This manuscript was compiled by KT and carefully reviewed and finalized by all authors.

Acknowledgements

We thank Drs Regina Wetzer of Natural History Museum of Los Angeles County and Brenda L. Doti of Universidad de Buenos Aires for their critical reading and valuable comments on our manuscript. This work was partly supported by the Japan Society for the Promotion of Science KAKENHI grants JP21H00910, JP22H01011, JP22K06373, and JP22K06371 to KT. This work was also supported by grants from the Nakdonggang National Institute of Biological Resources (NNIBR) funded by the Ministry of Environment (MOE) of the Republic of Korea (NNIBR202302102) to CL. We would like to thank Editage (www.editage.com) for English language editing.

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