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Description of 56 unrecorded bacterial species in Korea, isolated from freshwater in 2022

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Here we describe indigenous prokaryotic species in Korea, a total of 56 bacterial strains were isolated from freshwater and soil from the Republic of Korea. From the high 16S rRNA gene sequence similarity (>98.7%) and formation of a robust phylogenetic clade with the closest species, it was determined that each strain belonged to predefined bacterial species. There is no official report that these 56 species belonged to 4 phyla, 8 classes, 18 orders, 26 families, and 36 genera, which were assigned to *Pedobacter*, *Dyadobacter*, and *Chryseobacterium* of the phylum *Bacteroidota*; *Aeromicrobium*, *Cryobacterium*, *Isoptericola*, *Kitasatospora*, *Leifsonia*, *Microbacterium*, *Nonomuraea*, and *Streptomyces* of the phylum *Actinomycetota*; *Acinetobacter*, *Bradyrhizobium*, *Caballeronia*, *Chenggangzhangella*, *Chitinilyticum*, *Klebsiella*, *Lysobacter*, *Massilia*, *Mesorhizobium*, *Microvirga*, *Oxalicibacterium*, *Pantoea*, *Paraburkholderia*, *Phenylobacterium*, *Polaromonas*, *Pseudoxanthomonas*, *Sphingomonas*, *Tahibacter*, and *Undibacterium* of the phylum *Pseudomonadota*; *Bacillus*, *Paenibacillus*, *Lysinibacillus*, *Ureibacillus*, and *Virgibacillus* of the phylum *Bacillota*. Gram-staining results, colony and cell morphologies, basic biochemical characteristics, isolation sources, and strain IDs are also described in the species description section.

Keywords: 16S rRNA, bacterial diversity, freshwater, indigenous prokaryotic species in Korea, soil, unrecorded species

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Introduction

Freshwater accounts for 2.5% of the aquatic environment, while seawater makes up 97.5%. Freshwater, containing only about 3.5% salt, differs slightly from seawater and encompasses various ecosystems, including rivers and streams, where water flows depending on gradients and altitude differences. It exists in forms such as rivers, lakes, and groundwater, comprising approximately 0.6% of Earth's total water system excluding glaciers, yet remains a vital resource for human survival (Wetzel, 2001). In freshwater environments, most dominant prokaryotes remain uncultured except for certain groups. Microorganisms are more abundant in downstream water compared to upstream, with upstream waters hosting many low-nutrient microorganisms while downstream waters support the proliferation of high-nutrient microorganisms. While most bacterial groups typically form biofilms, fungi and algae are also commonly found. Microorganisms in oligotrophic and eutrophic lakes exhibit differences, with photosynthetic bacteria such as Cyanobacteria thriving in eutrophic lakes during summer. To date, approximately 20 phyla of bacterial clusters have been identified in freshwater environments, with *Actinomycetota*, *Bacteroidota*, *Pseudomonadota*, *Cyanobacteriota*, and *Verrucomicrobiota* being predominant (Newton *et al*., 2011).

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According to GOLD statistics, environmental bacteria contribute to about 10% of all genetic projects, with the majority of ecological research biased towards the digestive tract, oceans, and soil, while freshwater research accounts for 9.2% (Mukherjee *et al*., 2022). Similar to marine and terrestrial environments, freshwater-derived prokaryotes primarily function as decomposers, converting organic matter into inorganic substances through microfood networks and playing a crucial role in greenhouse gas cycling. Therefore, research analyzing microbial diversity derived from freshwater, despite its significant impact on human life, remains relatively underexplored. Such research could contribute to understanding the roles of freshwater-specific prokaryotes and further expand domestic microbial species discovery efforts.

In 2022, we collected environmental samples from freshwater in Korea and isolated many novel and unrecorded bacterial species during a research program supported by NNIBR (Nakdonggang National Institute of Biological Resources) of Korea. The identified bacterial species belongs to the classes *Bacilli*, *Actinomycetia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Shpingobacteriia*, *Cytophagia*, and *Flavobacteriia*. Here we shortly describe 56 unrecorded bacterial species in the 8 classes belonging to 18 orders.

Materials and Methods

A total of 56 bacterial strains affiliated to the classse *Bacilli*, *Actinomycetia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Shpingobacteriia*, *Cytophagia*, and *Flavobacteriia* were isolated from freshwater and soil samples. Freshwater and soil samples were processed separately, spread onto diverse culture media including AIA, R2A, five folds-diluted R2A, nutrient, five folds-diluted nutrient, and incubated at 20, 25 and 30°C for 5−10 days (Table 1). The designated strain IDs, sources, culture media, and incubation conditions are summarized in Table 1. All strains were purified as single colonies and stored as 20−25% (w/v) glycerol suspension −80°C as well as lyophilized ampoules.

The morphology was studied on agar plates for 2−5 days under their optimum temperature. Cell size and shape were examined either by transmission electron microscopy or scanning electron microscopy. Gram staining was performed using a Gram-staining kit of the standard procedures. Using API 20NE (bioMérieux) the biochemical characteristics were performed according to the manufacturer's instructions.

Bacterial DNA extraction, PCR amplification and 16S rRNA gene sequencing were performed using the standard procedures described by Lane (1991). The 16S rRNA gene sequences of the strains assigned to the 56 bacterial strains were compared with the sequences held in GenBank by BLASTN and also analyzed using the EzBioCloud blast (https://www.ezbiocloud.net) (Yoon *et al*., 2017). For phylogenetic analyses, multiple alignments were performed using the Clustal_X program (Thompson *et al*., 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic trees were constructed by using the neighbor-joining (Saitou and Nei, 1987) methods with the MEGA X Program (Kumar *et al*., 2018) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Results and Discussion

The 56 strains were distributed in 8 classes of the *Bacilli*, *Actinomycetia, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Gammaproteobacteria, Shpingobacteriia, Cytophagia,* and *Flavobacteriia*; 5 strains for the class *Bacilli*, 17 strains for the *Actinomycetia*, 10 strains for the *Alphaproteobacteria*, 12 strains for the *Betaproteobacteria*, 8 strains for the *Gammaproteobacteria*, 2 strains for the *Shpingobacteriia*, 1 strain for the *Cytophagia* and 1 strain for the *Flavobacteriia* (Table 1). These strains were Gram-staining-negative or positive, chemoheterotrophic, rod, short-rod and coccoid shaped showing in Fig. 1.

The strains in the phylum *Actinomycetota* (Fig. 2) were found to belong to 1 class 9 separate genera: *Streptomyces* (8 species), *Nonomuraea* (1 species), *Mycolicibacterium* (1 species), *Microbacterium* (2 species), *Leifsonia* (1 species), *Kitasatospora* (1 species), *Isoptericola* (1 species), *Cryobacterium* (1 species), *Aeromicrobium* (1 species).

Fig. 3 shows phylogenetic assignment of 30 strains of the phylum *Pseudomonadota* belong to 3 classes 19 separate genera: *Acinetobacter* (1 species), *Bradyrhizobium* (1 species), *Caballeronia* (1 species), *Chenggangzhangella* (1 species), *Chitinilyticum* (1 species), *Klebsiella* (1 species), *Lysobacter* (1 species), *Massilia* (3 species), *Mesorhizobium* (1 species), *Microvirga* (2 species), *Oxalicibacterium* (1 species), *Pantoea* (2 species), *Paraburkholderia* (3 species), *Phenylobacterium* (1 species), *Pseudoxanthomonas* (2 species), *Sphingomonas* (4 species), *Tahibacter* (1 species), and *Undibacterium* (2 species).

Fig. 4 shows phylogenetic assignment of 9 strains of the phyla *Bacillota* and *Bacteroidota* belong to 4 classes 8 separate genera: *Bacillus* (1 species), *Paenibacillus* (1 species), *Lysinibacillus* (1 species), *Ureibacillus* (1 species), *Virgibacillus* (1 species), *Pedobacter* (2 species), *Dyadobacter* (1 species), and *Chryseobacaterium* (1 species).

Here we report 56 unrecorded bacterial species in Korea belonging to 8 classes of 4 phyla.

Description of *Microvirga tunisiensis* AS1R-43

Cells are Gram-staining-negative, non-flagellated and oval-shaped. Colonies are circular, raised, entire and light pink color after 2 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase. Utilize D-glucose, L-arabinose, D-mannose and potassium gluconate. Does not utilize D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain AS1R-43 (=NNIBR 2022642BA5) has been isolated from a fresh water sample, Heukseok-dong, Dongjak-gu, Seoul, Republic of Korea (37°30′32.1″N 126°58′07.2″E).

Description of *Caballeronia mineralivorans* AS1R-58

Cells are Gram-staining-negative, flagellated and oval-shaped. Colonies are circular, convex, smooth and white color after 2 days on R2A at 25°C. In API 20NE, positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Does not utilize D-maltose and adipic acid. Strain AS1R-58 (=NNIBR2022642BA6) has been isolated from a fresh water sample, Heukseok-dong, Dongjak-gu, Seoul, Republic of Korea (37°30′32.1″N 126°58′07.2″E).

Description of *Paraburkholderia steynii* AS2R-57

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and white color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, β-galactosidase, but negative for, indole production, glucose fermentation, urease, esculin hydrolysis, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Does not utilize D-maltose and adipic acid. Strain AS2R-57 (=NNIBR2022642BA7) has been isolated from a fresh water sample, Heukseok-dong, Dongjak-gu, Seoul, Republic of Korea (37°30′32.1″N 126°58′07.2″E).

Description of *Bacillus massiliogabonensis* AS3N-2

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire and cream color after 2 days on R2A at 25°C. In API 20NE, positive for urease, esculin hydrolysis, gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, β-galactosidase. Utilize D-mannitol, adipic acid and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, malic acid and phenylacetic acid. Strain AS3N-2 (=NNIBR2022642BA8) has been isolated from a fresh water sample, Heukseok-dong, Dongjak-gu, Seoul, Republic of Korea (37°30′32.1″N 126°58′07.2″E).

Description of *Paraburkholderia acidiphila* AS3R-50

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and white color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, malic acid and phenylacetic acid. Does not utilize D-maltose, adipic acid and trisodium citrate. Strain AS3R-50 (=NNIBR2022642BA9) has been isolated from a fresh water sample, Heukseok-dong, Dongjak-gu, Seoul, Republic of Korea (37°30′32.1″N 126°58′07.2″E).

Description of *Massilia rivuli* AS3R-65

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, raised, entire and cream color after 2 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase. Utilize D-glucose, L-arabinose, D-mannose, D-maltose and potassium gluconate. Does not utilize D-mannitol, *N*-acetyl-D-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain AS3R-65 (=NNIBR2022642BA10) has been isolated from a fresh water sample, Heukseok-dong, Dongjak-gu, Seoul, Republic of Korea (37°30′32.1″N 126°58′07.2″E).

Description of *Bradyrhizobium centrosematis* AS4R-44

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, raised, entire and white color after 2 days on R2A at 25°C. In API 20NE, positive for urease, esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase, β-galactosidase Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, adipic acid and malic acid. Does not utilize *N*-acetyl-D-glucosamine, D-maltose, capric acid,

Table 1. The taxonomic affiliations of isolated strains belong to the class *Alphaproteobacteria*.

Class	Order	Family	Genus	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation source	Isolation site	Medium	Incubation conditions (°C, days)
Bacilli	*Bacillales*	*Bacillaceae*	*Bacillus*	AS3N-2	NNIBR2022642BA8	*Bacillus massiliogabonensis*	99.25	Freshwater	Seoul-si	R2A	25°C, 2d
	Bacillales	*Paenibacillaceae*	*Paenibacillus*	HS3R-17	NNIBR2022642BA13	*Paenibacillus helianthi*	98.82	Freshwater	Hongcheon-gun	R2A	25°C, 2d
	Bacillales	*Planococcaceae*	*Lysinibacillus*	PS1N-9	NNIBR2022642BA17	*Lysinibacillus capsici*	100	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Bacillales	*Planococcaceae*	*Ureibacillus*	SS4R-24	NNIBR2022642BA36	*Ureibacillus manganicus*	99.93	Freshwater	Chuncheon-si	R2A	25°C, 2d
	Bacillales	*Bacillaceae*	*Virgibacillus*	MMS22-TA20	NNIBR2022642BA45	*Virgibacillus necropolis*	98.9	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
Actinomycetia	*Microbacteriales*	*Microbacteriaceae*	*Leifsonia*	HS2N-18	NNIBR2022642BA12	*Leifsonia flava*	98.83	Freshwater	Hongcheon-gun	R2A	25°C, 2d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	HS4R-32	NNIBR2022642BA15	*Streptomyces lydicamycinicus*	100	Freshwater	Hongcheon-gun	R2A	25°C, 2d
	Microbacteriales	*Microbacteriaceae*	*Cryobacterium*	PS1N-12	NNIBR2022642BA18	*Cryobacterium tepidiphilum*	98.89	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Microbacteriales	*Microbacteriaceae*	*Microbacterium*	PS2R-23	NNIBR2022642BA21	*Microbacterium imperiale*	98.96	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	PS3R-31	NNIBR2022642BA26	*Streptomyces viridochromogenes*	99.79	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Propionibacteriales	*Nocardioidaceae*	*Aeromicrobium*	SS2R-19	NNIBR2022642BA34	*Aeromicrobium flavum*	99.93	Freshwater	Chuncheon-si	R2A	25°C, 2d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	SS3R-15	NNIBR2022642BA35	*Streptomyces griseoaurantiacus*	100	Freshwater	Chuncheon-si	R2A	25°C, 2d
	Cellulomonadales	*Promicromonosporaceae*	*Isoptericola*	MMS22-DV25	NNIBR2022642BA51	*Isoptericola salitolerans*	99.5	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
	Streptosporangiales	*Streptosporangiaceae*	*Nonomuraea*	MMS22-SW7	NNIBR2022642BA52	*Nonomuraea cavernae*	99.23	Freshwater	Namyangju-si	R2A	30°C, 3d
	Mycobacteriales	*Mycobacteriaceae*	*Mycolicibacterium*	MMS22-SW13	NNIBR2022642BA53	*Mycolicibacterium cosmeticum*	99.37	Freshwater	Namyangju-si	R2A	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS22-GS18	NNIBR2022642BA57	*Streptomyces lushanensis*	99.23	Freshwater	Gongju-si	R2A	30°C, 3d
	Microbacteriales	*Microbacteriaceae*	*Microbacterium*	MMS22-SSW31	NNIBR2022642BA43	*Microbacterium album*	98.81	Freshwater	Namyangju-si	R2A	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Kitasatospora*	MMS22-YZ4	NNIBR2022642BA44	*Kitasatospora mediocidica*	99.79	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS22-GA10	NNIBR2022642BA37	*Streptomyces carpaticus*	99.86	Freshwater	Gapyeong-gun	AIA	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS22-GM13	NNIBR2022642BA38	*Streptomyces dioscori*	99.15	Freshwater	Gapyeong-gun	MA	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS22-R20	NNIBR2022642BA39	*Streptomyces qaidamensis*	99.37	Freshwater	Gapyeong-gun	R2A	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS22-R51	NNIBR2022642BA40	*Streptomyces nigra*	99.79	Freshwater	Gapyeong-gun	R2A	30°C, 3d
Alphaproteobacteria	*Rhizobiales*	*Methylobacteriaceae*	*Microvirga*	AS1R-43	NNIBR2022642BA5	*Microvirga tunisiensis*	98.73	Freshwater	Seoul-si	R2A	25°C, 2d
	Rhizobiales	*Nitrobacteraceae*	*Bradyrhizobium*	AS4R-44	NNIBR2022642BA11	*Bradyrhizobium centrosematis*	99.93	Freshwater	Seoul-si	R2A	25°C, 2d
	Caulobacterales	*Caulobacteraceae*	*Phenylobacterium*	PS1R-109	NNIBR2022642BA19	*Phenylobacterium kunshanense*	99.14	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Rhizobiales	*Methylobacteriaceae*	*Microvirga*	PW3R-10	NNIBR2022642BA32	*Microvirga brassicacearum*	98.79	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Sphingomonadales	*Sphingomonadaceae*	*Sphingomonas*	MMS22-ER13	NNIBR2022642BA59	*Sphingomonas pokkalii*	98.79	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Sphingomonas*	MMS22-DV15	NNIBR2022642BA60	*Sphingomonas corticis*	99.43	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
	Rhizobiales	*Methylopila_f*	*Chenggangzhangella*	MMS22-DV23	NNIBR2022642BA50	*Chenggangzhangella methanolivorans*	98.7	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Sphingomonas*	MMS22-SW14	NNIBR2022642BA54	*Sphingomonas carotinifaciens*	99.06	Freshwater	Namyangju-si	R2A	30°C, 3d
	Rhizobiales	*Phyllobacteriaceae*	*Mesorhizobium*	MMS22-YZ104	NNIBR2022642BA49	*Mesorhizobium sophorae*	99.79	Freshwater	Namyangju-si	R2A	30°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Sphingomonas*	MMS22-W6L	NNIBR2022642BA48	*Sphingomonas spermidinifaciens*	99.14	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Caballeronia*	AS1R-58	NNIBR2022642BA6	*Caballeronia mineralivorans*	99.66	Freshwater	Seoul-si	R2A	25°C, 2d
	Burkholderiales	*Burkholderiaceae*	*Paraburkholderia*	AS2R-57	NNIBR2022642BA7	*Paraburkholderia steynii*	99.86	Freshwater	Seoul-si	R2A	25°C, 2d
	Burkholderiales	*Burkholderiaceae*	*Paraburkholderia*	AS3R-50	NNIBR2022642BA9	*Paraburkholderia acidiphila*	99.31	Freshwater	Seoul-si	R2A	25°C, 2d
	Burkholderiales	*Oxalobacteraceae*	*Massilia*	AS3R-65	NNIBR2022642BA10	*Massilia rivuli*	98.76	Freshwater	Seoul-si	R2A	25°C, 2d
	Burkholderiales	*Oxalobacteraceae*	*Massilia*	HS4N-43	NNIBR2022642BA14	*Massilia tieshanensis*	98.9	Freshwater	Hongcheon-gun	R2A	25°C, 2d
	Burkholderiales	*Comamonadaceae*	*Polaromonas*	HW3N-3	NNIBR2022642BA16	*Polaromonas naphthalenivorans*	98.76	Freshwater	Hongcheon-gun	R2A	25°C, 2d
	Burkholderiales	*Oxalobacteraceae*	*Undibacterium*	PS2R-86	NNIBR2022642BA23	*Undibacterium oligocarboniphilum*	100	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Burkholderiales	*Oxalobacteraceae*	*Oxalicibacterium*	PS2R-116	NNIBR2022642BA24	*Oxalicibacterium faecigallinarum*	98.97	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Burkholderiales	*Burkholderiaceae*	*Paraburkholderia*	PS3R-121	NNIBR2022642BA28	*Paraburkholderia paradisi*	99.85	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Neisseriales	*Neisseriaceae*	*Chitinilyticum*	PS4R-81	NNIBR2022642BA31	*Chitinilyticum aquatile*	99.93	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Burkholderiales	*Oxalobacteraceae*	*Undibacterium*	MMS22-C1	NNIBR2022642BA55	*Undibacterium crateris*	99.38	Freshwater	Hongcheon-gun	R2A	30°C, 3d
	Burkholderiales	*Oxalobacteraceae*	*Massilia*	MMS22-ST2	NNIBR2022642BA41	*Massilia umbonata*	98.77	Freshwater	Namyangju-si	R2A	30°C, 3d

Table 1. Continued.

Class	Order	Family	Genus	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation source	Isolation site	Medium	Incubation conditions (°C, days)
Gammaproteobacteria	*Lysobacterales*	*Lysobacteraceae*	*Tahibacter*	PS2R-18	NNIBR2022642BA20	*Tahibacter caeni*	99.11	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Moraxellales	*Moraxellaceae*	*Acinetobacter*	PS2R-27	NNIBR2022642BA22	*Acinetobacter pragensis*	100	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Lysobacterales	*Lysobacteraceae*	*Lysobacter*	PS3R-13	NNIBR2022642BA25	*Lysobacter gilvus*	99.59	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Lysobacterales	*Lysobacteraceae*	*Pseudoxanthomonas*	SS2R-1	NNIBR2022642BA33	*Pseudoxanthomonas gei*	99.58	Freshwater	Chuncheon-si	R2A	25°C, 2d
	Enterobacterales	*Enterobacteriaceae*	*Klebsiella*	MMS22-SI17	NNIBR2022642BA58	*Klebsiella huaxiensis*	98.74	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
	Lysobacterales	*Lysobacteraceae*	*Pseudoxanthomonas*	MMS22-K1	NNIBR2022642BA56	*Pseudoxanthomonas winnipegensis*	100	Freshwater	Hongcheon-gun	R2A	30°C, 3d
	Enterobacterales	*Erwiniaceae*	*Pantoea*	MMS22-MW13	NNIBR2022642BA47	*Pantoea anthophila*	99.57	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
	Enterobacterales	*Erwiniaceae*	*Pantoea*	MMS22-MW43	NNIBR2022642BA46	*Pantoea dispersa*	99.26	Freshwater	Yangpyeong-gun	R2A	30°C, 3d
Sphingobacteriia	*Sphingobacteriales*	*Sphingobacteriaceae*	*Pedobacter*	PS4R-28	NNIBR2022642BA29	*Pedobacter mongoliensis*	100	Freshwater	Hwaseong-si	R2A	25°C, 2d
	Sphingobacteriales	*Sphingobacteriaceae*	*Pedobacter*	PS4R-55	NNIBR2022642BA30	*Pedobacter planticolens*	100	Freshwater	Hwaseong-si	R2A	25°C, 2d
Cytophagia	*Cytophagales*	*Spirosomaceae*	*Dyadobacter*	PS3R-65	NNIBR2022642BA27	*Dyadobacter bucti*	99.37	Freshwater	Hwaseong-si	R2A	25°C, 2d
Flavobacteriia	*Flavobacteriales*	*Weeksellaceae*	*Chryseobacterium*	MMS22-YWC9	NNIBR2022642BA42	*Chryseobacterium timonianum*	99.01	Freshwater	Namyangju-si	R2A	30°C, 3d

trisodium citrate and phenylacetic acid. Strain AS4R-44 (=NNIBR2022642BA11) has been isolated from a fresh water sample, Heukseok-dong, Dongjak-gu, Seoul, Republic of Korea (37°30′32.1″N 126°58′07.2″E).

Description of *Leifsonia flava* HS2N-18

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire and yellow color after 2 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose and potassium gluconate. Does not utilize *N*-acetyl-D-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain HS2N-18 (=NNIBR2022642BA12) has been isolated from a fresh water sample, Seo-myeon, Hongcheon-gun, Gangwon-do, Republic of Korea (37°41′20.0″N 127°41′22.7″E).

Description of *Paenibacillus helianthi* HS3R-17

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire and cream color after 2 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase. Utilize D-glucose and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium, gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain HS3R-17 (=NNIBR2022642BA13) has been isolated from a fresh water sample, Seo-myeon, Hongcheon-gun, Gangwon-do, Republic of Korea (37°41′20.0″N 127°41′22.7″E).

Description of *Massilia tieshanensis* HS4N-43

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and pale-yellow color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, urease, esculin hydrolysis, gelatinase, but negative for indole production, glucose fermentation, arginine dihydrolase, β-galactosidase. Utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, malic acid and phenylacetic acid. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid and trisodium citrate. Strain HS4N-43 (=NNIBR2022642BA14) has been isolated from a fresh water sample, Seo-myeon, Hongcheon-gun, Gangwon-do, Republic of Korea (37°41′20.0″N 127°41′22.7″E).

Description of *Streptomyces lydicamycinicus* HS4R-32

Cells are Gram-staining-positive, non-flagellated and

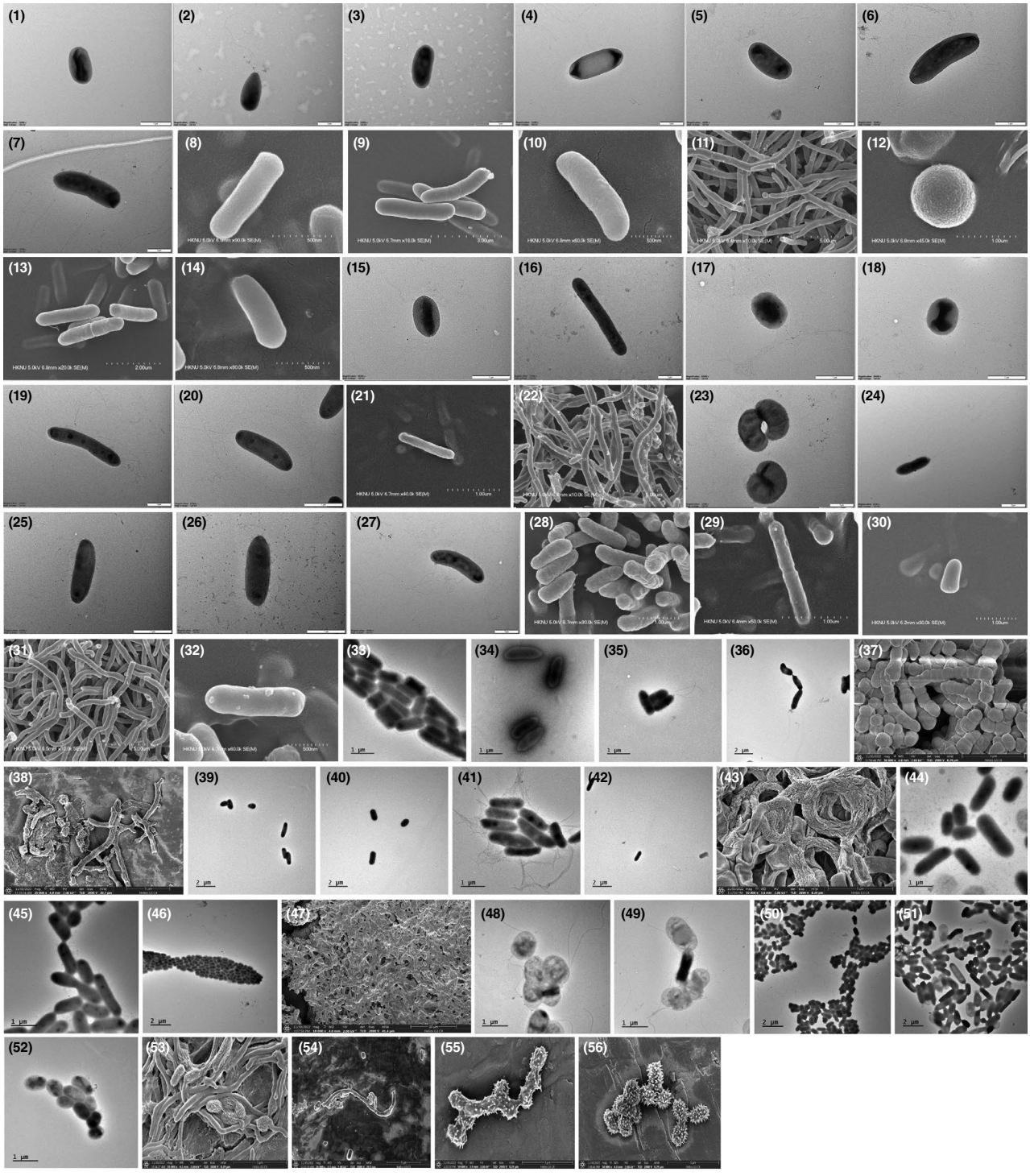


Fig. 1. Transmission and scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, AS1R-43; 2, AS1R-58; 3, AS2R-57; 4, AS3N-2; 5, AS3R-50; 6, AS3R-65; 7, AS4R-44; 8, HS2N-18; 9, HS3R-17; 10, HS4N-43; 11, HS4R-32; 12, HW3N-3; 13, PS1N-9; 14, PS1N-12; 15, PS1R-109; 16, PS2R-18; 17, PS2R-23; 18, PS2R-27; 19, PS2R-86; 20, PS2R-116; 21, PS3R-13; 22, PS3R-31; 23, PS3R-65; 24, PS3R-121; 25, PS4R-28; 26, PS4R-55; 27, PS4R-81; 28, PW3R-10; 29, SS2R-1; 30, SS2R-19; 31, SS3R-15; 32, SS4R-24; 33, MMS22-SI17; 34, MMS22-ER13; 35, MMS22-DV15; 36, MMS22-DV23; 37, MMS22-DV25; 38, MMS22-SW7; 39, MMS22-SW13; 40, MMS22-SW14; 41, MMS22-C1; 42, MMS22-K1; 43, MMS22-GS18; 44, MMS22-ST2; 45, MMS22-YWC9; 46, MMS22-SSW31; 47, MMS22-YZ4; 48, MMS22-MW13; 49, MMS22-MW43; 50, MMS22-YZ104; 51, MMS22-TA20; 52, MMS22-W6L; 53, MMS22-GA10; 54, MMS22-GM13; 55, MMS22-R20; 56, MMS22-R51.

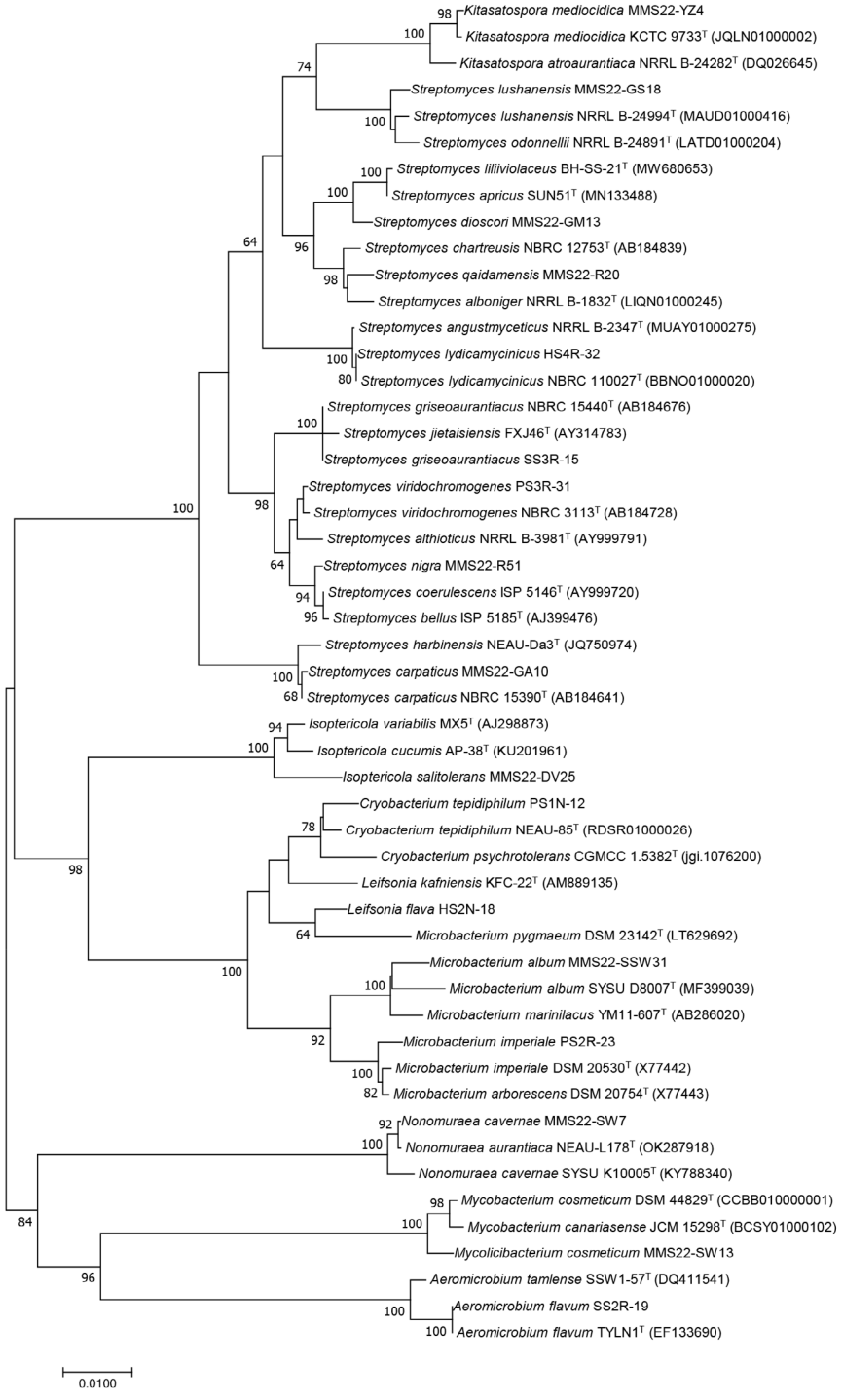


Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Actinomycetota*. Bootstrap values (>60%) based on 500 replications are shown in the neighbor-joining method. Bar, 0.02 substitutions per nucleotide position.

filamentous-shaped. Colonies are filamentous, umbonate and pale-gray color after 2 days on R2A at 25°C. In API 20NE, positive for urease, esculin hydrolysis, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase. Utilize D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize L-arabinose, capric

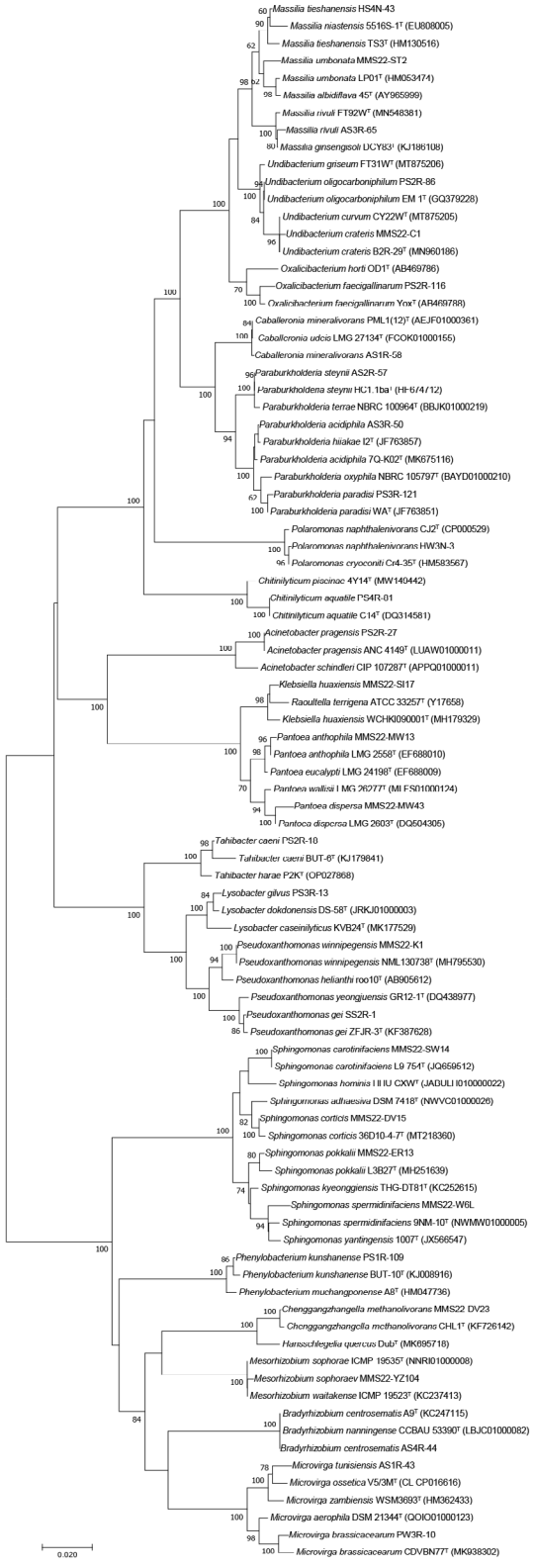


Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Pseudomonadota*. Bootstrap values (>60%) based on 500 replications are shown in the neighbor-joining method. Bar, 0.02 substitutions per nucleotide position.

acid, adipic acid, trisodium citrate and phenylacetic acid. Strain HS4R-32 (=NNIBR2022642BA15) has been isolated from a fresh water sample, Seo-myeon, Hongcheon-gun, Gangwon-do, Republic of Korea (37°41′20.0″N 127°41′22.7″E).

Description of *Polaromonas naphthalenivorans* HW3N-3

Cells are Gram-staining-negative, non-flagellated and coccoid-shaped. Colonies are circular, raised, entire and cream color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, gelatinase, but negative for indole production, glucose fermentation, urease, esculin hydrolysis, β-galactosidase. Utilizes trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid and phenylacetic acid. Strain HW3N-3 (=NNIBR2022642BA16) has been isolated from a fresh water sample, Seo-myeon, Hongcheon-gun, Gangwon-do, Republic of Korea (37°41′20.0″N 127°41′22.7″E).

Description of *Lysinibacillus capsici* PS1N-9

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire and cream color after 2 days on R2A at 25°C. In API 20NE, positive for arginine, dihydrolase urease, but negative for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis gelatinase, β-galactosidase. Utilize *N*-acetyl-D-glucosamine, potassium gluconate, malic acid and trisodium citrate, Does not utilize D-glucose, L-arabinose, D-mannose, D-mannito,l D-maltose, capric acid, adipic acid and phenylacetic acid. Strain PS1N-9 (=NNIBR2022642BA17) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Cryobacterium tepidiphilum* PS1N-12

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire and pale-yellow color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol and malic acid. Does not utilize *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain PS1N-12 (=NNIBR2022642BA18) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

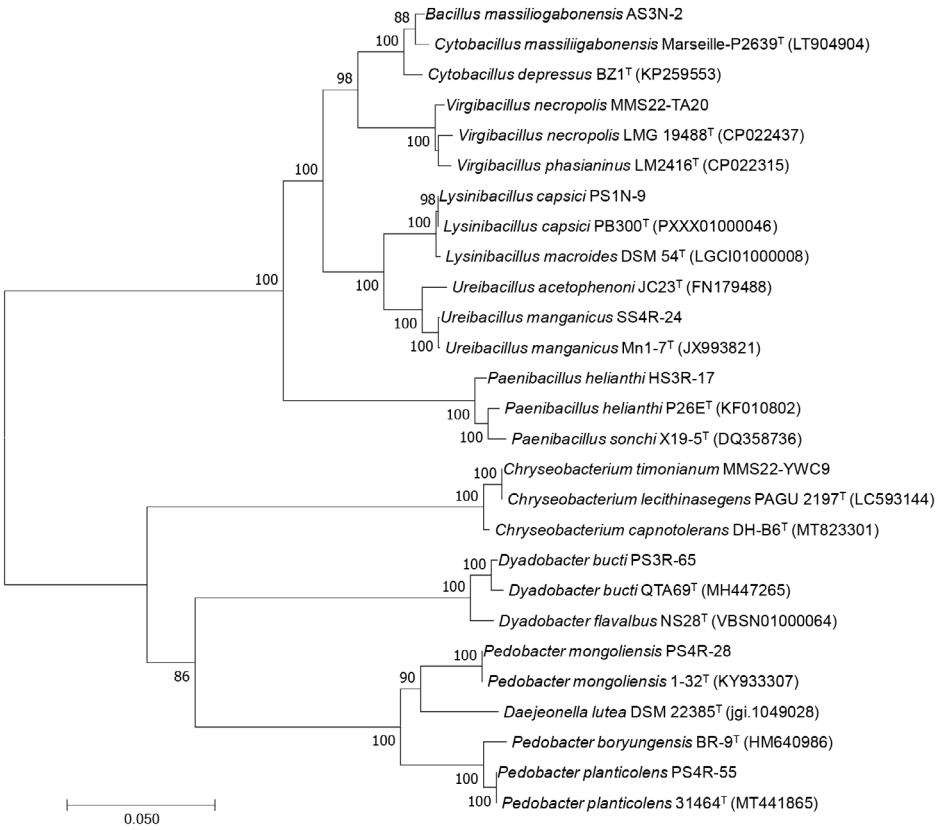


Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Bacillota* and *Bacteroidota*. Bootstrap values (>60%) based on 500 replications are shown in the neighbor-joining method. Bar, 0.02 substitutions per nucleotide position.

Description of *Phenylobacterium kunshanense* PS1R-109

Cells are Gram-staining-negative, flagellated and oval-shaped. Colonies are circular, raised, entire and cream color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, β-galactosidase. Utilize potassium gluconate and adipic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain PS1R-109 (=NNIBR2022642BA19) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Tahibacter caeni* PS2R-18

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and pale-yellow color after 2 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase. Utilize *N*-acetyl-D-glucosamine, capric acid, malic acid, trisodium citrate and phenylacetic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate and adipic acid. Strain PS2R-18 (=NNIBR2022642BA20) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Microbacterium imperiale* PS2R-23

Cells are Gram-staining-positive, non-flagellated and oval-shaped. Colonies are circular, raised, entire and yellow color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain PS2R-23 (=NNIBR2022642BA21) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeong-

gi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Acinetobacter pragensis* PS2R-27

Cells are Gram-staining-negative, non-flagellated and oval-shaped. Colonies are circular, raised, entire and cream color after 2 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase. Utilize capric acid, trisodium citrate and phenylacetic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid and malic acid. Strain PS2R-27 (=NNIBR2022642BA22) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Undibacterium oligocarboniphilum* PS2R-86

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, raised, entire and ivory color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, gelatinase, D-glucose, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase. Utilize D-glucose, D-maltose and potassium gluconate. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain PS2R-86 (=NNIBR2022642BA23) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Oxalicibacterium faecigallinarum* PS2R-116

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, flat, entire and pale-yellow color after 2 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase. Utilizes malic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain PS2R-116 (=NNIBR2022642BA24) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Lysobacter gilvus* PS3R-13

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, flat, entire and yellow color after 2 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, β-galactosidase. Utilizes D-glucose. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain PS3R-13 (=NNIBR2022642BA25) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Streptomyces viridochromogenes* PS3R-31

Cells are Gram-staining-positive, non-flagellated and filamentous-shaped. Colonies are filamentous, umbonate and gray color after 2 days on R2A at 25°C. In API 20NE, positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid and trisodium citrate. Does not utilize capric acid and phenylacetic acid. Strain PS3R-31 (=NNIBR2022642BA26) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Dyadobacter bucti* PS3R-65

Cells are Gram-staining-negative, non-flagellated and curved-shaped. Colonies are circular, convex, entire and yellow color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, urease, esculin hydrolysis, β-galactosidase, but negative for, indole production, glucose fermentation, arginine dihydrolase, gelatinase. Utilize D-glucose, D-mannose, *N*-acetyl-D-glucosamine and D-maltose. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain PS3R-65 (=NNIBR2022642BA27) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Paraburkholderia paradisi* PS3R-121

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, raised, entire and ivory color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, gelatinase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase. Utilize D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine,

D-maltose, potassium gluconate, capric acid, malic acid and phenylacetic acid. Does not utilize L-arabinose, adipic acid and trisodium citrate. Strain PS3R-121 (=NNIBR2022642BA28) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Pedobacter mongoliensis* PS4R-28

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, smooth and light pink color after 2 days on R2A at 25°C. In API 20NE, positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. Utilize D-glucose and L-arabinose. Does not utilize D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain PS4R-28 (=NNIBR2022642BA29) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Pedobacter planticolens* PS4R-55

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, smooth and ivory color after 2 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilize D-glucose, L-arabinose, D-mannitol and *N*-acetyl-D-glucosamine. Does not utilize D-mannose, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain PS4R-55 (=NNIBR2022642BA30) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Chitinilyticum aquatile* PS4R-81

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, raised, entire and ivory color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, glucose fermentation, gelatinase, but negative for indole production, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase. Utilize D-mannose, *N*-acetyl-D-glucosamine and potassium gluconate. Does not utilize D-glucose, L-arabinose, D-mannitol, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain PS4R-81 (=NNIBR2022642BA31) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Microvirga brassicacearum* PW3R-10

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and ivory color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase. Utilize D-mannitol. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain PW3R-10 (=NNIBR2022642BA32) has been isolated from a fresh water sample, Jangan-myeon, Hwaseong-si, Gyeonggi-do, Republic of Korea (37°01′17.4″N 126°48′50.7″E).

Description of *Pseudoxanthomonas gei* SS2R-1

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, raised, entire and yellow color after 2 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, gelatinase, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease. Utilize D-glucose, *N*-acetyl-D-glucosamine and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SS2R-1 (=NNIBR2022642BA33) has been isolated from a fresh water sample, Geunhwa-dong, Chuncheon-si, Gangwon-do, Republic of Korea (37°53′37.8″N 127°43′26.7″E).

Description of *Aeromicrobium flavum* SS2R-19

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, raised, entire and yellow color after 2 days on R2A at 25°C. In API 20NE, positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase. Utilize, D-glucose, D-maltose, potassium gluconate and adipic acid. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain SS2R-19 (=NNIBR2022642BA34) has been isolated from a fresh water sample, Geunhwa-dong, Chuncheon-si, Gangwon-do, Republic of Korea (37°53′37.8″N 127°43′26.7″E).

Description of *Streptomyces griseoaurantiacus* SS3R-15

Cells are Gram-staining-positive, non-flagellated and filamentous-shaped. Colonies are filamentous, umbonate and pale-brown color after 2 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, esculin hydro-

lysis, β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, adipic acid and malic acid. Does not utilize D-maltose, capric acid, trisodium citrate and phenylacetic acid. Strain SS3R-15 (=NNIBR2022642BA35) has been isolated from a fresh water sample, Geunhwa-dong, Chuncheon-si, Gangwon-do, Republic of Korea (37°53′37.8″N 127°43′26.7″E).

Description of *Ureibacillus manganicus* SS4R-24

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, flat, entire and ivory color after 2 days on R2A at 25°C. In API 20NE, positive for gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain SS4R-24 (=NNIBR2022642BA36) has been isolated from a fresh water sample, Geunhwa-dong, Chuncheon-si, Gangwon-do, Republic of Korea (37°53′37.8″N 127°43′26.7″E).

Description of *Klebsiella huaxiensis* MMS22-SI17

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, smooth, convex and white color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, arginine dihydrolase, esculin hydrolysis, β-galactosidase, but negative for indole production, urease, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and trisodium citrate. Does not utilize capric acid, adipic acid, malic acid and phenylacetic acid. Strain MMS22-SI17 (=NNIBR2022642BA58) has been isolated from a fresh water sample, Gangsang-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea (37°28′19.1″N 127°29′39.0″E).

Description of *Sphingomonas pokkalii* MMS22-ER13

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, sticky and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase, but negative for indole production, glucose fermentation. Utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, malic acid and trisodium citrate. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain MMS22-ER13 (=NNIBR2022642BA59) has been isolated from a fresh water sample, Gangsang-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea (37°28′19.1″N 127°29′39.0″E).

Description of *Sphingomonas corticis* MMS22-DV15

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, smooth, convex and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, esculin hydrolysis, gelatinase, but negative for indole production, glucose fermentation, urease, β-galactosidase. Utilize D-glucose, potassium gluconate, malic acid and trisodium citrate. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, capric acid, adipic acid and phenylacetic acid. Strain MMS22-DV15 (=NNIBR2022642BA60) has been isolated from a fresh water sample, Okcheon-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea (37°32′52.5″N 127°30′01.2″E).

Description of *Chenggangzhangella methanolivorans* MMS22-DV23

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are punctiform, convex, entire and white color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, but negative for indole production, glucose fermentation, β-galactosidase. Utilize D-glucose, L-arabinose, *N*-acetyl-D-glucosamine and malic acid. Does not utilize D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain MMS22-DV23 (=NNIBR2022642BA50) has been isolated from a fresh water sample, Okcheon-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea (37°32′52.5″N 127°30′01.2″E).

Description of *Isoptericola salitolerans* MMS22-DV25

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are punctiform, convex, entire and yellow green color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase, β-galactosidase, but negative for indole production, arginine dihydrolase, urease. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose and potassium gluconate. Does not utilize capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS22-DV25 (=NNIBR2022642BA51) has been isolated from a fresh water sample, Okcheon-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea

(37°32′52.5″N 127°30′01.2″E).

Description of *Nonomuraea cavernae* MMS22-SW7

Cells are Gram-staining-positive, non-flagellated and filamentous-shaped. Colonies are circular, smooth, entire and beige color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase, but negative for indole production, glucose fermentation. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium, gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS22-SW7 (=NNIBR2022642BA52) has been isolated from a fresh water sample, Joan-myeon, Namyangju-si, Gyeonggi-do, Republic of Korea (37°31′01.5″N 127°18′11.1″E).

Description of *Mycolicibacterium cosmeticum* MMS22-SW13

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and pink color after 3 days on R2A at 30°C. In API 20NE, positive for arginine dihydrolase, urease, esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, gelatinase, β-galactosidase. Utilize D-mannitol and malic acid. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain MMS22-SW13 (=NNIBR2022642BA53) has been isolated from a fresh water sample, Joan-myeon, Namyangju-si, Gyeonggi-do, Republic of Korea (37°31′01.5″N 127°18′11.1″E).

Description of *Sphingomonas carotinifaciens* MMS22-SW14

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid and trisodium citrate. Does not utilize D-mannitol, capric acid, phenylacetic acid. Strain MMS22-SW14 (=NNIBR2022642BA54) has been isolated from a fresh water sample, Joan-myeon, Namyangju-si, Gyeonggi-do, Republic of Korea (37°31′01.5″N 127°18′11.1″E).

Description of *Undibacterium crateris* MMS22-C1

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, convex, entire and white color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, gelatinase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, β-galactosidase. Utilize D-glucose, D-mannose, *N*-acetyl-D-glucosamine and D-maltose. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS22-C1 (=NNIBR2022642BA55) has been isolated from a fresh water sample, Seoseok-myeon, Hongcheon-gun, Gangwon-do, Republic of Korea (37°42′20.0″N 128°12′25.6″E).

Description of *Pseudoxanthomonas winnipegensis* MMS22-K1

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, convex, entire and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase, but negative for indole production. Utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate. Does not utilize D-mannitol, capric acid, adipic acid and phenylacetic acid. Strain MMS22-K1 (=NNIBR2022642BA56) has been isolated from a fresh water sample, Seoseok-myeon, Hongcheon-gun, Gangwon-do, Republic of Korea (37°42′20.0″N 128°12′25.6″E).

Description of *Streptomyces lushanensis* MMS22-GS18

Cells are Gram-staining-positive, non-flagellated and filamentous-shaped. Colonies are filamentous, raised, undulate and brown to green color after 3 days on R2A at 30°C. In API 20NE, positive for urease, esculin hydrolysis, gelatinase, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS22-GS18 (=NNIBR2022642BA57) has been isolated from a fresh water sample, Banpo-myeon, Gongju-si, Chungcheongnam-do, Republic of Korea (36°23′23.3″N 127°15′09.8″E).

Description of *Massilia umbonata* MMS22-ST2

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, convex, entire and light-yellow color after 3 days on R2A at 30°C. In API 20NE, positive for urease, esculin hydrolysis, gelatinase, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase.

Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, malic acid and trisodium citrate. Does not utilize D-maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain MMS22-ST2 (=NNIBR2022642BA41) has been isolated from a fresh water sample, Joan-myeon, Namyangju-si, Gyeonggi-do, Republic of Korea (37°31′01.5″N 127°18′11.1″E).

Description of *Chryseobacterium timonianum* MMS22-YWC9

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, indole production, urease, esculin hydrolysis, gelatinase, but negative for glucose fermentation, arginine dihydrolase, β-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS22-YWC9 (=NNIBR2022642BA42) has been isolated from a riverside soil sample, Joan-myeon, Namyangju-si, Gyeonggi-do, Republic of Korea (37°31′01.5″N 127°18′11.1″E).

Description of *Microbacterium album* MMS22-SSW31

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and light-yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, esculin hydrolysis, but negative for indole production, arginine dihydrolase, urease, gelatinase, β-galactosidase. Utilize D-glucose, L-arabinose, D-mannitol, D-maltose, adipic acid, malic acid and trisodium citrate. Does not utilize D-mannose, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid and phenylacetic acid. Strain MMS22-SSW31 (=NNIBR2022642BA43) has been isolated from a riverside soil sample, Joan-myeon, Namyangju-si, Gyeonggi-do, Republic of Korea (37°31′01.5″N 127°18′11.1″E).

Description of *Kitasatospora mediocidica* MMS22-YZ4

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and white color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, but negative for indole production, glucose fermentation, gelatinase. Utilize D-glucose and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS22-YZ4 (=NNIBR2022642BA44) has been isolated from a riverside soil sample, Joan-myeon, Namyangju-si, Gyeonggi-do, Republic of Korea (37°31′01.5″N 127°18′11.1″E).

Description of *Pantoea anthophila* MMS22-MW13

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, convex, entire and light-yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase, β-galactosidase, but negative for indole production, arginine dihydrolase, urease. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate. Does not utilize capric acid, adipic acid and phenylacetic acid. Strain MMS22-MW13 (=NNIBR2022642BA47) has been isolated from a riverside soil sample, Okcheon-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea (37°32′52.5″N 127°30′01.2″E).

Description of *Pantoea dispersa* MMS22-MW43

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, convex, entire and light-yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase, β-galactosidase, but negative for indole production, arginine dihydrolase, urease. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, malic acid and trisodium citrate. Does not utilize capric acid, adipic acid and phenylacetic acid. Strain MMS22-MW43 (=NNIBR2022642BA46) has been isolated from a riverside soil sample, Okcheon-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea (37°32′52.5″N 127°30′01.2″E).

Description of *Mesorhizobium sophorae* MMS22-YZ104

Cells are Gram-staining-negative, flagellated and rod-shaped. Colonies are circular, convex, entire and white color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, β-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS22-YZ104 (=NNIBR2022642BA49) has been isolated from a riverside soil sample, Joan-myeon, Namyangju-si, Gyeonggi-do, Republic of Korea (37°31′01.5″N 127°18′11.1″E).

Description of *Virgibacillus necropolis* MMS22-TA20

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and light pink color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, indole production, esculin hydrolysis, gelatinase, but negative for glucose fermentation, arginine dihydrolase, urease, β-galactosidase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain MMS22-TA20 (=NNIBR2022642BA45) has been isolated from a riverside soil sample, Okcheon-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea (37°32′52.5″N 127°30′01.2″E).

Description of *Sphingomonas spermidinifaciens* MMS22-W6L

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for esculin hydrolysis, gelatinase, β-galactosidase, but negative for, nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease. Utilize D-glucose and trisodium citrate. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid and phenylacetic acid. Strain MMS22-W6L (=NNIBR2022642BA48) has been isolated from a riverside soil sample, Gangsang-myeon, Yangpyeong-gun, Gyeonggi-do, Republic of Korea (37°28′19.1″N 127°29′39.0″E).

Description of *Streptomyces carpaticus* MMS22-GA10

Cells are Gram-staining-positive, non-flagellated and filamentous-shaped. Colonies are filamentous, umbonate and brown color after 3 days on R2A at 30°C. In API 20NE, positive for urease, esculin hydrolysis, gelatinase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, β-galactosidase. Utilize potassium gluconate, malic acid and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, capric acid, adipic acid and phenylacetic acid. Strain MMS22-GA10 (=NNIBR2021642BA37) has been isolated from a fresh water sample, Seorak-myeon, Gapyeong-gun, Gyeonggi-do, Republic of Korea (37°42′35.0″N 127°27′27.0″E).

Description of *Streptomyces dioscori* MMS22-GM13

Cells are Gram-staining-positive, non-flagellated and filamentous-shaped. Colonies are filamentous, umbonate and brown color after 3 days on R2A at 30°C. In API 20NE, positive for glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, β-galactosidase but negative for nitrate reduction, indole production. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, potassium gluconate, adipic acid and malic acid. Does not utilize D-maltose, capric acid, trisodium citrate and phenylacetic acid. Strain MMS22-GM13 (=NNIBR2021642BA38) has been isolated from a fresh water sample, Seorak-myeon, Gapyeong-gun, Gyeonggi-do, Republic of Korea (37°42′35.0″N 127°27′27.0″E).

Description of *Streptomyces qaidamensis* MMS22-R20

Cells are Gram-staining-positive, non-flagellated and filamentous-shaped. Colonies are filamentous, umbonate and brown color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, esculin hydrolysis, gelatinase, β-galactosidase, but negative for indole production, glucose fermentation, urease. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate, adipic acid, malic acid. Does not utilize capric acid, trisodium citrate and phenylacetic acid. Strain MMS22-R20 (=NNIBR2021642BA39) has been isolated from a fresh water sample, Seorak-myeon, Gapyeong-gun, Gyeonggi-do, Republic of Korea (37°42′35.0″N 127°27′27.0″E).

Description of *Streptomyces nigra* MMS22-R51

Cells are Gram-staining-positive, non-flagellated and filamentous-shaped. Colonies are filamentous, umbonate and brown color after 3 days on R2A at 30°C. In API 20NE, positive for esculin hydrolysis, gelatinase, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain MMS22-R51 (=NNIBR2022642BA40) has been isolated from a fresh water sample, Seorak-myeon, Gapyeong-gun, Gyeonggi-do, Republic of Korea (37°42′35.0″N 127°27′27.0″E).

Conflicts of Interest

The author of this paper have no affiliation with any interests and is solely responsible for the paper.

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First report of four unrecorded macrofungal species in two national parks of South Korea

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During a mycological field survey conducted from 2023, 4 specimens of mushrooms belonging to different species were collected from national parks in South Korea. Based on molecular phylogenetic analyses and morphological examinations, four species (*Entoloma cornicolor*, *Homophron spadiceum*, *Pluteus chrysophlebius*, and *Xeromphalina enigmatica*) were reported for the first time in South Korea. This study presents morphological descriptions, color photographs, and phylogenetic analyses of these species.

Keywords: morphology, mushrooms, phylogeny, taxonomy

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Introduction

Macrofungi are a group of fungi with large, easily observable, spore-bearing structures (Niego *et al*., 2023). Approximately 14,000 species of this genus are globally distributed (Lu *et al*., 2020). They were classified under the phylum Ascomycota Caval.-Sm. and Basidiomycota R.T. Moore, based on how sexual spores are formed, according to Kinge *et al*. (2020). Ascomycota include cup, flask, and truffle-like fungi. Most mushrooms, jelly fungi, Gasteromycetes, and boletes belong to Basidiomycota. Macrofungi are an integral part of forest ecosystems and provide various ecosystem services, including innovative biotechnological, medicinal, and ecological applications (Hyde *et al*., 2019).

According to the Korean National Park Service, South Korea has 22 national parks. In this study, four basidiomycete fungi were identified in Juwangsan National Park (Gyeongsangbuk-do) and Taebaeksan National Park (Gangwon-do) of Korea. Based on their morphology and phylogeny, these species were identified as *Entoloma cornicolor* O.V. Morozova, Reschke & Noordel, *Homophron spadiceum* (P. Kumm.) Örstadius and E. Larss., *Pluteus chrysophlebius* (Berk. & M.A. Curtis) and *Xeromphalina enigmatica* K.W. Hughes & R.H. Petersen, respectively. Detailed descriptions, photographs, and phylogenetic analyses were provided here.

Materials and Methods

Sampling and morphological examination

In 2023, Wild mushroom samples were collected to assess mycological diversity in two national parks in South

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Korea, namely Juwangsan National Park and Taebaeksan National Park. The species were identified based on their macroscopic and microscopic characteristics. The dried collected samples were then mounted in distilled water and 5% KOH and observed using a Zeiss Axio Imager A1 microscope equipped with an Axiocam 503 color camera (Zeiss, Germany). The systematics of the taxa in this study correspond to those of the Index Fungorum (http://www.indexfungorum.org). The dried specimens used in this study were maintained at the National Institute of Biological Resources in Incheon, Korea.

DNA extraction, polymerase chain reaction (PCR), and phylogenetic analyses

Genomic DNA was extracted from the specimens using a TaKaRa MiniBEST Universal Genomic DNA Extraction Kit (Takara, Japan). The internal transcribed spacer (ITS) region of rDNA was amplified using ITS1 and ITS4 primers. PCR amplicons were purified and sequenced using the Macrogen sequencing service (Seoul, Korea). The dataset was aligned using MAFFT version 7 (Katoh and Standley, 2013), and all other parameters were set to their default values. A phylogenetic tree was constructed using RAxML-HPC2 on XSEDE (v. 8.2.4) in the CIPRES Science Gateway (https://www.phylo.org). The relative robustness of the individual branches was estimated using ML bootstrap support values. GenBank accession numbers were obtained from the National Center for Biotechnology Information (NCBI), and the sequences generated in this study were assigned to NCBI (accession numbers PQ187058−PQ187061).

Results

Phylogenetic analysis

Phylogenetic tree analyses of the ITS sequences revealed the presence of four different species, namely *En-*

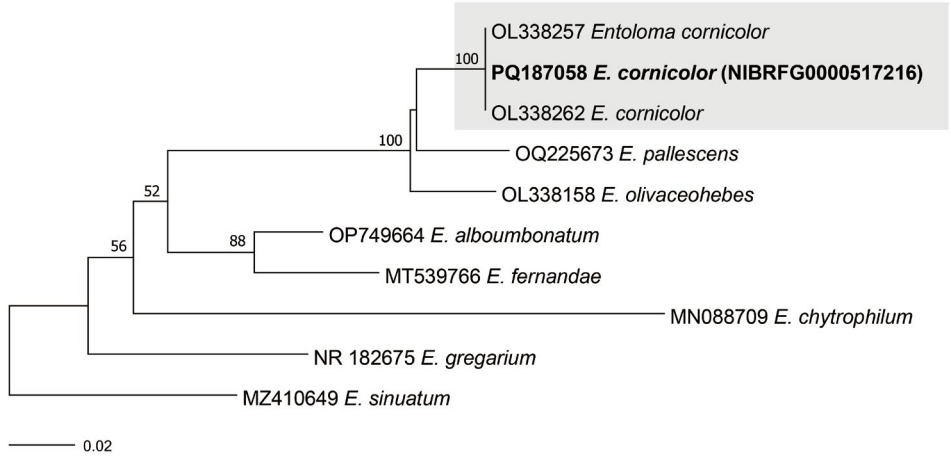


Fig. 1. RAxML phylogenetic tree based on ITS sequences of *Entoloma cornicolor*: Sequences generated in this study are indicated in bold text.

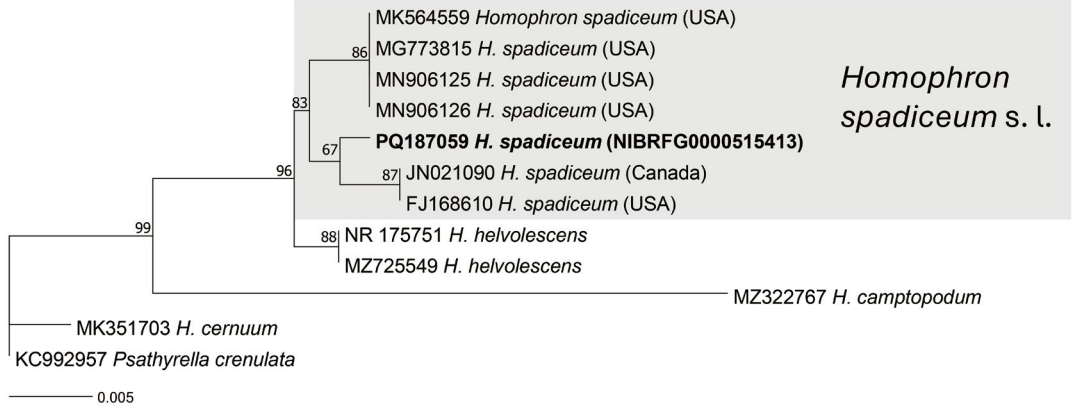


Fig. 2. RAxML phylogenetic tree based on ITS sequences of *Homophron spadiceum*: Sequences generated in this study are indicated in bold text.

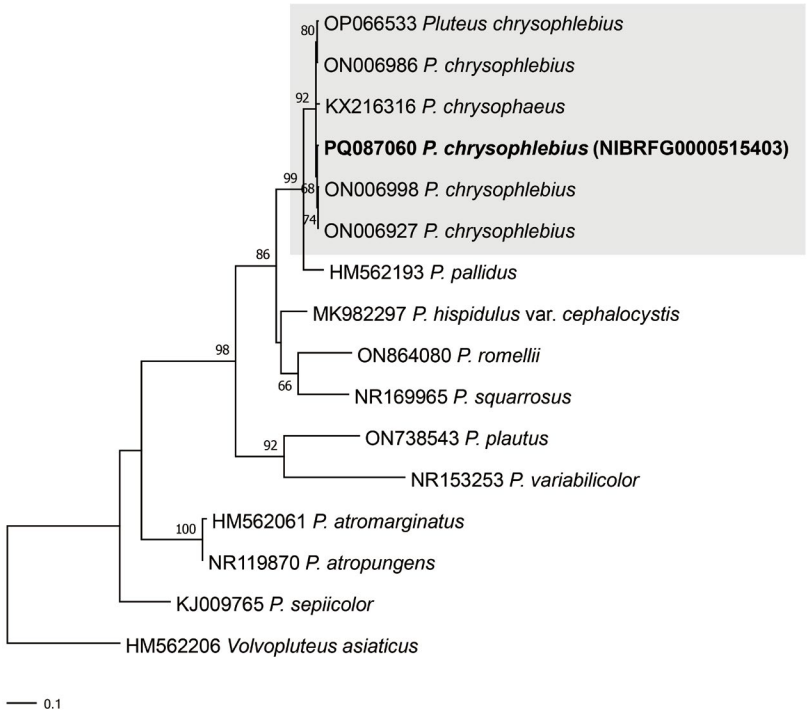


Fig. 3. RAxML phylogenetic tree based on ITS sequences of *Pluteus chrysophlebius*: Sequences generated in this study are indicated in bold text.

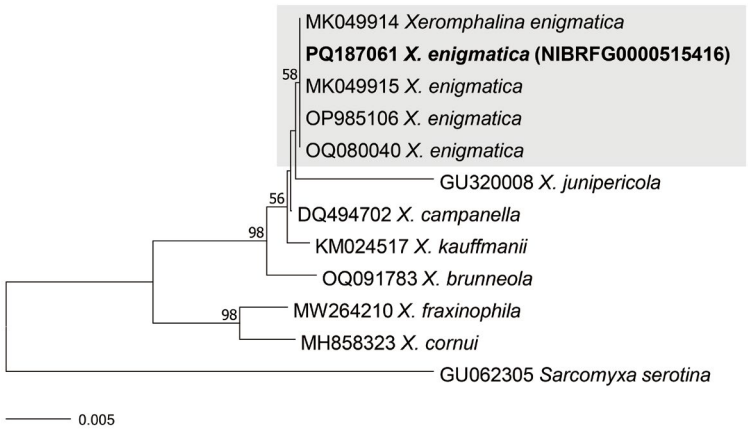


Fig. 4. RAxML phylogenetic tree based on ITS sequences of *Xeromphalina enigmatica*: Sequences generated in this study are indicated in bold text.

toloma cornicolor, *Homophron spadiceum*, *Pluteus chrysophlebius*, and *Xeromphalina enigmatica* (Figs. 1–4). The Korean collection of *E. cornicolor* clustered with *E. cornicolor*, with a bootstrap (BS) value of 100%. The Korean collection of *H. spadiceum* was found to belong to *H. spadiceum sensu lato* (Eberhardt *et al*., 2022). Although *H. spadiceum* is a type species of the genus, it was not possible to determine its species limits phylogenetically. Therefore, in this study, the Korean collection was identified as *H. spadiceum*. The Korean collection of *P. chrysophlebius* clustered within the *P. chrysophlebius* clade. This result is phylogenetically consistent with that of a previous study (Qi *et al*., 2024). In this study (Qi *et al*., 2024), the phylogenetic position of *P. chrysophlebius* was studied based on the ITS + TEF1-a sequence data, and this clade was described as the *P. chrysophlebius* clade. Therefore, the Korean collection was identified as *P. chrysophlebius*. Finally, the Korean collection of *X. enigmatica* clusters with the other sequences of *X. enigmatica*.



Fig. 5. Morphological characters of *Entoloma cornicolor*: A, B, CKU20230731-02; C, basidium; D, basidiospores. Scale bars: 3 cm (A, B); 10 μm (C, D).

Taxonomy

1. *Entoloma cornicolor* O.V. Morozova, Reschke & Noordel., in Reschke, Morozova, Dima, Cooper, Corriol, Biketova, Piepenbring & Noordeloos, Persoonia 49: 158 (2022) (Figs. 1, 5)

Diagnostic characters. Pileus 15−30 mm in diameter, conic, broadly conic to hemispherical with small umbo when young, convex and papillulate when old; upper surface yellowish-brown or grayish brown when young, pale ochraceous, yellowish beige, with paler margin, darker toward center when old, smooth, glabrous; context thin, concolorous with upper surface or paler. **Lamellae** adnate, ventricose, moderately distant, whitish when young and pinkish when old. **Stipe** 40−60 × 1.5−3.0 mm, cylindrical, slightly tapering upward, yellowish-brown, smooth, polished, basal mycelium white, tomentose. **Basidiospores** 7.6−9.7 × 5.6−7.0 μm, heterodiametrical, with 5−7 angles. **Basidia** 28.5−35.5 × 10.5−12.5 μm, clavate, thin-wall, with 4-sterigmata.

Habitat. Solitary or small groups on litter and soil in mixed forests.

Material examined. KOREA, Taebaeksan National Park, Taebaek-si, Gangwon-do, 37°12′24.97″N 128°54′58.60″ E, 31 vii 2023, NK Kim, NIBRFG0000517216.

Remarks. This species is characterized by its relatively small mycenoid basidiocarps, with deeply translucently striated, beige to yellowish-brown pileus with a contrasting dark center, and polished, small, heterodiametrical basidiospores (Reschke *et al*., 2022). Their morphology closely resembled that of the Korean collection.

2. *Homophron spadiceum* (P. Kumm.) Örstadius & E. Larss., in Örstadius, Ryberg & Larsson, Mycol. Progr. 14(no. 25): 35 (2015) (Figs. 2, 6)

Diagnostic characters. Pileus 25–50 mm, convex, with inflexed margin when young, plane when old; upper surface pale pinkish when young, pale reddish-brown with dark brown center when old, covered with minute hairs at margin; context flesh colored or pink. **Lamellae** adnate, very crowded, and pinkish to pale brown when young, and brown with red tinges when old. **Stipe** 30–80 × 5–11 mm, cylindrical, equal to or slightly tapering upward, white when young, pale brown when old, flocculose at apex. **Basidiospores** 6.7–10.5 × 4.2–5.5 µm, ovoid, oblong, subcylindrical, rarely amygdaloid, thin-walled. **Basidia** 16–35 × 6.5–8.0 µm, thin-wall, with 4-sterigmata. **Pleurocystidia** 30–85 × 10–30 µm, fusiform to conical, and abundant.
Habitat. Small groups on soil in mixed forests.
Material examined. KOREA, Taebaeksan National Park, Taebaek-si, Gangwon-do, 37°5′35.1″N 128°56′8.96″E, 4 vii 2023, NK Kim, NIBRFG0000515413.
Remarks. The phylogenetic tree analyzed in this study showed that this species formed an *H. spadiceum* s.l. clade consisting of three subclades (Fig. 6). In addition, this species appears to have no restricted geographical distribution. The phylogenetic results were supported by previous molecular results (Eberhardt *et al*., 2022).

3. *Pluteus chrysophlebius* (Berk. & M.A. Curtis) Sacc., Syll. fung. (Abellini) 5: 678 (1887) (Figs. 3, 7)

Diagnostic characters. Pileus 15–25 mm, bell-shaped to broadly conic when young, broadly convex to flat when old; upper surface bright yellow when young, dull yellow or brownish-yellow when old, veined, margin finely lined. **Lamellae** free, close, or crowded, whitish when young, pinkish when old. **Stipe** 20–45 × 1–3 mm, cylindrical, equal or slightly tapering upward, pale yellow, fragile, bald, basal mycelia white. **Basidiospores** 5.1–6.9 × 4.5–6.1 µm, subglobose to ellipsoid, smooth; hyaline, inamyloid. **Basidia** 20–30 × 7.5–11 µm, fusiform to clavate, thin-walled, 4-sterigmata. **Pleurocystidia** 35–60 × 15–20 µm, widely lageniform, thick-walled, and hyaline.
Habitat. Solitary on litter and soil in mixed forests.
Material examined. KOREA, Juwangsan National Park, Cheongsong-gun, Gyeongsangbuk-do, 36°24′1.78″N 129° 10′43.8″E, 20 vi 2023, NK Kim, NIBRFG0000515403.
Remarks. As shown in a previous study (Justo *et al*., 2011), the sequences of *P. chrysophlebius* were grouped into three distinct subclades. According to Malysheva *et al*. (2016), *Pluteus* sect. *Celluloderma* can recognize ten taxa including three species complexes, namely *P. chrysophlebius*, *P. phlebophorus*, and *P. cinereofuscus*. Therefore, *P. chrysophlebius* is recognized as a species complex, and further studies are required for the taxonomic identification of this species.



Fig. 6. Morphological characters of *Homophron spadiceum*: A, CKU20230704-01; B, basidium; C, cystidia; D, basidiospores. Scale bars: 3 cm (A); 10 µm (B–D).

4. *Xeromphalina enigmatica* K.W. Hughes & R.H. Petersen, in Aldrovandi, Johnson, O'Meara, Petersen & Hughes, Mycologia 107(6): 1280 (2015) (Figs. 4, 8)

Diagnostic characters. Pileus 7–15 mm in diameter, convex to plano-convex with a depressed center; upper surface orange-brown to yellow-brown, with stria margin. **Lamellae** decurrent, distant, bright yellow to orange, with

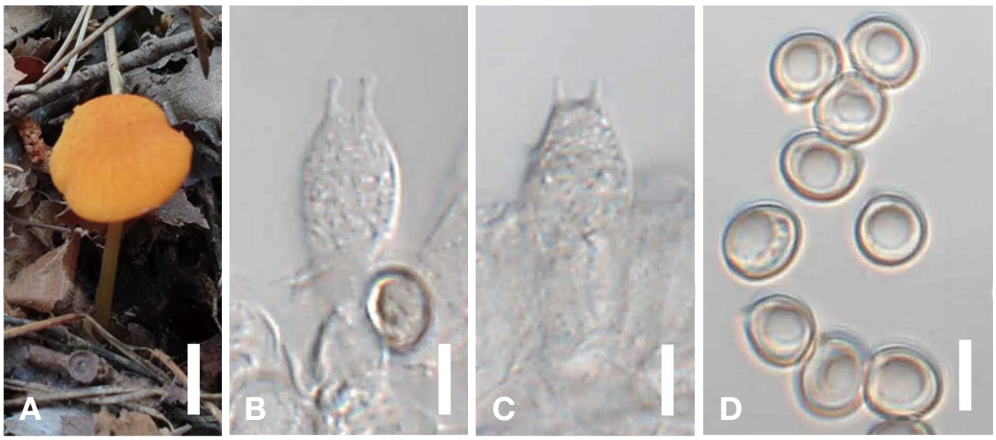


Fig. 7. Morphological characters of *Pluteus chrysophlebius*: A, CKU20230620-12; B, C, basidia; D, basidiospores. Scale bars: 1 cm (A); 5 μm (B–D).

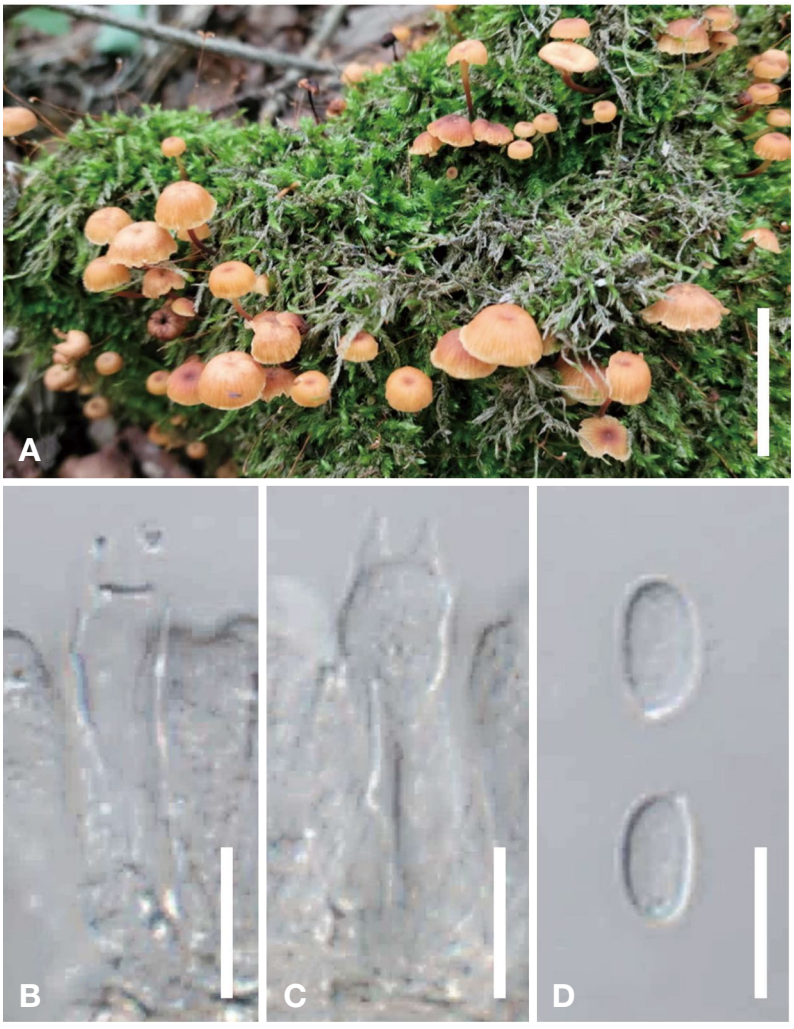


Fig. 8. Morphological characters of *Xeromphalina enigmatica*: A, CKU20230704-08; B, C, Basidia; D, basidiospores. Scale bars: 2 cm (A); 10 μm (B–D).

intervenose. **Stipe** 10–40 × 0.5–2.0 mm, cylindrical, equal to tapering downward, curved, yellowish above, reddish-brown to dark brown with hairs, smooth. **Basidiospores** 5.2–8.2 × 3.1–4.2 µm, ellipsoid, smooth, thin-walled, hyaline. **Basidia** 19–32 × 3.9–7.5 µm, clavate, hyaline, smooth, with 4-sterigmata. **Pleurocystidia** 32–62 × 5.0–14 µm, clavate to cylindrical, hyaline, thin-walled.

Habitat. Gregarious on moss in mixed forests.

Material examined. KOREA, Taebaeksan National Park, Taebaek-si, Gangwon-do, 37°5′ 32.08″N 128°55′57.76″E, 4 vii 2023, NK Kim, NIBRFG0000515416.

Remarks. In a previous phylogenetic analysis based on ITS, 28S, and *RPB2* sequences, this species comprised several clades (Aldrovandi *et al*., 2015). The phylogeny in this study showed that the Korean collection is clustered with a well-supported monophyletic clade (Fig. 8). Therefore, the Korean collection was identified as *X. enigmatica*.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowlegements

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First novel microsatellite marker sets developed for Korean Plecoptera: Development and characterization for *Nemoura geei* (Nemouridae) in South Korea

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Plecoptera, commonly known as stoneflies, play crucial roles in stream ecosystems, either as herbivores or predators, and are one of the key aquatic insects for assessing water quality. A total of 104 species are known from South Korea, and their weak flight ability and habitat specificity make them vulnerable, warranting studies on their population genetic features. However, population-level analyses of genetic diversity and structure using polymorphic microsatellite markers have not been conducted in any of these species. Here, we first developed and characterized novel microsatellite markers for *Nemoura geei*, a dominant stonefly species in Korea, to investigate its genetic diversity and population structure. Using high-throughput sequencing, we developed the first set of microsatellite markers for a Korean Plecoptera species. The markers revealed low genetic diversity across three populations, suggesting small effective population sizes and high conservation risks. These novel microsatellite markers will be highly useful for understanding the population genetics and conservation status of *N. geei* from broader distributions in Asia.

Keywords: aquatic insect, high-throughput sequencing, microsatellites, population genetics, spring stonefly

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Introduction

Plecoptera, commonly known as stoneflies, are a small order of hemimetabolous insects whose nymphs are entirely aquatic, typically inhabiting diverse streams (Stewart *et al*., 2009; DeWalt *et al*., 2015). Most species inhabit mountainous regions at high altitudes, while relatively lower species diversity is found in lowland areas. They mostly live in permanent, oxygenated streams and play crucial roles in stream ecosystems, either as herbivores or predators, depending on their developmental stage (DeWalt *et al*., 2015). Stoneflies are also a key component of the EPT (Ephemeroptera, Plecoptera, Trichoptera) index, which is widely used to the assessment of water quality (DeWalt *et al*., 2015). Approximately 4,500 species of stoneflies have been identified worldwide (DeWalt *et al*., 2024), with 104 species known in South Korea (Hwang and Muranyi, 2020; Hwang *et al*., 2023). Some species are highly vulnerable in lotic ecosystems and are also considered endangered in different regions (Fochetti and Tierno de Figueroa, 2006; 2008). Their vulnerability is amplified by their weak flight ability, which could limit their dispersal to new habitats, making them particularly important for studies on how dispersal ability shapes population genetic structures in aquatic insects (Elbrecht *et al*., 2014; Phillipsen *et al*., 2015). However, the level

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of genetic diversity, conservation status based on genetic data at the population levels or population genetic structure (e.g. gene flow, inter-population differentiation) in any Korean plecopteran species have not been investigated. In addition, misidentification often occurs in Plecoptera species, highlighting species-specific markers being needed for *Nemoura* species.

To address the genetic diversity and population structure of these vulnerable species, microsatellite markers, which are highly polymorphic and co-dominant, can serve as one of the most efficient genetic toolkits (Moritz, 1999; Abdul-Muneer, 2014). These markers can assess polymorphic information content, gene diversity, and heterozygosity (Abdurakhmonov, 2016). They are widely used to investigate the level of genetic diversity, population structures, phylogeography, and phylogenetics of diverse insect taxa (Miller *et al*., 2002; Theissinger *et al*., 2013; Schröder *et al*., 2022). Furthermore, the origin and dispersal route of invasive populations can be analyzed based on this species-specific genetic marker (Kang *et al*., 2023). A recent study on the population genetics of Ephemeroptera, Plecoptera, and Amphipoda species showed that microsatellite datasets are more suited for investigating recent ongoing gene flow and small-scale population structure, whereas mitochondrial haplotype data are better for large-scale phylogeographic patterns (Schröder *et al*., 2022).

In the context of Korean plecopteran taxa, the genus *Nemoura*, belonging to the family Nemouridae, is particularly worth to study since *Nemoura* spp. were reported as one of the most dominant species groups, accounting for 32.24% of total abundance from 412 sampling sites across Korea (Lee *et al*., 2022). The genus *Nemoura* Latreille, 1796, encompasses 226 species worldwide (DeWalt *et al*., 2024), with 16 *Nemoura* species reported in Korea (Hwang and Muranyi, 2020). According to Baumann (1975), the Asian species of the genus *Nemoura* are suggested to form two species complexes based on the structure of the male terminalia. *Nemoura geei* Wu, 1929, originally described from Beijing and widely distributed in Korea, China, Japan, and the Russian Far East (Zwick, 2010; Murányi and Park, 2011), can be a good candidate species for developing the first microsatellite makers of Korean Plecoptera due to its high abundance in Korea.

Here, we developed novel microsatellite markers for *N. geei* using high-throughput sequencing methods for the first time on Plecoptera species in Korea. We also validated the polymorphism of these novel microsatellite markers with Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) analyses. Furthermore, the level of genetic diversity and differentiation among three populations of *N. geei* were tested. This new set of microsatellite loci will be used as an important tool to investigate the level of genetic diversity, population genetic structures, and conservation status for one of the vulnerable and not well-studied stonefly populations in Korea.

Materials and Methods

Sample collection and DNA extraction

A total of 32 *N. geei* adult individuals were collected from four sampling sites in South Korea (Fig. 1). One individual from Mulgok 2 Bridge, Bongsu-ri, Sangmyeon, Gapyeong-gun, Gyeonggi-do 37°49′40.31″N 127°19′5.51″E (alt. 200 m) (2020-III-20) (n = 1) was used for microsatellite development and 31 individuals from three sites were used for population genetic analyses. The species was identified using key characters (e.g., epiproct) by comparison with the original description in Wu (1929) (Fig. 1C). All specimens were stored in 100% ethanol until use. Total genomic DNA was isolated from the thorax

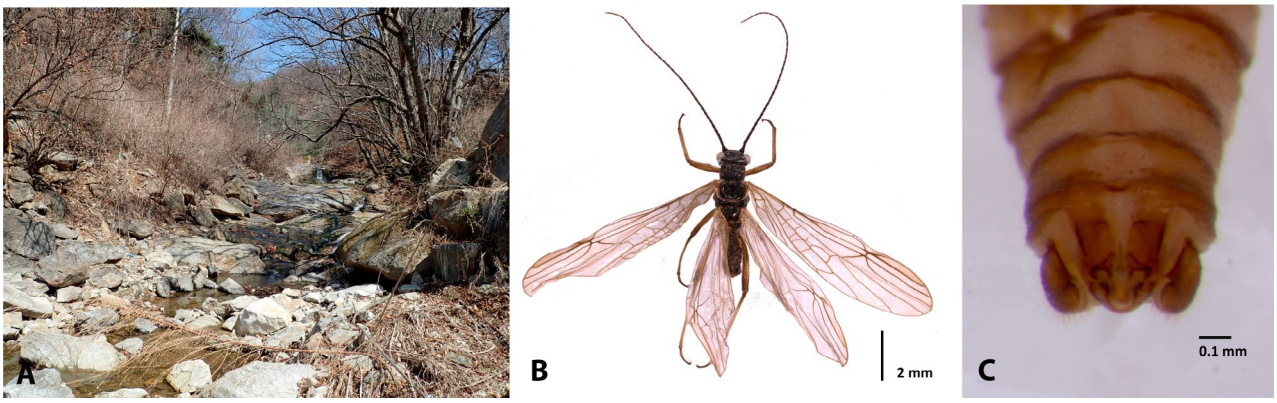


Fig. 1. Habitat (A), adult male (B) and male genitalia (C) of *Nemoura geei* in Korea.

and legs of specimens using DNeasy blood & tissue kit (Qiagen, USA).

Novel microsatellite marker development using high-throughput sequencing

Whole genome shotgun sequencing was performed for a *N. geei* species collected from Gapyeong-gun. Total genomic DNA was extracted from the thorax of one specimen using DNeasy blood & tissue kit (Qiagen, USA) according to the manufacturer's protocol. Integrity, concentration and purity of DNA was assessed using a bioanalyzer 2100 (Agilent, USA). The sheared genomic DNA was processed to construct an MGI paired-end library using a MGIEasy DNA Library Prep Kit and 150 bp of paired-end reads were generated through the MGI MGISEQ2000 platform (Illumina, USA). Sequencing artifact such as sequencing adapters were removed using Trimmomatic v 0.39 (Bolger *et al*., 2014) with custom sequencing adapter information used in MGISEQ library. Genome assembly for microsatellite identification was conducted using megahit v1.2.9 (Dinghua *et al*., 2015) and microsatellite marker identified using Krait v1.3.3. (Du *et al*., 2018). Identified microsatellite markers were filtered using type > 1 and start > 99 for proper primer design and amplification specificity of primers for genotyping were tested to assembled genome using MFEprimer-3.0 (Wang *et al*., 2019). The primers were designed with their lengths ranging from 20−26 nucleotides, annealing temperatures ranging from 50−52°C, and product sizes ranging from 133−204 bp. A set of 20 candidate microsatellite primer pairs was initially tested for polymorphism, of which eight microsatellite markers were chosen. GenBank accession numbers of the eight loci are listed in Table 2.

Microsatellite genotyping

One of each primer pairs for eight novel microsatellite markers were labeled the fluorescent dyes 5'-FAM. The PCR was performed in a 20-μL reaction volume containing 30 ng DNA, 10 mM dNTPs, 100 nM each primer, and 5U HANLAB Taq polymerase (HANLAB, Korea) in an ABI 2720 Thermo cycler (Applied Biosystems, USA). The following PCR cycling conditions were used: an initial denaturation at 95°C for 2 min, 35 cycles of 20 s at 95°C, 30 s at 50°C, and 30 s at 72°C, and a final extension step at 72°C for 5 min. Amplified PCR products were electrophoresed on an ABI 3730x. automated DNA sequencer and fragment sizes were analyzed using GENEMAPPER software 5 (Applied Biosystems, USA).

Genetic diversity of microsatellite markers

Microsatellite diversity was accessed with mean number of alleles per locus (N_A), the polymorphic information content (PIC), allelic richness (*AR*), expected (H_E) and observed heterozygosity (H_O), and inbreeding coefficient (F_{IS}) were estimated using GENEPOP v4.0 (Rousset, 2008) and FSTAT v.2.9.3.2 (Goudet, 2002). Presence of null alleles was assessed using MICROCHECKER v. 2.2.3 (Van Oosterhout *et al*., 2004) with 1000 randomizations at the 95% confidence level. The multi-locus tests for Hardy-Weinberg equilibrium (HWE) and null allele frequency at each locus were estimated using GENEPOP v4.0.

To examine inter-population differentiation, exact tests for population differentiation and calculation of population pairwise of F_{ST} were conducted at both markers using GENEPOP v4.0. The pairwise population comparisons were tested for significance with a Bonferroni correction.

Results and Discussion

Accurate species identification is essential for developing novel microsatellite loci as species-specific markers. Identifying species accurately using larvae from 16 *Nemoura* species in Korea is highly challenging. Therefore, *Nemoura* spp. as a group rather than specifying each species, has been used for ecological studies (Lee *et al*., 2022). Microsatellite marker development based on precise species identification of *N. geei* adult males by examination of distinct male epiproct characteristics is crucial.

Microsatellite loci of *N. geei* were identified using a high throughput sequencing method. A total of 327,418,478 reads with 407,685 scaffolds containing di-, tri- and tetra-nucleotide repeat motifs, with an average length of 22.34 bp were generated. The number of perfect microsatellite sequences, which are suitable for primer designing, was 80,661 containing 8,292 di- (10.28%), 8,627 tri- (10.7%) and 6,435 tetra-nucleotides (7.98%) repeat motif. Eight polymorphic microsatellite makers out of 20 initially screened markers were successfully amplified with stable and reproducible amplicon patterns with distinct peaks in capillary electrophoresis. The numbers of allele for each locus ranged from 3 to 15, and the PIC across loci ranged from 0.243 to 0.865, with its highest value of 0.865 found at the NG1380 locus (Table 1). The H_O per locus ranged from 0.065 to 0.806 with an average 0.501, while H_E was from 0.266 and 0.893 with average of 0.703. Although a single population would be ideal for microsatellite marker assessment to minimize the potential population bias, we used 31 individuals from three different populations as it was difficult to sample more than 20 individuals per population. However, these three populations are not genetically divergent and can thus be considered a single genetic group, as indicated

Table 1. Characteristics of eight polymorphic microsatellite loci developed and validated in of 31 *Nemoura geei* individuals from three sites in this study.

Loci	GenBank accession	Primer Sequence (Fw)	Repeat motif	Tm (C)	Allele size (bp)	N_A	*AR*	PIC	H_E	H_O	F_{IS}	*HWE*
NG246	PP188157	F: ATGTGGATTAAGTGTCCG R:CTCCAACAAGCTATAAGC	(CT)8	51.6	133	11	10.740	0.780	0.823	0.355	0.569	High. sign.
NG256	PP188158	F: CACGTTTTCTAACCTACC R: AGGAAGGATGAGTATTGG	(TGG)5	50.0	201	3	3.000	0.243	0.266	0.065	0.758	0.002
NG445	PP188159	F: GCATATATCACTCAAGAGC R: GTAGTCCTACTTTCTTTAGG	(AC)7	50.5	182	10	10.000	0.733	0.775	0.269	0.652	High. sign.
NG802	PP188160	F: GCTCCGTATAGTACATGG R: TGCTAGATAGATATACGACC	(TG)7	51.3	204	6	5.952	0.637	0.697	0.774	−0.11	0.803
NG808	PP188161	F: ACATCGAATGACACTTCC R: CTCGCAATTCTAAATACTGG	(TCG)5	51.9	186	3	2.839	0.390	0.508	0.516	−0.016	0.996
NG1380	PP188162	F: GACACTTCCAGTATCACC R: ACAGAGAGTTAGAATGAGG	(CT)8	51.6	159	15	14.139	0.865	0.893	0.774	0.133	0.066
NG2062	PP188163	F: GTTTAATGGGTTTGAGTCC R: TATACCATGGGTAAAGTCC	(TGC)10	51.3	171	8	7.677	0.784	0.824	0.806	0.021	0.738
NG3390	PP188164	F: GAATAGAGTAACCGTTTCC R: CTATGCTTGATGCTTATGC	(TG)15	50.3	162	7	6.999	0.787	0.834	0.448	0.462	0.002

F forward primer, *R* reverse primer, N_A number of allele, *AR* average allelic richness, PIC polymorphism information content, H_E expected heterozygosity, H_O observed heterozygosity, F_{IS} inbreeding coefficient *HWE* exact *P* value of Hardy-Weinberg Equilibrium (*$P < 0.05$)

Table 2. Summary of genetic diversity statistics in three populations of *Nemoura geei* in South Korea with eight novel microsatellite loci.

Populations	Sites	GPS	*N*	N_A	*AR*	H_E (SD)	H_O (SD)	F_{IS}	H-W tests (*P*)
ST01	Gaeunsan (Mt.), Seongbuk-gu, Seoul	37°35′44.00″N 127°01′42.23″E	10	5.38	5.020	0.702 (0.135)	0.569 (0.327)	0.198	0.007
ST02	Tapdong-dong, Dongducheon-si, Gyeonggi-do	37°52′27.80″N 127°6′39.01″E	9	5.75	5.565	0.700 (0.239)	0.453 (0.253)	0.367	**
ST03	National endangered species restoraton center, 23, Gowol-gil, Yeongyang-eup, Yeongyang-gun, Gyeongsangbuk-do	36°38′21.32″N 129°9′9.70″E	12	5.63	5.105	0.668 (0.272)	0.476 (0.304)	0.298	**

N sample size, N_A number of allele across eight loci, *AR* allelic richness, H_E expected heterozygosity, H_O observed heterozygosity, F_{IS} observed inbreeding coefficient, H-W tests (*P*) *P* value for multi-locus tests for Hardy-Weinberg equilibrium (HWE).

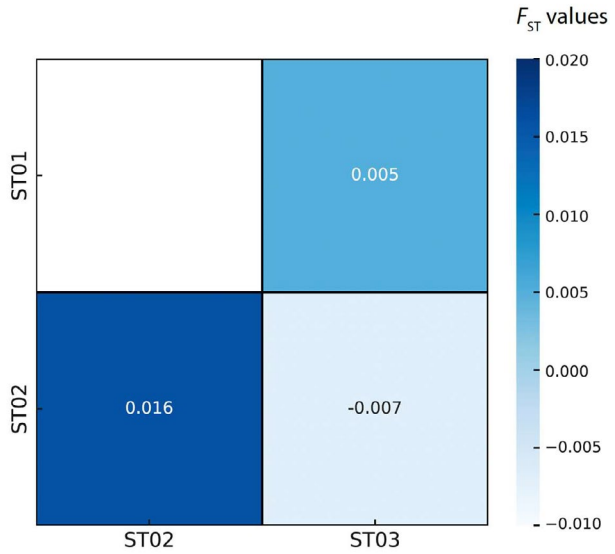


Fig. 2. Pairwise F_{ST} values among *Nemoura geei* populations in South Korea.

by low, non-significant F_{ST} values, making them suitable for marker assessment. Some microsatellite markers (e.g., NG246 and NG445) in this study show significant deviation from Hardy-Weinberg equilibrium, which may indicate homozygote excess potentially due to the presence of null alleles. Additionally, heterozygote deficiency can be influenced by factors such as inbreeding, the Wahlund effect, and selection, though it is generally attributed to non-amplifying PCR null alleles (Selkoe and Toonen, 2006; Wang *et al*., 2014). Nonetheless, all loci in this study were subject to variation in SSR motif number and were identified as reliable and repeatable.

Novel microsatellite markers were successfully validated in 31 individuals from three *N. geei* populations, providing information for the level of genetic diversity and population differentiation. Genetic diversity within populations of Korean *N. geei* was relatively lower with a range of H_E of 0.668−0.702 and H_O of 0.453−0.569. The highest H_E and H_O values were found in ST01 (Table 2). N_A across the eight loci per population ranged from 5.38 to 5.75 (Table 2) and the level of *AR* ranged from 5.020 to 5.565. In particular, two populations (ST02 and ST03) showed significant deviation from HWE (Table 2) indicating nonrandom mating, probably inbreeding within these groups. The level of F_{IS} ranged from 0.198 to 0.367 and its highest value was observed from ST02 (Table 2). All pairwise F_{ST} values calculated among the three populations were statistically non-significant ($p<0.05$) with a range of -0.007 to 0.016 after being corrected for multiple tests with the Bonferroni correction method (Fig. 2). Although Plecoptera species are generally known for their low dispersal ability (Schröder *et al*., 2022), the lack of population genetic structure indicated by small F_{ST} values might reflect the distinct connectivity characteristics of *N. geei* populations in Korea. Despite only analyzing three populations with novel microsatellite markers in this study, the findings can provide valuable insights into the level of ongoing gene flow among the *N. geei* populations. Furthermore, the observed low level of genetic diversity suggests a small effective population size, indicating a high risk of conservation concern or vulnerability for this species. Future research needs to be expanded using these markers with more samples and also more populations from other geographic distributions, including China, Japan, and the Russian Far East in order to understand its current population connectivity and genetic structures covering its whole distribution. The novel microsatellite markers developed for *N. geei* will be versatile in understanding the population genetics related to their ecological characteristics and will significantly contribute to assessing the vulnerability of a Plecoptera species as well in the Asian region.

Conflicts of Interest

The author of this paper have no affiliation with any interests and is solely responsible for the paper.

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Ten new species of siphonostomatoid copepods (Crustacea) associated with marine invertebrates from Korea

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Ten new species in three families (Asterocheridae, Dinopontiidae, and Entomolepididae) of siphonostomatoid copepods are described as associates of marine invertebrates from Korea. These new species are *Acontiophorus decamerus* n. sp., *A. enneamerus* n. sp., *A. acuminatus* n. sp., *Asteropontoides parvus* n. sp., *Rhynchomyzon rostrale* n. sp., *Scottocheres jungi* n. sp., and *Thermocheres viridis* n. sp. in the Asterocheridae, *Stenopontius brevicauda* n. sp. and *S. alatus* n. sp. in the Dinopontiidae, and *Entomopsyllus aplanatus* n. sp. in the Entomolepididae. The three new species of *Acontiophorus* differ from one another primarily in the segmentation of female antennule, the shape of genital double-somite, and the relative length of the caudal ramus. As diagnostic features of *Asteropontoides parvus* n. sp., the caudal ramus is 1.43 times longer than wide and the antennule is 19-segmented in the female. *Rhynchomyzon rostrale* n. sp. resembles *R. rubrovittatum* Sars, 1915, yet can be distinguished by its relatively shorter caudal ramus, which is about 2.8 times longer than wide in the female and by a larger seta on the basis of the maxilliped. In *Scottocheres jungi* n. sp., the female's caudal ramus is 1.25 times longer than wide, with a 17-segmented antennule. *Thermocheres viridis* n. sp. distinctively possesses a small mandibular palp. In *Stenopontius brevicauda* n. sp., the female caudal ramus is slightly wider than it is long, with the inner coxal seta present on legs 2 to 4. *Stenopontius alatus* n. sp. uniquely features a wing-like lateral expansion on the genital double-somite and first abdominal somite. *Entomopsyllus aplanatus* n. sp. stands out among its congeners by having four spines and four setae on the third exopodal segment of both legs 1 and 4.

Keywords: Asterocheridae, Dinopontiidae, Entomolepididae, symbiotic copepods, taxonomy

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Introduction

In the "national species list of Korea" (National Institute of Biological Resources, 2019), 135 species of siphonostomatoid copepods recorded in Korean waters up to that time were listed. These siphonostomatoid copepods included 93 fish-parasitic species and 42 species associated with marine invertebrates. New species and records of siphonostomatoids added to the Korean fauna since then comprise two new species of Asterocheridae (*Acontiophorus estivalis* and *Thermocheres pacificus*) reported by Lee *et al.* (2022); four new species of Asterocheridae (*Amalomyzon elongatum*, *Dokdocheres rotundus*, *Asterocheres banderae*, and *Scottocheres nesobius*) by Kim & Lee (2023); eight new species (*Artotrogus muricatus*, *Artotrogus parmatus*, *Cryptopontius bilobatus*, *Chejupontius variabilis*, *Sewellopontius pterophorus*, *S. macrochelatus*, *Bradypontius culicis*, *Neopon-*

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tius rectus) and one new record (*Pteropontius cristatus* Giesbrecht, 1895) by Lee & Kim (2023); and 14 new species (*Asterocheres geminus*, *A. processus*, *A. culicis*, *A. eurychelatus*, *A. scutellatus*, *A. spiniventer*, *A. fici*, *A. serratus*, *A. tridentatus*, *A. nodulosus*, *A. tetraodontis*, *A. dokdoicus*, *A. exilis*, and *A. zodius*) and one new record (*A. siphonatus* Giesbrecht, 1897) by Kim & Lee (2024). In total, the siphonostomatoid copepods associated with marine invertebrates in Korean seas now number 72 species. This paper describes ten additional new species of siphonostomatoid copepods associated with marine invertebrates found along the southern coasts of Korea.

Materials and Methods

Collected copepod specimens were preserved in 80% ethanol. Before microscopic examination, selected copepod specimens were immersed in lactic acid for approximately 10 minutes. Dissections and microscopic observations employed the reversed slide technique described by Humes and Gooding (1964). Drawings were created using a microscope equipped with a drawing apparatus. The lengths of copepod appendage segments represent the average of the longest and shortest margins. The terminology used in this paper primarily follows that of Huys and Boxshall (1991). Type specimens have been deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon, and the Honam National Institute of Biological Resources (HNIBR), Mokpo.

Results and Discussion

Class Copepoda Milne Edwards, 1840
Order Siphonostomatoida Burmeister, 1835
Family Asterocheridae Giesbrecht, 1899
Genus *Acontiophorus* Brady, 1880

***Acontiophorus decamerus* n. sp. (Figs. 1, 2)**
https://zoobank.org/urn:lsid:zoobank.org:act:81ECF01E-F385-4006-96E6-37C9C19C78DE

Type material. Holotype (intact ♀; MABIK CR00257785), intact paratypes (10 ♀♀; MABIK CR00257786) and dissected paratype (1 ♀) collected from sponge washings at Munseom, Seogwipo, Jeju Island (33°13′39.4″N, 126°33′48.2″E), SCUBA, at a depth of 56 m, by Taekjun Lee, on 25 April 2023. The holotype and intact paratypes have been deposited at the National Marine Biodiversity Institute of Korea (MABIK), Seocheon. The dissected paratype is maintained in the collection of I.-H. Kim.

Etymology. The name derives from the Greek *deca* (= ten) and *mero* (= part), referring to the 10-segmented antennule in the female.

Female. Body (Fig. 1A) narrowing from anterior to posterior. Body length of figured and described specimen 536 μm. Prosome 375 μm long, consisting of cephalothorax and second to fourth pedigerous somites. Cephalothorax 236 × 261 μm. Second pedigerous somite with angular posterolateral corners; other prosomal somites with rounded posterolateral corners. Urosome (Fig. 1B) 4-segmented. Fifth pedigerous somite 74 μm wide. Genital double-somite 0.81 times longer than wide (55 × 68 μm); genital apertures positioned dorsolaterally in middle of double-somite; lateral margins of genital region roundly protruded. Two free abdominal somites 25 × 42 and 27 × 40 μm, respectively. Anal somite (Fig. 1D) with pair of longitudinal rows of 5 or 6 thick setules on ventral surface; posterior margin unornamented. Caudal ramus (Fig. 1C) 2.19 times longer than wide (35 × 19 μm), armed with 6 setae, but no setules or spinules present on ventral surface and distal margin; setae II and VII positioned at distal quarter of ramus length; seta VI inserted on digitiform process.

Rostrum (Fig. 1K) small, strongly tapering. Antennule (Fig. 1E) short, 88 μm long, 10-segmented; articulation between 2 terminal segments incomplete, discernible only on one side; armature formula 2, 13, 7, 2, 7, 4, 1 + aesthetasc, 1, 3, and 8; setae densely arranged; aesthetasc on seventh segment large, longer than total of segments, with constriction at distal quarter. Antenna (Fig. 1F) consisting of coxa, basis, 1-segmented exopod, and 2-segmented endopod; coxa short and unarmed; basis unarmed but ornamented with 3 long setules at inner distal corner; exopod digitiform, 4.0 times longer than wide (24 × 6 μm), armed with 3 setae (1 proximal, 1 small subdistal, and 1 sparsely pinnate, large distal); first endopodal segment 19 μm long, unarmed and unornamented; second endopodal segment 24 μm long, armed with 5 setae (1 proximal, 1 large distal, 3 unequal subdistal) and ornamented subdistally with transverse row of several spinules.

Oral siphon (Fig. 1G) bendable, extending to posterior margin of genital double-somite, consisting of tapering proximal one-fifth and thin distal four-fifths. Mandible (Fig. 1H) consisting of thread-like stylet and short, 1-segmented palp tipped with 1 minute seta and 1 large, heavily pinnate seta. Maxillule (Fig. 1I) bilobed; smaller outer lobe with 1 small, naked and 3 pinnate setae; inner lobe with 5 unequal distal setae (2 pinnate setae, 2 equally long, naked setae, and 1 small naked seta). Maxilla (Fig. 1J) 2-segmented; proximal segment (syncoxa) unarmed; distal segment (basis) arched, about 1.5 times longer than proximal segment, ornamented with rows of spinules. Maxilliped (Fig. 2A) 5-segmented; first segment (syncoxa) 52 μm long, partially divided by faint line into precoxal and coxal regions, with 1 seta subdistally on inner margin and row of spinules on outer margin of coxal region; basis 47 μm

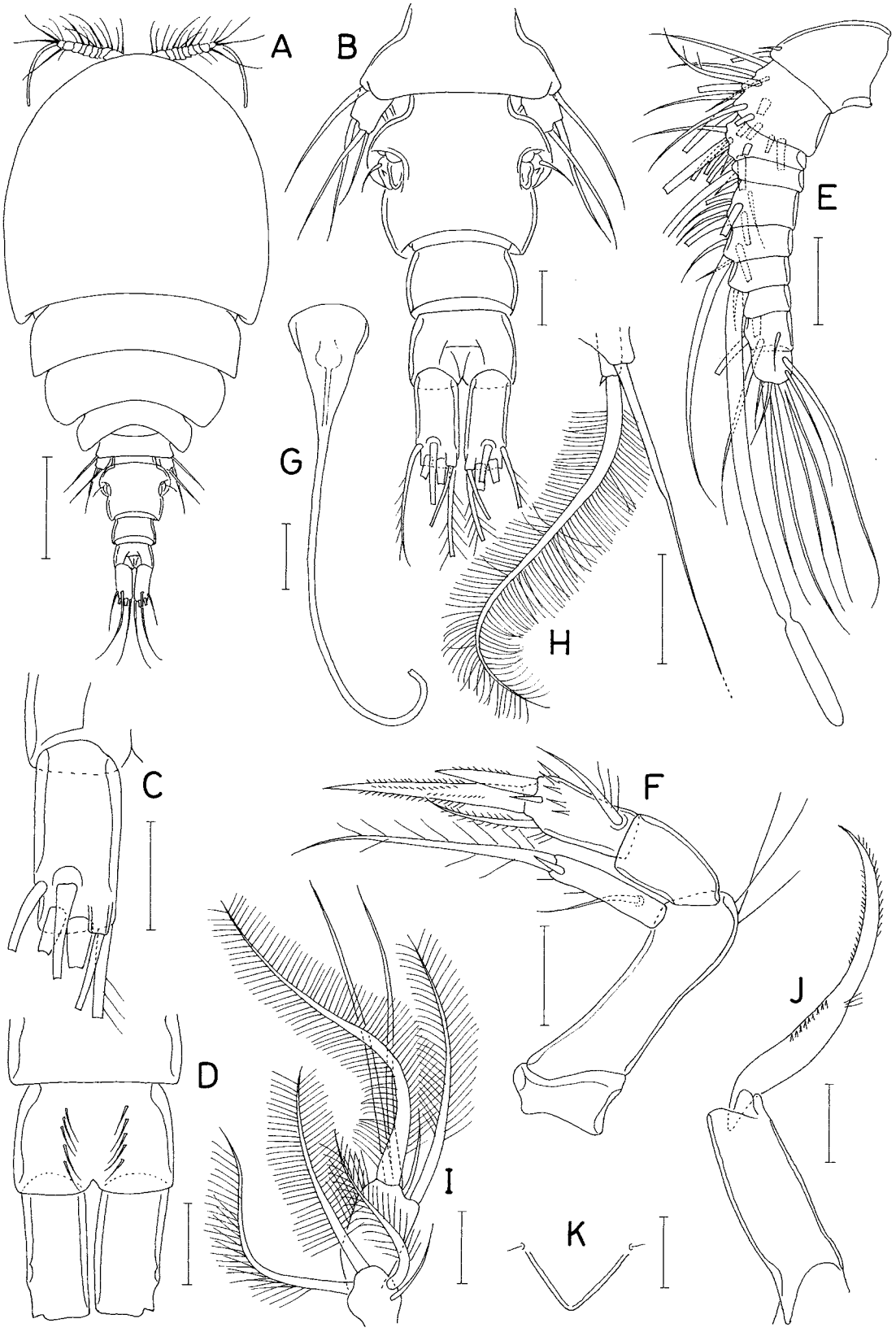


Fig. 1. *Acontiophorus decamerus* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, left caudal ramus, dorsal; D, anal somite and caudal rami, ventral; E, antennule; F, antenna; G, oral siphon; H, mandible; I, maxillule; J, maxilla; K, rostrum. Scale bars: A, 0.1 mm; B–F, I, J, 0.02 mm; G, H, 0.05 mm; K, 0.01 mm.

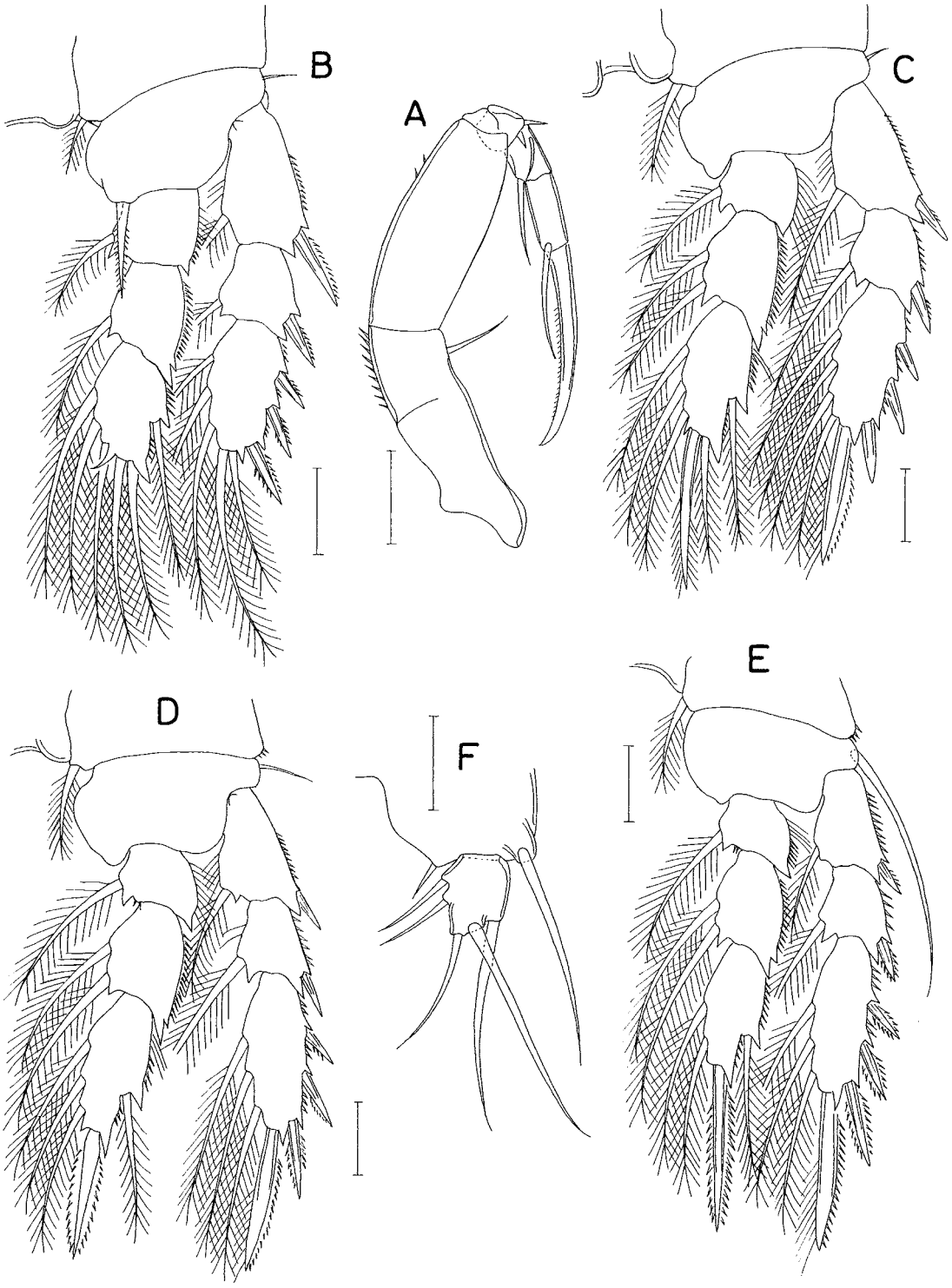


Fig. 2. *Acontiophorus decamerus* n. sp., female. A, maxilliped; B, leg 1; C, leg 2; D, leg 3; E, leg 4; F, leg 5. Scale bars: 0.02 mm.

long, unarmed, but with few spinules on outer margin; armature formula of 3 endopodal segments 2, 2, and 1 + claw; terminal claw arched, 44 μm long, about 2.8 times longer than terminal segment, ornamented with minute spinules along concave inner margin.

Legs 1−4 (Fig. 2B−E) biramous, with 3-segmented rami. Outer seta on basis small in legs 1−3, but large in leg 4. Inner distal spine on basis of leg 1 setiform, with minute spinules on margins. Second endopodal segment of legs 1−4 with bicuspid outer distal corner. Distal spine on third

endopodal segment pinnate in leg 2 but serrate in legs 3 and 4. Armature formula for legs 1–4 as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-I	I-1; I-1; III, 2, 3	0-1; 0-1; 1, 2, 3
Leg 2	0-1	1-0	I-1; I-1; III, I, 4	0-1; 0-2; 1, 1 + I, 3
Leg 3	0-1	1-0	I-1; I-1; III, I, 3	0-1; 0-2; 1, I, 3
Leg 4	0-1	1-0	I-1; I-1; III, I, 3	0-1; 0-2; 1, I, 2

Leg 5 (Fig. 2F) consisting of protopod and 1-segmented exopod; protopod not articulated from somite, wider than long, with 1 large outer and 1 small inner setae, both setae naked; exopodal segment 1.13 times longer than wide (17 × 15 μm), armed with 5 naked setae (1 outer subdistal, 2 distal, and 2 inner). Leg 6 (Fig. 1B) represented by 1 seta and 1 spinule on genital operculum.

Male. Unknown.

Remarks. *Acontioporus decamerus* n. sp. is most closely related to *A. estivalis* Lee, Chang & Kim, 2022 also found in Korean waters. Both species exhibit many similar morphological features, including proportional lengths of caudal rami and oral siphon, segmentation and ornamentation of the maxilliped, and identical armature formulae for legs 1 to 5. However, the new species is not conspecific with *A. estivalis*, due to several differences: (1) the body length of the female distinctly shorter, 536 μm versus 938 μm in *A. estivalis* as reported by Lee *et al*. (2022); (2) the female's cephalothorax is wider than it is long, contrasting with the equally proportioned cephalothorax of *A. estivalis*; (3) the genital double-somite of the new species features a centrally positioned genital aperture and the rounded posterolateral corners, whereas *A. estivalis* has its genital apertures at the anterior of the double-somite with angular posterolateral corners; (4) the new species has an unornamented caudal ramus compared to the ornamaneted caudal ramus of *A. estivalis*; and (5) the female's antennule is 10-segmented in the new species, in contrast to the 11-segmented antennule in *A. estivalis*.

Acontiophorus enneamerus n. sp. (Figs. 3, 4)

https://zoobank.org/urn:lsid:zoobank.org:act:423DB17F-1DE3-4D75-83A7-77B939367B20

Type material. Holotype (intact ♀; MABIK CR00257787), intact paratype (♀; MABIK CR00257788), and dissected paratype (♀) from washings of mixed species of sponges, at Munseom, Seogwipo, Jeju Island (33°13′39.4″N, 126°33′48.2″E), at a depth of 56 m, collected by Taekjun Lee, on 25 April 2023. The holotype and intact paratype have been deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon. The dissected paratype is held in the collection of I.-H. Kim.

Etymology. The specific name is derived from the Greek *enne* (= nine) and *mero* (= part), alluding to the nine-segmented antennule in the female.

Female. Body (Fig. 3A) tapering posteriorly. Body length of dissected paratype 665 μm. Prosome 448 μm long. Cephalothorax 300 × 348 μm, distinctly wider than pedigerous somites. All prosomal somite with rounded posterolateral corners. Urosome (Fig. 3B) 4-segmented. Fifth pedigerous somite 95 μm wide. Genital double-somite 0.84 times as long as wide (79 × 94 μm), widest at anterior 34% region, followed by evenly tapering posterior part; genital apertures positioned dorsolaterally at region just posterior to widest region; posterolateral region of genital apertures bearing pointed process (Fig. 4G). Two free abdominal somites 34 × 48 and 28 × 47 μm, respectively. Anal somite (Fig. 3D) ornamented with 2 oblique rows of 9 thick setules on ventral surface. Caudal ramus (Fig. 3C) 1.91 times longer than wide (42 × 22 μm), armed with 6 setae and ornamented with several small spinules on mediodorsal surface and 6 thick setules on medioventral surface (Fig. 3D); seta VI tipped on digitiform process.

Rostrum absent. Antennule (Fig. 3E) short, 102 μm long, 9-segmented; armature formula 2, 14, 6, 2, 6, 3, 1 + aesthetasc, 5, and 7; setae densely arranged; aesthetasc on seventh segment shorter than total length of segments. Antenna (Fig. 3F) with unarmed coxa; basis bearing long setules at inner distal corner and on subdistal inner margin; exopodal segment 3.0 times longer than wide (33 × 11 μm), bearing 1 small seta near middle and 1 large, pinnate seta and 1 spiniform process distally; first endopodal segment 17 μm long, unarmed; second endopodal segment 23 μm long, armed with 5 very unequal setae consisting of 1 proximal pinnate seta, 2 broad, spiniform setae covered with numerous spinules all over (longer distal one 65 μm long and shorter subdistal one 43 μm long), 1 small distal seta, and 1 finely spinulose subdistal seta.

Oral siphon (Fig. 3G) bendable, extremely long, 622 μm long, extending to distal margin of caudal rami, consisting of tapering proximal 15% and thin distal 85%. Mandible (Fig. 3H) consisting of thread-like stylet and unsegmented palp; palp bearing 1 small spinule-like setule and tipped with large, plumose seta; latter seta not articulated from palp segment. Maxillule (Fig. 3I) bilobed; outer lobe 15 × 15 μm, with 1 small, naked seta and 3 larger pinnate seta; inner lobe 22 × 18 μm, with 5 unequal setae, 2 of them pinnate, remaining 3 naked. Maxilla (Fig. 3J) 2-segmented, ornamented as in *A. decamerus* n. sp. Maxilliped (Fig. 4A) 5-segmented; armature formula 1, 0, 2, 2, and 1; first segment (completely fused precoxa and coxa) 64 μm long, with spinules on outer margin; second segment (basis) also 64 μm long, bearing spinules on outer margin; terminal segment 27 μm long, terminal claw 57 μm long, bearing fine spinules along concave margin.

Leg 1 (Fig. 4B) lacking inner coxal seta. Armature formula of legs 2–4 (Fig. 4C–E) as in *A. decamerus* n. sp. Leg 4 with moderately long outer seta on basis. Leg 5 (Fig. 4F)

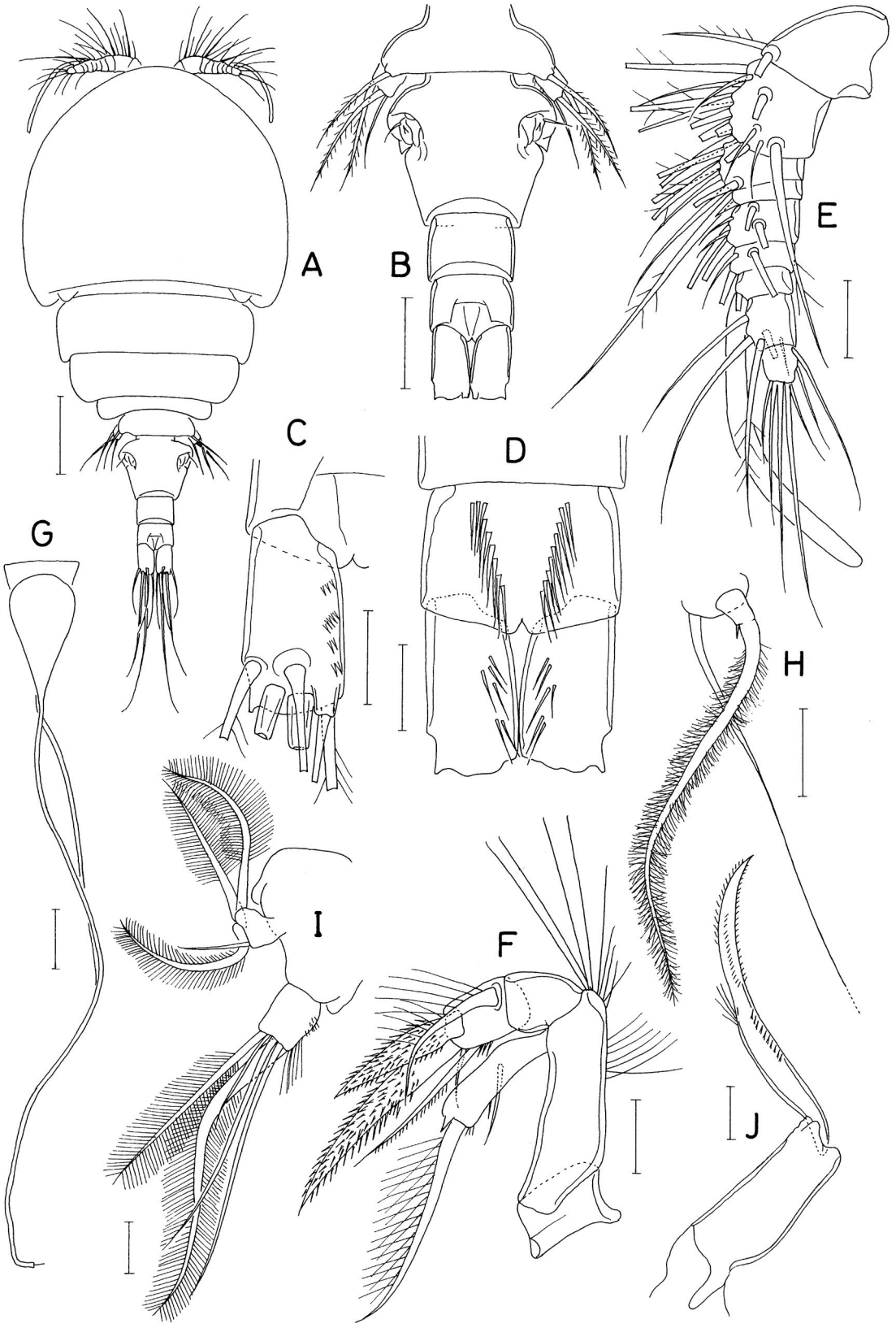


Fig. 3. *Acontiophorus enneamerus* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, left caudal ramus, dorsal; D, anal somite and caudal rami, ventral; E, antennule; F, antenna; G, oral siphon; H, mandible; I, maxillule; J, maxilla. Scale bars: A, 0.1 mm; B, G, 0.05 mm; C−F, H−J, 0.02 mm.

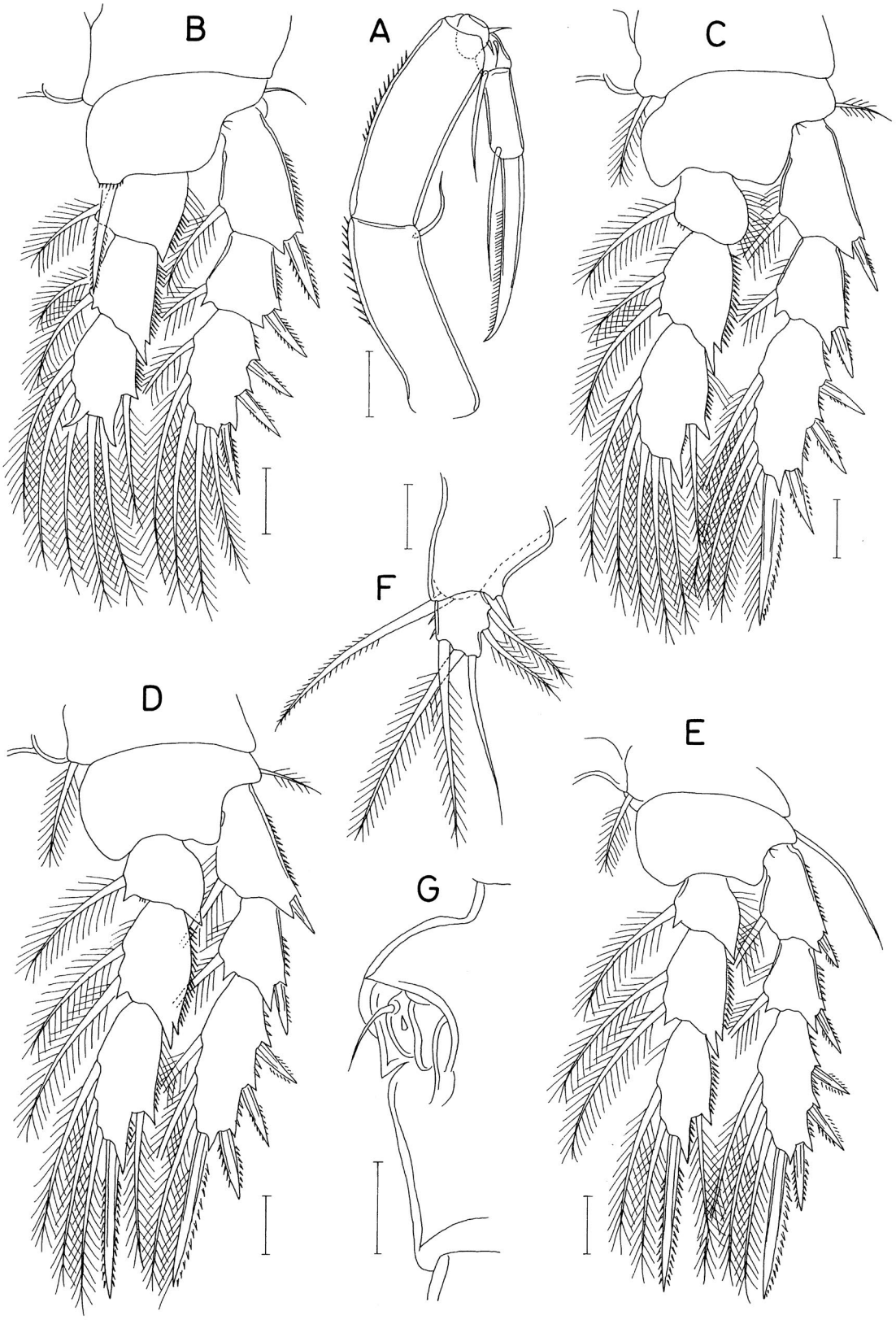


Fig. 4. *Acontiophorus enneamerus* n. sp., female. A, maxilliped; B, leg 1; C, leg 2; D, leg 3; E, leg 4; F, leg 5; G, left side of genital double-somite, dorsal. Scale bars: 0.02 mm.

consisting of protopod and free exopod; protopod fused with somite as long as wide, with 1 weakly pinnate outer distal seta and 1 small, naked inner distal seta; exopodal segment small, 1.11 times longer than wide (20 × 18 μm) armed with 5 setae (1 pinnate outer subdistal, 1 pinnate and 1 naked distal, and 2 shorter pinnate inner). Leg 6 (Fig. 4G) as 1 naked seta and 1 spinule on genital operculum.

Male. Unknown.

Remarks. *Acontiophorus enneamerus* n. sp. closely resembles *A. pilosus* Kim & Je, 2000, which is associated with a bryozoan in Korea (Kim & Je, 2000). Both species share the absence of the inner seta on the coxa of leg 1, and have a nine-segmented female antennule, a feature possessed only by them and *A. usuaiensis* Johnsson, 2001. They also have similarly proportioned female caudal rami. However, they cannot be identified as the same species due to several significant distinctions: (1) the presence of a pointed process on the lateral margin near the genital aperture in *A. enneamerus* n. sp., which is absent in *A. pilosus*; (2) a smooth lateral margin of the caudal ramus in *A. enneamerus* n. sp., as opposed to a pinnate margin in *A. pilosus*; (3) the two large terminal spiniform elements of the antenna are uniformly spinulose in *A. enneamerus* n. sp., whereas in *A. pilosus* they display two or more rows of spinules; (4) the largest terminal spiniform element of the antenna in *A. enneamerus* n. sp. is about 2.9 times longer than its terminal segment, while it is only about 2.0 times longer in *A. pilosus*; and (5) the female adult body of *A. enneamerus* n. sp. measures approximately 0.67 mm, smaller than the 0.92 mm reported for *A. pilosus* in the original description (Kim & Je, 2000). These differences have been validated through re-examination of newly collected specimens of *A. pilosus*.

Acontiophorus acuminatus n. sp. (Figs. 5, 6)

https://zoobank.org/urn:lsid:zoobank.org:act:E4B7321E-4F22-4C78-A40B-FB6E001F2617

Type material. Holotype (intact ♀, HNIBR IV7753), intact paratype (♀, HNIBR IV7754), and dissected paratype (♀) from washings of mixed species of sponges, near Biyang-do, Jeju Island (33°24′17.0″N, 126°12′16.5″E), SCUBA, depth 5−10 m, collected by Tae Won Jung and Jong Guk Kim, on 13 June 2023. Both the holotype and intact paratype have been deposited in the Honam National Institute of Biological Resources (HNIBR), Mokpo. The dissected paratype is maintained in the collection of I.-H. Kim.

Etymology. The specific name of the new species is from the Latin *acumin* (= a point), alluding to the sharply pointed posterolateral corners of the genital double-somite and first free abdominal somite.

Female. Body (Fig. 5A) moderately broad. Body length 770 μm in dissected and figured specimen. Prosome 524 μm long, consisting of cephalothorax and second to fourth pedigerous somites. Cephalothorax 378 × 415 μm, with pointed posterolateral corners. Second pedigerous somite with acutely pointed posterolateral corners. Third and fourth pedigerous somites with nipple-shaped posterolateral corners. Urosome (Fig. 5B) 4-segmented. Fifth pedigerous somite 121 μm wide. Genital double-somite slightly wider than long (100 × 105 μm), consisting of slightly expanded anterior third and narrower posterior two-thirds bearing parallel lateral margins; genital apertures located dorsolaterally at posterior region of anterior expansion. Genital double-somite and first free abdominal somites bearing acutely pointed posterolateral corners. Anal somite with minute spinules along posteroventral margin. Caudal ramus (Fig. 5C) short, 1.10 times longer than wide (32 × 29 μm), armed with 6 setae and ornamented with several spinules on lateral surface, 4 transverse rows of fine spinules on mediodorsal surface, and row of fine spinules along posteroventral margin; seta VI tipped on digitiform process.

Rostrum absent. Antennule (Fig. 5D) 212 μm long, 16-segmented; armature formula 2, 2, 11, 7, 2, 2, 2, 2, 2, 2, 2, 1, 1 + aesthetasc, 2, 4, and 7; aesthetasc on thirteenth segment half as long as total segments. Antenna (Fig. 5E) slender; coxa unarmed; basis elongate, 4.9 times longer than wide (68 × 14 μm long), unarmed; exopodal segment 3.86 times longer than wide (27 × 7 μm), as long as first endopodal segment, armed with 1 naked seta in middle and distally with 1 small, naked and 1 large, pinnate setae; first endopodal segment 27 μm long, unarmed; second endopodal segment 31 μm long, armed with 1 proximal pinnate seta, 1 small, pinnate subdistal seta, 1 naked distal seta, and 1 large spiniform distal seta of 93 μm long.

Oral siphon (Fig. 5F) bendable, extending to posterior margin of genital double-somite, consisting of tapering proximal quarter and thin distal three quarters. Mandible (Fig. 5G) as in *A. enneamerus* n. sp. Maxillule bilobed; outer lobe (Fig. 5I) bearing 3 pinnate setae; inner lobe (Fig. 5H) with protruded medial margin bearing fine spinules, armed distally with 2 large naked setae, 2 large pinnate setae and 1 small naked seta. Maxilla (Fig. 5J) slender; proximal segment unarmed; distal segment arched, longer than proximal segment, characteristically bearing 1 large naked seta at proximal third, followed by row of minute spinules along concave margin. Maxilliped (Fig. 6A) slender, 6-segmented, but articulation between proximal 2 segments incomplete, discernible only on one side; armature formula 0, 1, 1, 2, 1, 1 + claw; basis 114 μm long, ornamented with fine spinules along outer margin, its inner seta small, positioned at proximal third of segment length; terminal segment 32 μm long; terminal claw 77 μm long.

Legs 1−4 (Fig. 6B−E) segmented and armed as in *A. decamerus* n. sp.; outer seta on basis pinnate, well-developed.

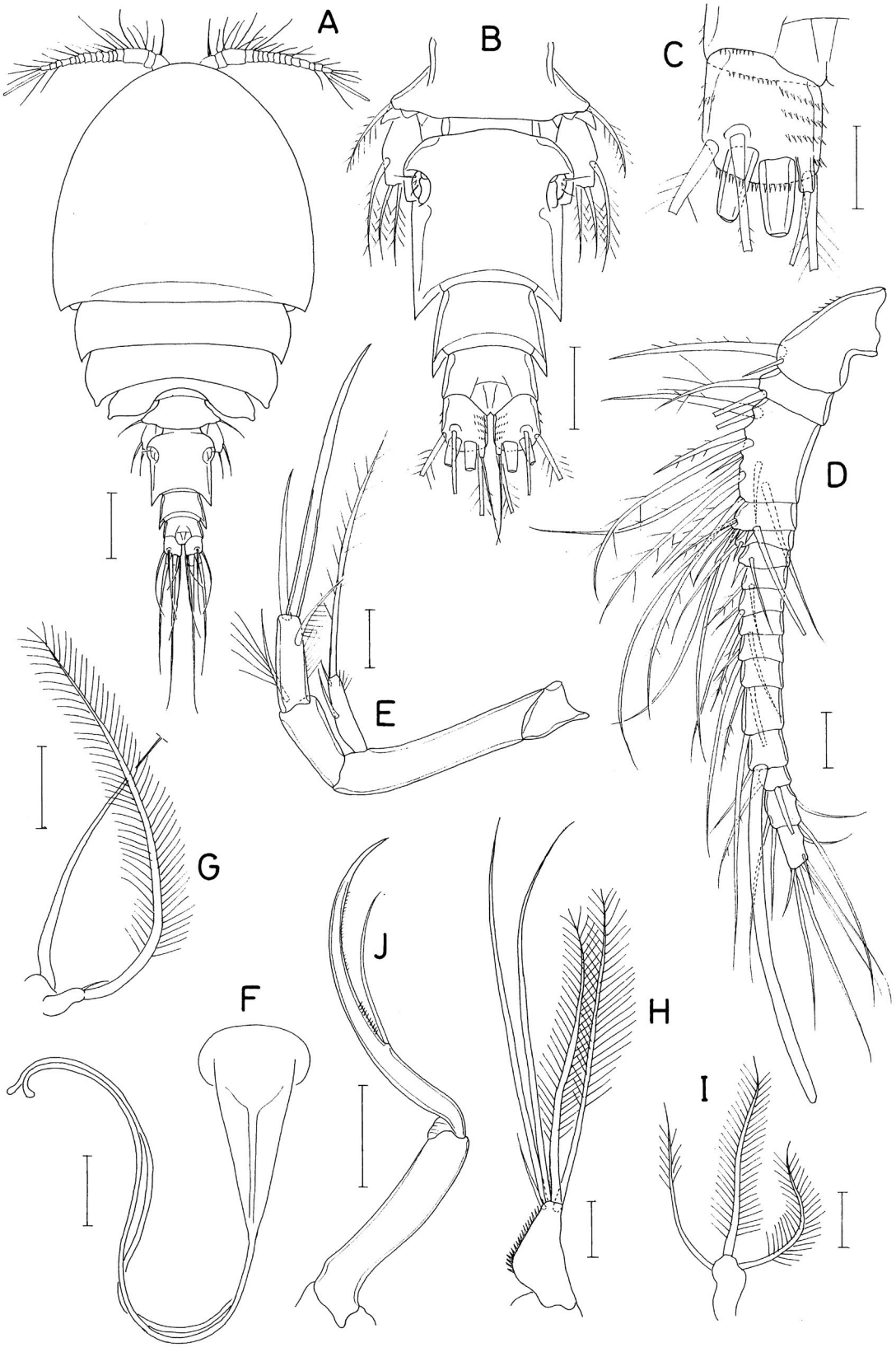


Fig. 5. *Acontiophorus acuminatus* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, left caudal ramus, dorsal; D, antennule; E, antenna; F, oral siphon; G, mandible; H, inner lobe of maxillule; I, outer lobe of maxillule; J, maxilla. Scale bars: A, 0.1 mm; B, F, G, J, 0.05 mm; C−E, H, I, 0.02 mm.

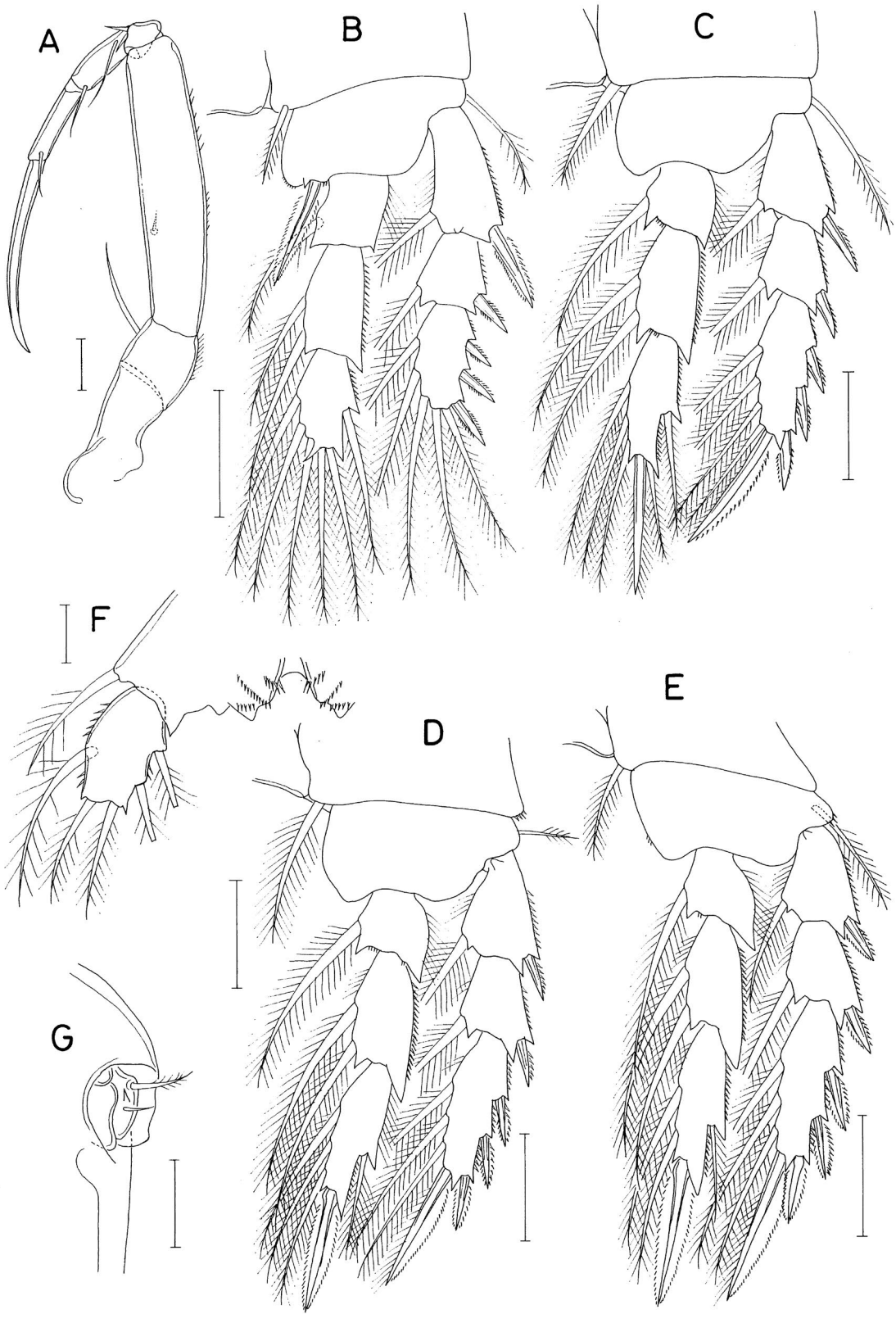


Fig. 6. *Acontiophorus acuminatus* n. sp., female. A, maxilliped; B, leg 1; C, leg 2; D, leg 3; E, leg 4; F, leg 5; G, right genital aperture, dorsal. Scale bars: A, F, G, 0.02 mm; B–E, 0.05 mm.

Leg 5 (Fig. 6F) consisting of protopod and free exopod; protopod completely fused with somite, armed only with 1 pinnate outer distal seta, ornamented with 3 rows of minute spinules on serrate inner margin; inner distal corner of protopod acutely projected; exopodal segment 1.72 times longer than wide, armed with 5 pinnate setae (1 outer, 2 distal, and 2 inner) and ornamented with several spinules on outer and inner margins. Leg 6 (Fig. 6G) represented by 1 pinnate and 1 smaller naked setae and 1 spinule on genital operculum.

Male. Unknown.

Remarks. Existing species of *Acontiophorus* can be classified into two groups. The first group consists of eight species including *A. scutatus* (Brady & Robertson, 1873), while the second group also comprises eight species including *A. ornatus* (Brady & Robertson, 1876) and *A. acuminatus* n. sp. In the "*ornatus* group", the second segment (basis) of the maxilla bears a large seta, the caudal ramus is relatively short, typically not exceeding 1.1 times its width, and the inner margin of the protopod of leg 5 is serrate. In contrast, in the "*scutatus* group", there is no seta on the second segment of the maxilla, the caudal ramus is longer, at least 1.6 times its width, and the inner margin of the protopod of leg 5 is smooth.

The segmentation of the antennule appears to be a reliable taxonomic character in *Acontiophorus*. The female antennule of existing species is nine-segmented in three species, ten-segmented in two species, 11-segmented in four species, 13-segmented in two species, and 16-segmented in four congeners. *Acontiophorus acuminatus* n. sp. has a 16-segmented female antennule, aligning it with four other species, namely, *A. armatus* Brady, 1880, *A. brevifurcatus* Stock, 1966, *A. maldivensis* Sewell, 1949, and *A. ornatus*. All these five species pertain to the *ornatus* group. Despite the incomplete descriptions of most *ornatus* group species, they can be distinguished from *A. acuminatus* n. sp. based on distinct differences.

In *A. armatus*, the body is significantly larger, nearly twice as long as that of *A. acuminatus* n. sp., measuring 1.5 mm in females according to its original description by Brady (1880). The maxillule bears three "pinnate" setae on the inner lobe and two setae on the outer lobe. In *A. brevifucatus*, the cephalothorax and the second pedigerous somite have blunt or rounded posterolateral corners. The caudal ramus is wider than long, the second endopodal segment of legs 1, 3 and 4 features a bicuspid outer distal corner, the outer lobe of the maxillule has 1 pinnate and 2 naked setae, and the exopod of leg 5 is armed with four setae, as Stock (1966) described or illustrated. In *A. maldivensis*, the genital double-somite and the first free abdominal somite exhibit blunt posterolateral corners. The second segment (basis) of the antenna is three times longer than it is wide, according to Sewell (1949) (compared to approximately five times in *A. acuminatus* n. sp.). The second endopodal segment of the antenna is shorter than the first (contrary to the reverse condition in *A. acuminatus* n. sp.), and the exopod of leg 5 is armed with 4 setae. *Acontiophorus acuminatus* n. sp. more closely resembles *A. ornatus* than other species. Sars (1921) illustrated the more extensively studied the European species *A. ornatus*, which has a genital double-somite longer than it is wide, an exopodal segment of the antenna shorter than the first endopodal segment, a distal seta on the exopod of the antenna significantly shorter than that of the new species, extending only slightly beyond the distal margin of the second segment, and the protopod of leg 5 is articulated from the fifth pedigerous somite. These distinctions establish *A. acuminatus* as a new species.

Genus *Asteropontoides* Stock, 1975

***Asteropontoides parvus* n. sp. (Figs. 7–9)**

https://zoobank.org/urn:lsid:zoobank.org:act:AF84D087-77E3-4E80-AD9D-5B8C1F6EC506

Type material. Holotype (intact ♀, HNIBR IV2396), intact paratypes (1 ♀, 1 ♂, HNIBR IV2397), and dissected paratypes (1 ♀, 1 ♂) from washings of various sponge species, near Beomseom, Seogwipo, Jeju Island (33°13′28.7″N, 126°30′46.9″E), at a depth of 20 m, SCUBA, collected by Tae Won Jung, on 20 July 2022. The holotype and intact paratypes have been deposited in the Honam National Institute of Biological Resources (HNIBR), Mokpo. The Dissected paratypes are kept in the collection of I.-H. Kim.

Etymology. The name reflects the small body size, derived from the Latin word *parv*, meaning 'small'.

Female. Body (Fig. 7A) narrow. Body length 634 μm in dissected an figured specimen. Prosome 366 μm long, fusiform, consisting of cephalothorax and second to fourth pedigerous somites. Cephalothorax almost as long as wide (264 × 261 μm). Cephalothorax and second and third pedigerous somites with pointed or angular posterolateral corners; fourth pedigerous somite with strongly concave posterior margin and rounded posterolateral corners. Urosome (Fig. 7B) 4-segmented. Fifth pedigerous somite 81 μm wide. Genital double-somite 1.30 times longer than wide (95 × 73 μm), consisting of broadened proximal half and narrower distal half, widest at proximal one-fifth; genital apertures positioned dorsally at 36% region of double-somite length. Two free abdominal somites 34 × 43 and 34 × 45 μm, respectively. Anal somite with fine spinules along posteroventral margin (Fig. 7D). Caudal ramus (Fig. 7C, D) 1.43 times longer than wide (30 × 21 μm), almost rectangular, armed with 6 pinnate setae and ornamented with fine spinules along posteroventral margin.

Rostrum (Fig. 7E) longer than wide, beaklike, with pointed distal apex. Antennule (Fig. 7F) slender, 19-segmented, but articulations between second and sixth segments in-

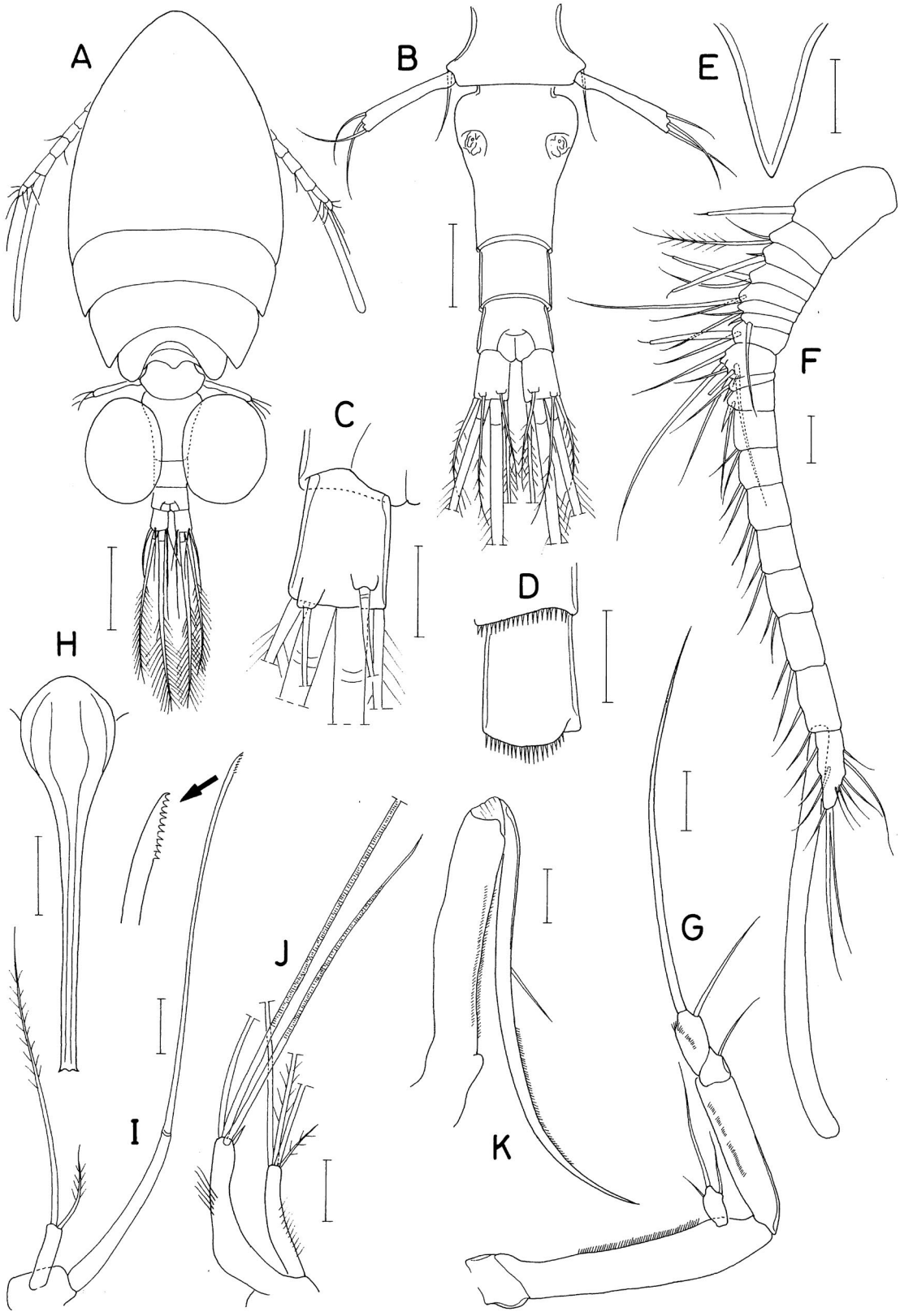


Fig. 7. *Asteropontoides parvus* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, left caudal ramus, dorsal; D, left caudal ramus, ventral; E, rostrum; F, antennule; G, antenna; H, oral siphon; I, mandible; J, maxillule; K, maxilla. Scale bars: A, 0.1 mm; B, H, 0.05 mm; C–G, I–K, 0.02 mm.

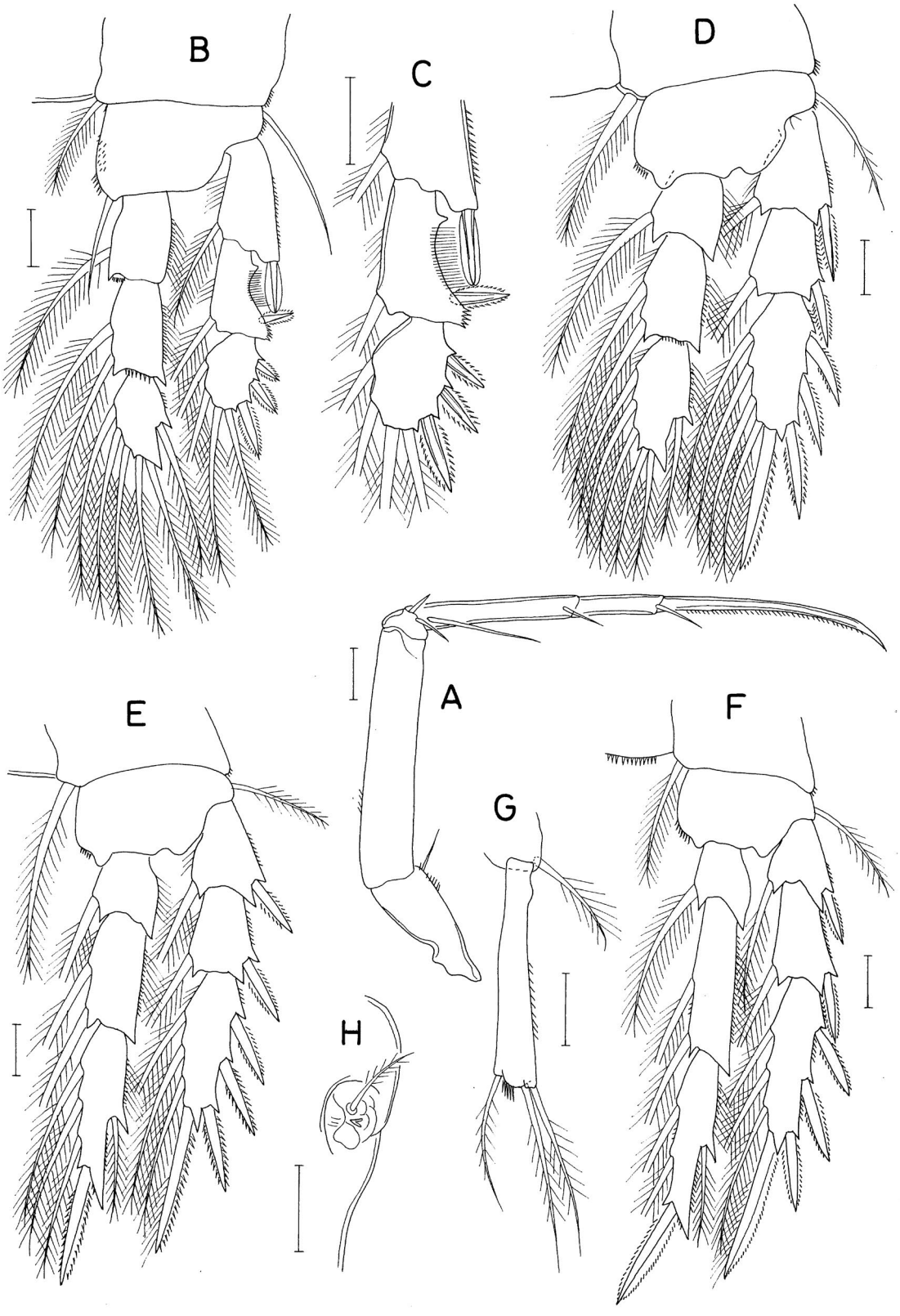


Fig. 8. *Asteropontoides parvus* n. sp., female. A, maxilliped; B, leg 1; C, exopod of leg 1; D, leg 2; E, leg 3; F, leg 4; G, leg 5; H, right genital aperture, dorsal. Scale bars: 0.02 mm.

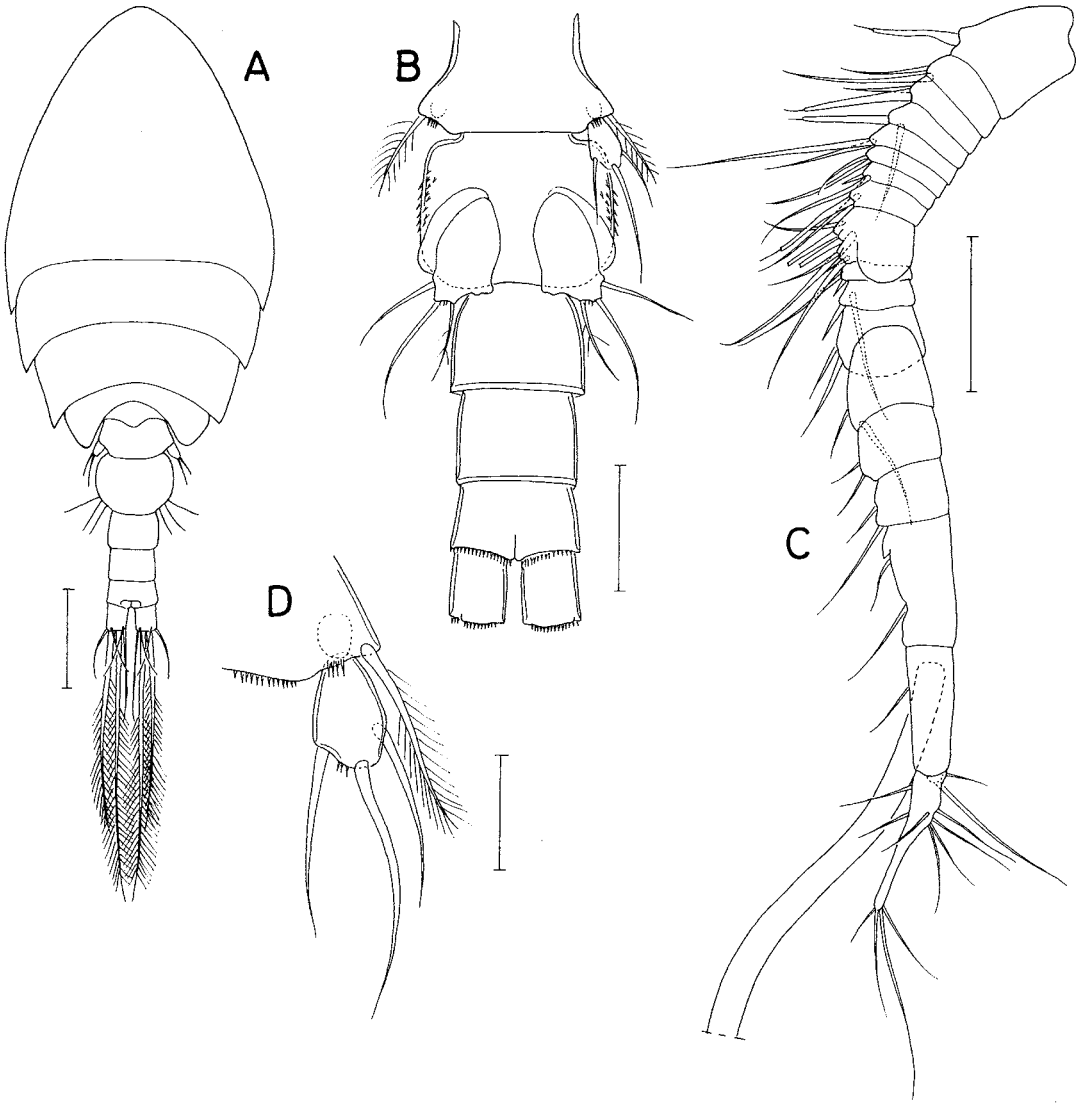


Fig. 9. *Asteropontoides parvus* n. sp., male. A, habitus, dorsal; B, urosome, ventral; C, antennule; D, leg 5. Scale bars: A, 0.1 mm; B, C, 0.05 mm; D, 0.02 mm.

complete, recognizable only on one side; first, nineth, eighteenth, and terminal segments with 1 seta, 7 setae, 2 setae + aesthetasc, and 12 setae, respectively; other segments with 2 setae each; aesthetasc on penultimate segment about 60% as long as total segments. Antenna (Fig. 7G) slender, consisting of coxa, basis, small exopod, and 3-segmented endopod; coxa short, unarmed; basis unarmed, but ornamented with minute spinules on outer margin; exopodal segment about twice longer than wide (15 × 7 μm), bearing 3 setae; first endopodal segment unarmed, 52 × 13 μm, with 1 longitudinal row of minute spinules; second endopodal segment short, with 1 seta; third endopodal segment slightly longer than wide, with 1 large distal seta (115 μm long), 1 shorter subdistal seta, and 1 row of minute spinules.

Oral siphon (Fig. 7H) consisting of expanded proximal quarter and slender distal three quarters, extending between legs 1 and 2. Mandible (Fig. 7I) consisting of stylet and 1-segmented palp; stylet thin, articulated at proximal third, 220 μm long, bearing 11 minute teeth at distal region, as arrowed in Fig. 7I; palp 25 μm long, tipped with 2 unequal, pinnate setae, larger seta 117 μm long. Maxillule (Fig. 7J) consisting of 2 slender lobes; outer lobe 38 μm long, with 4 distal setae and setulose outer margin; inner lobe 55 μm long, bearing 1 minute and 3 large distal setae and several setules on inner margin. Maxilla (Fig. 7K) 2-segmented; proximal segment unarmed, but bearing 2 longitudinal rows of minute spinules on inner margin; distal segment thin, arched, about 1.4 times longer than proximal segment, bearing 1 seta at proximal 40% region, fol-

lowed by row of minute spinules along concave margin. Maxilliped (Fig. 8A) slender, 5-segmented; armature formula 1, 0, 2, 2, and 1 + claw; second segment 100 μm long; fourth and fifth segments 58 and 33 μm long, respectively; terminal claw 91 μm long, with fine spinules along concave margin.

Legs 1–4 (Fig. 8B–F) with 3-segmented rami. Outer seta on basis naked in leg 1 but pinnate in legs 2–4. Inner distal element on basis of leg 1 as thin, naked seta. Second endopodal segment of legs 1–4 with monocuspid outer distal corner. Second exopodal segment of leg 1 with nipple-shaped outer proximal process, concave, setulose outer margin, and serrate outer distal corner (Fig. 8C). Second endopodal segment of legs 1–4 with 2 inner setae. Legs 3 and 4 with slender rami. Distal margin of intercoxal plate smooth in legs 1–3, but spinulose in leg 4. Armature formula for legs 1–4 as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-1	I-1; I-1; III, 2, 2	0-1; 0-2; 1, 2, 3
Leg 2	0-1	1-0	I-1; I-1; III, I, 4	0-1; 0-2; 1, 2, 3
Leg 3	0-1	1-0	I-1; I-1; III, I, 3	0-1; 0-2; 1, I, 3
Leg 4	0-1	1-0	I-1; I-1; III, I, 3	0-1; 0-2; 1, I, 2

Leg 5 (Fig. 8G) consisting of short protopod and elongate exopod; protopod completed fused with somite, bearing 1 pinnated dorsodistal seta; exopodal segment 5.73 times longer than wide (63 × 11 μm), gradually broadened distally, armed distally with 3 pinnated setae, spinules on outer margin, and patch of several spinules on distal margin. Leg 6 (Fig. 8H) represented by 1 pinnate seta and 1 spinule on genital operculum.

Male. Body (Fig. 9A) resembling that of female. Body length 620 μm. Prosome 409 μm long, Anterior part of cephalothorax tapering anteriorly. Urosome (Fig. 9B) 5-segmented. Fifth pedigerous somite 77 μm wide, bearing spinules on ventrodistal margin (Fig. 9D). Genital somite quadrate, 62 × 77 μm, with scattered spinules on lateral surfaces. Three abdominal somites 41 × 53, 36 × 48, and 28 × 51 μm, respectively. Caudal ramus 1.32 times longer than wide (29 × 22 μm).

Rostrum and antenna as in female. Antennule (Fig. 9C) 18-segmented; first, nineth, seventeenth, and terminal segments with 1 seta, 7 setae, 2 setae + aesthetasc, and 12 setae, respectively; other segments each bearing 2 setae; terminal segment characteristically attenuated distally; nineth segment inflated ventrodistally.

Antenna, oral siphon, mandible, maxillule, maxilla, maxilliped, and legs 1–4 as in female. Leg 5 (Fig. 9D) consisting of 1 pinnate lateral seta on fifth pedigerous somite and free exopod; exopodal segment ovoid, 20 × 13 μm, with 3 naked setae and several spinules on distal margin.

Remarks. The genus *Asteropontoides* comprises four acknowledged species: *A. attenuatus* (Thompson & Scott A., 1903), *A. elephatinus* Johnson, 1998, *A. nicobaricus* (Sewell, 1949), and *A. acutirostris* Kim, 2016. The ratios of length to width of the female caudal ramus in these species are 2.5 for *A. attenuatus*, 0.48 for *A. elephantinus*, 0.8 for *A. nicobaricus*, and 2.37 for *A. acutirostris*. The female antennule is 18-segmented in *A. attenuatus* and *A. nicobaricus*, 15-segmented in *A. elephantinus*, and 20-segmented in *A. acutirostris*. With a caudal ramus that is 1.43 times longer than wide in the female and a 19-segmented female antennule, *A. parvus* n. sp. is easily distinguishable from the four congeners. The unique possession by *A. parvus* n. sp. of a digitiform outer proximal process and a serrate outer distal corner on the second exopodal segment of leg 1 is unprecedented in *Asteropontoides*, further distinguishing *A. parvus* n. sp.

Genus *Rhynchomyzon* Giesbrecht, 1895

***Rhynchomyzon rostrale* n. sp. (Figs. 10, 11)**

https://zoobank.org/urn:lsid:zoobank.org:act:7465BAE7-783F-4886-8719-80C0AD6FBBCC

Type material. Holotype (♀, dissected and mounted on a slide; HNIBR IV7756) from washings of invertebrates, near Biyang-do, Jeju Island (33°24′13.8″N, 126°12′17.3″E), SCUBA, at a depth of 40 m, on a sandy bottom, collected by Tae Won Jung and Jong Guk Kim, on 23 June 2023. The holotype has been deposited in the Honam National Institute of Biological Resources (HNIBR), Mokpo.

Etymology. The specific name refers to the well-developed rostrum of this new species.

Female. Body (Fig. 10A) rather narrow, 920 μm long. Prosome 527 μm long, fusiform, consisting of cephalothorax and second to fourth pedigerous somites. Cephalothorax longer than wide (372 × 355 μm). Third pedigerous somite with acutely pointed, projected posterolateral corners. Fourth pedigerous somite with deeply concave posterodorsal margin. Urosome (Fig. 10B) 5-segmented. Fifth pedigerous somite 104 μm wide, with membranous fringe along posterodorsal margin. Genital double-somite 1.2 times longer than wide (143 × 120 μm), consisting of broadened anterior third and narrower posterior two-thirds; broader anterior part with beaklike posterolateral process on each side; genital apertures positioned dorsolaterally at posterior region of broad anterior part near base of beaklike process. Three free abdominal somites 45 × 71, 30 × 67, and 35 × 68 μm, respectively. Anal somite with membranous fringe along posteroventral margin (Fig. 10C). Caudal rami divergent; each ramus (Fig. 10C) nearly rectangular, 2.84 times longer than wide (91 × 32 μm), bearing pointed process at inner and outer distal corners, armed with 6 setae, ornamented with thin setules on inner margin, fringed with membrane of uneven width along posteroventral margin.

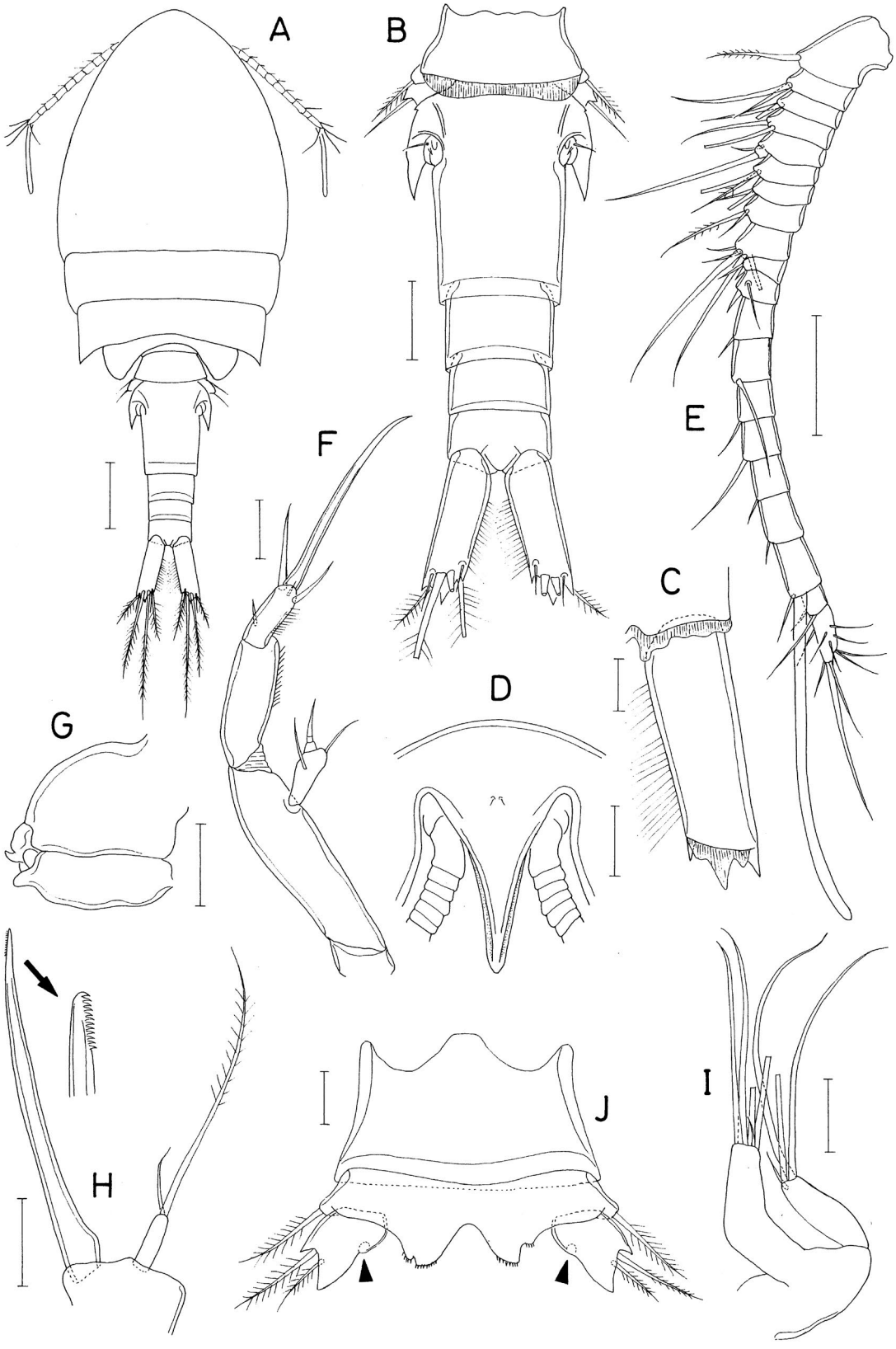


Fig. 10. *Rhynchomyzon rostrale* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, left caudal ramus, ventral; D, rostral area; E, antennule; F, antenna; G, oral cone, left; H, mandible; I, maxillule; J, fifth pedigerous somite and leg 5 pair, ventral. Scale bars: A, 0.1 mm; B, D, E, G, 0.05 mm; C, F, H–J, 0.02 mm.

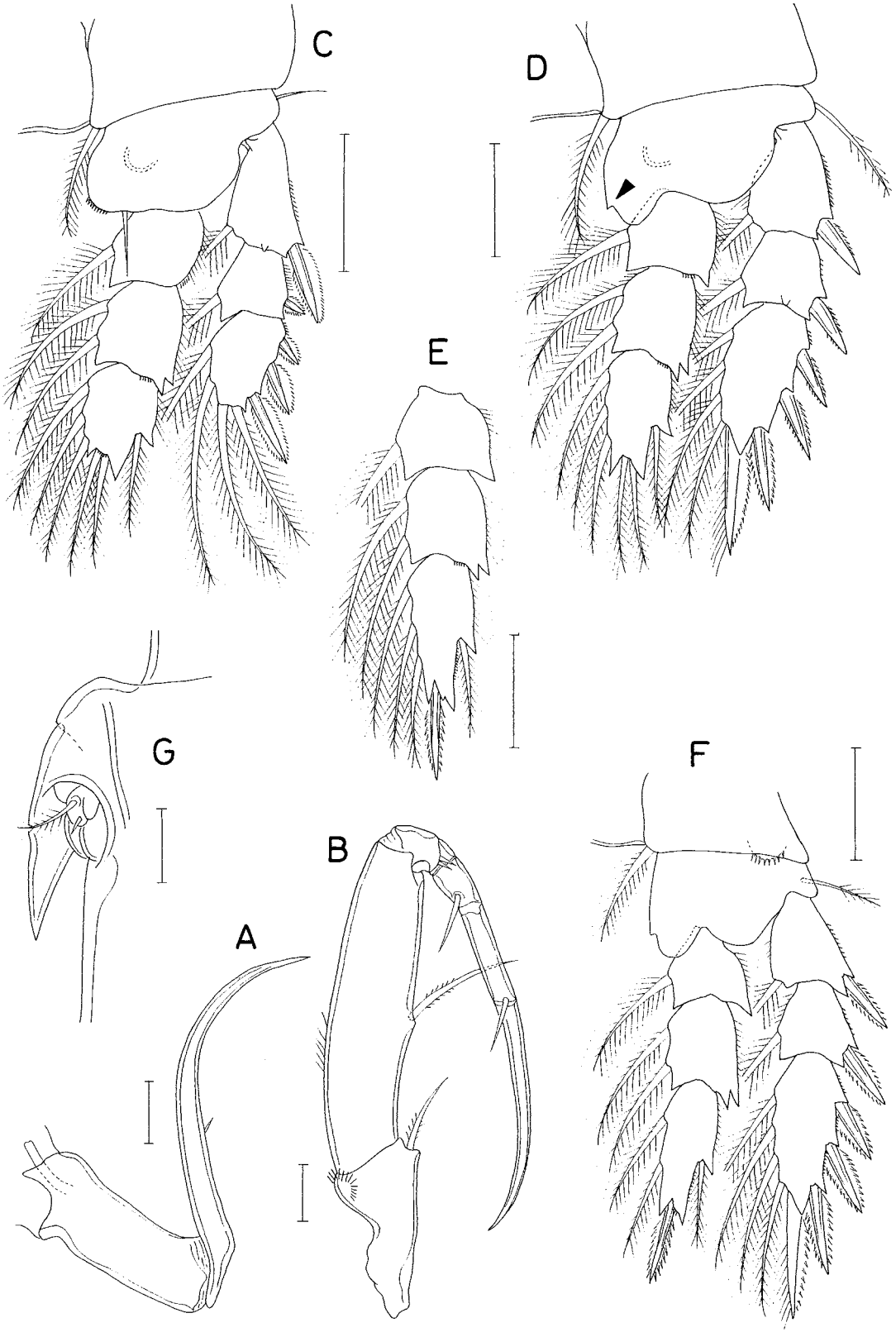


Fig. 11. *Rhynchomyzon rostrale*. n. sp., female. A, maxilla; B, maxilliped; C, leg 1; D, leg 2; E, endopod of leg 3; F, leg 4; G, left genital aperture, dorsal. Scale bars: A, B, G, 0.02 mm; C−F, 0.05 mm.

Rostrum (Fig. 10D) large, beaklike, longer than wide. Antennule (Fig. 10E) slender, 282 μm long, 20-segmented; first, twelfth to seventeenth segments each with 1 seta; nineth segment with 5 setae; second to eighth segments each with 2 setae; eighteenth segment with 1 seta plus 1 aesthetasc; and terminal segment with 10 setae; setae mostly small and thin. Antenna (Fig. 10F) consisting of coxa, basis, 1-segmented exopod, and 2-segmented endopod; coxa short, unarmed; basis also unarmed, 73 μm long; exopod 22 × 8 μm, gradually broadened distally, with 1 subdistal and 2 distal setae, inner one of latter 2 setae annulated proximally; first endopodal segment 42 μm long, with row of setules on outer margin; second endopodal segment 24 μm long, with 1 proximal seta, 3 unequal distal setae, and slender, spiniform terminal claw of 73 μm long.

Oral cone (Fig. 10G) short, about 100 μm long, as long as wide. Mandible (Fig. 10H) consisting of stylet and palp; stylet 82 μm long, bearing 12 minute teeth at distal region, as arrowed in Fig. 10H; palp 1-segmented, 13 μm long, tipped with 1 large pinnate seta (64 μm long) and 1 small naked seta. Maxillule (Fig. 10I) bilobed; outer lobe tipped with 1 rudimentary and 3 large, naked setae; inner lobe bent in middle, tipped with 1 rudimentary and 4 large, naked setae. Maxilla (Fig. 11A) 2-segmented; proximal segment with short excretory tube proximally; distal segment as arched claw bearing 1 small setule at 40% region of concave margin. Maxilliped (Fig. 11B) 6-segmented; armature formula 1, 1, 2, 0, 1, and 1 + claw; inner seta on second segment (basis) large; terminal segment 33 μm long; terminal claw 78 μm long.

Legs 1 (Fig. 11C), 2 (Fig. 11D), 3, and 4 (Fig. 11F) with 2-segmented protopod and 3-segmented rami; inner coxal seta well-developed, pinnate; outer seta on basis small, naked in leg 1 but distinct, pinnate in legs 2–4. Basis of leg 1 and 2 with tubercle on posterior surface. Basis of leg 1 with minute spinules at inner distal corner; inner distal seta thin, straight, spiniform. Basis of leg 2 with angle on subdistal inner margin (as indicated by arrowhead in Fig. 11D). Leg 3 similar to leg 2, except bearing 1 spine and 4 setae on third endopodal segment (Fig. 11E). Leg 4 with small, spinulose tubercle on posterior surface of coxa (Fig. 11F). Armature formula for legs 1–4 as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-1	I-1; I-1; III, 2, 2	0-1; 0-2; 1, 2, 3
Leg 2	0-1	1-0	I-1; I-1; III, I, 4	0-1; 0-2; 1, 2, 3
Leg 3	0-1	1-0	I-1; I-1; III, I, 4	0-1; 0-2; 1, I, 3
Leg 4	0-1	1-0	I-1; I-1; III, I, 4	0-1; 0-2; 1, I, 2

Leg 5 (Fig. 10J) consisting of protopod and free exopod; left and right protopod fused with each other, without any division between them, bearing 1 seta at outer distal corner and weakly bilobed, spinulose inner distal protrusion; exopodal segment slightly longer than wide, with pointed outer distal process, broad inner distal process, 2 pinnate seta on distal margin, and 1 scar of setal insertion on inner margin at places indicated by arrowheads in Fig. 10J. Leg 6 (Fig. 11G) represented by 1 pinnate and 1 small, naked setae on genital operculum.

Male. Unknown.

Remarks. The genus *Rhynchomyzon* comprises six known species. The closest relative of *R. rostrale* n. sp. is *R. rubrovittatum* Sars, 1915, known from Norwegian waters. These two species share the unique feature of a large, pointed process on the lateral margins of the genital double-somite (absent on the same place in other species) and the armature formula II, I, 4 (three spines plus four setae) on the third exopodal segment of leg 1 (contrasting with formula III, I, 4 in other species). However, they are distinct species: in the Norwegian species, the caudal ramus of the female is about 5.5 times longer than wide when measured the illustration of Sars (1915), the endopod of the antenna is 3-segmented, the inner seta on the basis of the maxilliped is small, and the basis of leg 1 lacks an inner distal seta. These significant differences clearly distinguish *R. rostrale* n. sp. from its most closely related species, *R. rubrovittatum*.

Genus *Scottocheres* Giesbrecht, 1897

***Scottocheres jungi* n. sp. (Figs. 12, 13)**

https://zoobank.org/urn:lsid:zoobank.org:act:B6E15D6C-DE19-4CA2-AC7B-7C1085A0562F

Type material. Holotype (intact ♀; HNIBR IV2386), intact paratypes (2 ♀♀; HNIBR IV2387), and dissected paratypes (1 ♀, 1 ♂) from mixed species of sponges, Chuja Island (33°47′513.6″N, 126°19′21.9″E), SCUBA, at a depth of 24 m, SCUBA, collected by Tae Won Jung, on 22 June 2022. The holotype and intact paratypes have been deposited in the Honam National Institute of Biological Resources (HNIBR), Mokpo. The dissected paratypes are retained in the colletion of I.-H. Kim.

Etymology. The species is named in tribute to Dr. Tae Won Jung, who collected these specimens.

Female. Body (Fig. 12A) narrow. Body length 927 μm in dissected and figured specimen. Prosome 606 μm long, with thin exoskeleton. Cephalothorax 358 × 321 μm, sub-circular. First 3 prosomal somites separated from one another by broad arthrodial membranes. Second to fourth pedigerous somites 276, 264, and 200 μm wide, respectively. Urosome (Fig. 12B) 4-segmented. fifth pedigerous somite 114 μm wide, with angular posterolateral corners. Genital double-somite broad, 160 × 150 μm, with convex lateral margins, dentiform angle in middle of lateral margin, followed by weak subsidiary angle; genital apertures positioned dorsally in middle of double-somite length.

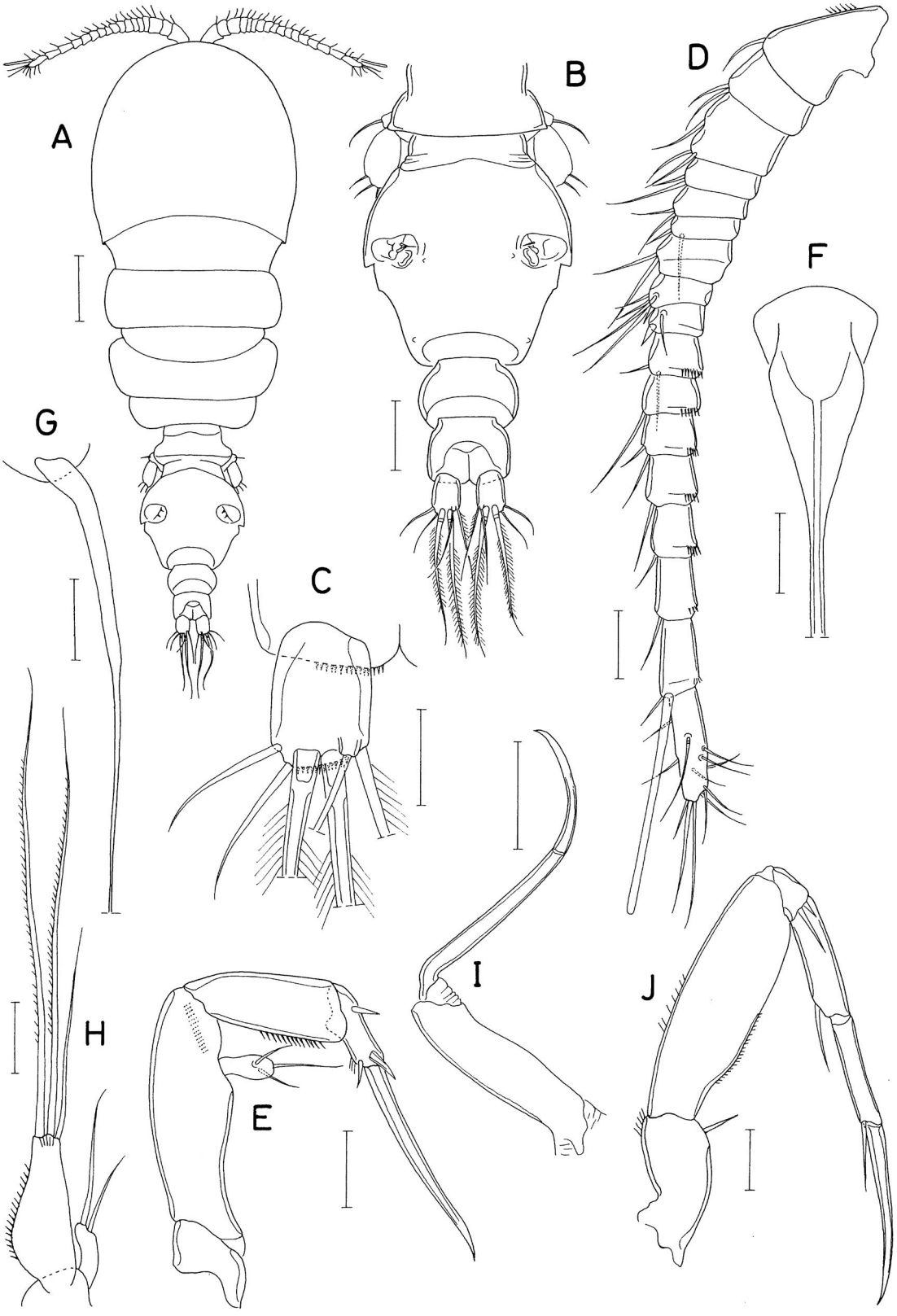


Fig. 12. *Scottocheres jungi* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, left caudal ramus, dorsal; D, antennule; E, antenna; F, oral siphon; G, mandible; H, maxillule; I, maxilla; J, maxilliped. Scale bars: A, 0.1 mm; B, F, I, 0.05 mm; C–E, G, H, J, 0.02 mm.

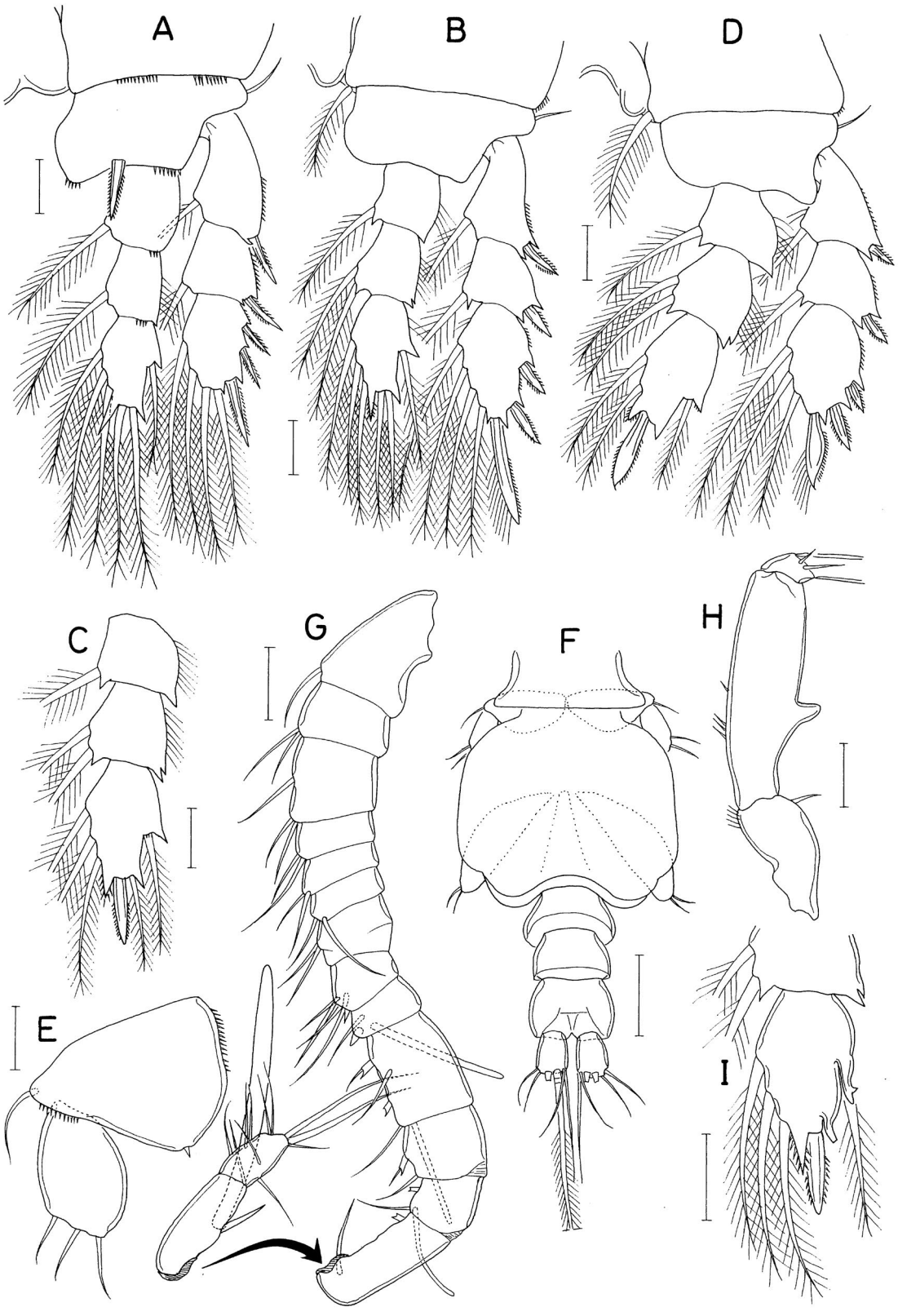


Fig. 13. *Scottocheres jungi* n. sp., female. A, leg 1; B, leg 2; C, endopod of leg 3; D, leg 4; E, leg 5. Male: F, urosome, dorsal; G, antennule; H, proximal part of maxilliped; I, third endopodal segment of leg 3. Scale bars: A–E, G–I, 0.02 mm; F, 0.05 mm.

First free abdominal somite 42 × 71 μm, with convex lateral margins. Anal somite 45 × 59 μm, with minute spinules along posteroventral margin. Caudal ramus (Fig. 12C) 1.25 times longer than wide (30 × 24 μm), with 6 setae and row of minute spinule along posteroventral margin; seta VII tipped on tubercle.

Rostrum absent. Antennule (Fig. 12D) 247 μm long, 17-segmented; armature formula 1, 2, 3, 2, 2, 2, 2, 5, 2, 2, 1, 2, 1, 1, 1, 1 + aesthetasc, and 13; first segment with several spinules on anterior margin; tenth to sixteenth segments bearing transverse row of minute spinules on posterior side near their distal margin; all setae small and naked. Antenna (Fig. 12E) with short, unarmed coxa; basis 63 μm long, with row of minute spinules subdistally; exopodal segment 14 × 7 μm, with inflated distal half and 3 naked setae (2 subdistal and 1 distal); endopod 2-segmented; first endopodal segment 37 × 16 μm, unarmed but with row of spinules on outer margin; second endopodal segment 19 × 7 μm, with 3 small, spiniform setae (1 proximal and 2 subdistal), few setules at outer subdistal region, and spiniform terminal claw of 58 μm long.

Oral siphon (Fig. 12F) consisting of slightly inflated proximal part and thin distal part, reaching insertions of legs 3. Mandible (Fig. 12G) represented by thin stylet, lacking palp. Maxillule (Fig. 12H) bilobed; outer lobe small, 15 μm long, bearing 2 naked setae (40 and 24 μm long, respectively); inner lobe 41 μm long, with spinulose, inflated inner margin, tipped with 3 setae (127, 120, and 60 μm long, respectively, from inner to outer). Maxilla (Fig. 12I) slender; proximal segment 95 μm long; distal segment slender, claw-like, divided by articulation-like annulation into proximal (89 μm long) and distal (60 μm long) parts. Maxilliped (Fig. 12J) consisting of syncoxa, basis, and 3-segmented endopod; armature formula 1, 0, 2, 1, and 1 + claw; minute spinules present on outer distal region of syncoxa and on proximal half of outer and inner margins of basis; third endopodal segment and terminal claw 36 and 59 μm long, respectively.

Legs 1 (Fig. 13A), 2 (Fig. 13B), 3, and 4 (Fig. 13D) with 2-segmented protopod and 3-segmented rami; inner coxal seta absent in leg 1, but present in legs 2–4; outer seta on basis small, naked. Inner distal spine on basis of leg 1 shorter than first endopodal segment, 18 μm long, spinulose. Leg 3 similar to leg 2, except bearing 1 spine plus 5 setae on third endopodal segment (Fig. 13C). Inner distal process of third endopodal segment of legs 2–4 spinulose. Distal spine on third exopodal and endopodal segments of leg 4 inflated. Armature formula for legs 1–4 as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-I	I-1; I-1; III, 2, 2	0-1; 0-1; 1, 2, 3
Leg 2	0-1	1-0	I-1; I-1; II, I, 4	0-1; 0-2; 1, 2, 3
Leg 3	0-1	1-0	I-1; I-1; II, I, 4	0-1; 0-2; 1, 1 + I, 3
Leg 4	0-1	1-0	I-1; I-1; II, I, 3	0-1; 0-2; 1, I, 2

Leg 5 (Fig. 13E) consisting of protopod and exopod; protopod 45 × 63 μm, broad, plate-like, articulated from somite, armed with 1 naked outer distal seta and rudimentary, spinule-like inner distal seta, and ornamented with 1 row of minute spinules on outer part of distal margin and proximal part of inner margin; exopodal segment 1.43 times longer than wide (40 × 28 μm), bearing 3 subequal, naked setae on oblique distal margin. Leg 6 represented by 1 small seta and 1 spinule on genital operculum

Male (with damaged prosome)**.** Urosome (Fig. 13F) 5-segmented. Fifth pedigerous somite 92 μm wide. Genital somite nearly quadrate, 110 × 136 μm. Three abdominal somites with convex lateral margins, 30 × 56, 30 × 47, and 37 × 53 μm, respectively. Caudal ramus 1.47 times longer than wide (28 × 19 μm).

Rostrum absent. Antennule (Fig. 13G) 15-segmented, with strong geniculation between thirteenth and fourteenth segments; armature formula 1, 2, 3, 2, 2, 2, 2, 5, 2 + aesthetasc, 4 + aesthetasc, 2, 2 + aesthetasc, 2, 2 + aesthetasc, and 11; some setae on tenth to fourteenth segments rod-shaped or tipped with setule. Antenna as in female.

Oral siphon, mandible, maxillule, and maxilla as in female. Maxilliped (Fig. 13H) bearing digitiform, prominent process on inner margin of basis.

Legs 1, 2, and 4 as in female. Third endopodal segment of leg 3 (Fig. 13I) with distally bifurcate, elongated process on outer margin and truncate, elongated outer distal process. Leg 5 armed as in female. Leg 6 (Fig. 13F) represented by 2 small, naked setae on genital operculum.

Remarks. In the genus *Scottocheres* the armature formula of legs 1–4 varies across species. The armature formulae for leg segments of *S. jungi* n. sp. are (1) III, 2, 2 for the third exopodal segment of leg 1 (shared by six congeners), (2) II, I, 4 for the third exopodal segment of leg 2 (shared by five congeners), (3) II, I, 4 for the third exopodal segment of leg 3 (shared by five species), (4) 1, 1 + I, 3 for the third endopodal segment of leg 3 (shared by six congeners), (5) II, I, 3 for the third exopodal segment of leg 4 (shared by five congeners), (6) 1, I, 2 for the third endopodal segment of leg 4 (shared by eight congeners), and (7) 0–1 for the coxa of legs 2–4 (but not for the coxa of leg 1) (shared by four congeners). Two species in the genus, *S. laubieri* Stock, 1967 and *S. mipoensis* Kim, 2016, share all eight armature formulae with *S. jungi* n. sp. *Scottocheres mipoensis* was recorded from Korea and can be excluded from further comparison with the new species due to having an 18-segmented female antennule and a 16-segmented male antennule (Kim, 2016). *Scottocheres laubieri* was discovered in association with sponges in the Mediterranean (Stock, 1967). Although *S. laubieri* shares the same armature formulae for legs and antennule segmentation with *S. jungi* n. sp., it differs from the new species in the following features: (1) the genital double-somite is narrow, approximately 1.56 times longer

than wide, according to the illustration by Stock (1967) (vs. the double somite is 1.07 times longer than wide in *S. jungi* n. sp.), (2) the first free abdominal somite of the female is distinctly longer than wide (vs. wider than long in *S. jungi* n. sp.), (3) the outer lobe of the maxillule is armed with three setae (vs. two setae in *S. jungi* n. sp.), (4) the male antennule bears aesthetascs on proximal segments (vs. no aesthetasc on the proximal segments in *S. jungi* n. sp.), and (5) the outer and distal processes of the third endopodal segment of male leg 3 are not transformed (vs. the processes are modified in *S. jungi* n. sp.).

In *Scottocheres* the length-to-width of caudal ramus varies across species. In *S. jungi* n. sp., the ratio is 1.25, which may uniquely characterize this new species, as in the 11 species of the genus, the ratio is approximately 1 or less, or exceeds 2.

Johnsson *et al.* (2001) reported *S. laubieri* from Brazilian waters, but their material needs confirmation of the species identity due to incongruences with the original description, e.g., an inner coxal seta on leg 1, a monocuspid outer distal corner on the second endopodal segment of legs 1, 3, and 4, no outer distal seta on the protopod of leg 5, and the exopod of leg 5 where the mid-terminal seta is longer than the other two nearby setae.

Genus *Thermocheres* Kim, 2010

***Thermocheres viridis* n. sp. (Figs. 14, 15)**

https://zoobank.org/urn:lsid:zoobank.org:act:B84E8AF3-1CC9-462A-B7C1-CD3A88BF661B

Type material. Holotype (♂, dissected and mounted on a slide; MABIK CR00257789) was collected from mixed species of sponges near Munseom, Seogwipo, Jeju Island (33°13′39.4″N, 126°33′48.2″E), SCUBA, at a depth of 56 m, by Taekjun Lee, on 25 April 2023. The holotype has been deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon.

Etymology. The name deriveds from Latin *virid* (= greenish) and refers to the greenish body color of the new species.

Male. Body (Fig. 14A) greenish, 1.03 mm long. Prosome 629 μm long. Cephalothorax 418 × 473 μm. All prosomal somite with acutely pointed posterolateral corners. Urosome (Fig. 14B) 6-segmented. Fifth pedigerous somite 130 μm wide. Genital somite nearly quadrangular, wider than long (109 × 165 μm), bearing acutely pointed posterolateral corners; genital operculum with cusp on subdistal inner margin. Four abdominal somites 55 × 125, 47 × 109, 31 × 93, and 45 × 95 μm, respectively; first and second abdominal somites with acutely pointed, posteriorly extended posterolateral corners. Anal somite with minute spinules on posteroventral margin. Caudal rami slightly divergent; each ramus (Fig. 14C) 2.02 times longer than wide (85 × 42 mm), slightly narrowing distally, armed with 6 setae and ornamented with thin setules on inner margin and membranous fringe on posteroventral margin; setae II and VII positioned subdistally and dorsally at same plane.

Rostrum (Fig. 14D) lightly longer than wide, tapering, with rounded distal apex. Antennule (Fig. 14E) 17-segmented, geniculated between fifteenth and sixteenth segments; First to fifth, seventh, eleventh, and fourteenth segments each with 2 setae + aesthetasc, sixth and tenth segments each with 2 setae, nineth segment with 7 setae + 3 aesthetascs, twelfth segment each with 4 setae + aesthetasc, thirteenth and fifteenth segments each with 1 seta; penultimate segment with 1 seta + aesthetasc, and terminal segment with 11 setae; all setae naked. Aesthetasc on penultimate segment about 0.6 times as long as entire segments; aesthetascs on other segments thin, generally as long as that of penultimate segment. Antenna (Fig. 14F) slender, consisting of coxa, basis, 1-segmented exopod, and 2-segmented endopod; coxa 27 μm long, unarmed; basis 64 μm long, unarmed but ornamented with longitudinal row of pectinated spinules along distal half; exopodal segment 17 × 5.5 μm, bearing 3 setae; first endopodal segment 35 μm long, unarmed but with thin spinules on inner and outer margins; second endopodal segment 29 μm long, terminated in elongated spine (120 μm long), with 3 pinnated setae (1 proximal and 2 distal) and setules on outer surface.

Oral siphon (Fig. 14J) 380 μm long, slender, evenly narrowing distally. Mandible (Fig. 14G) consisting of segment-like coxa, thin stylet, and small palp; stylet 354 μm long, bearing 11 teeth at distal region (Fig. 14I); palp (Fig. 14H) small, digitiform, unsegmented, not articulated from coxa, tipped with 2 small setae. Maxillule (Fig. 14G) bilobed; outer lobe small, 25 μm long, bearing 4 setae (2 distal and 2 subdistal); inner lobe 104 μm long, distally armed with 1 rudimentary and 3 extremely long setae, latter 3 setae subequal in length, blunt and spinulose at tip. Maxilla (Fig. 15A) slender, 2-segmented; both segments unarmed; distal segment terminated in spiniform claw, with 1 minute seta at distal third and several setules distally; claw spinulose, about 0.38 times as long as segment. Maxilliped (Fig. 15B) consisting of syncoxa, basis, and 3-segmented endopod; armature formula 1, 1, 2, 2, and 1 + claw; basis with rounded protrusion, its inner seta minute, positioned at distal region of protrusion; setae on second and third endopodal segments pinnate; third endopodal segment 54 μm long; terminal claw 95 μm long, bearing spinules along concave margin.

Legs 1–4 (Fig. 15C–F) with 2-segmented protopod and 3-segmented rami; all these legs with pinnate inner seta on coxa and pinnate outer seta on basis. Leg 1 basis with thin, naked inner distal seta and spinules on inner distal corner. Second endopodal segment of legs 1–4 with bicuspid outer distal corner. Leg 4 with rudimentary inner distal

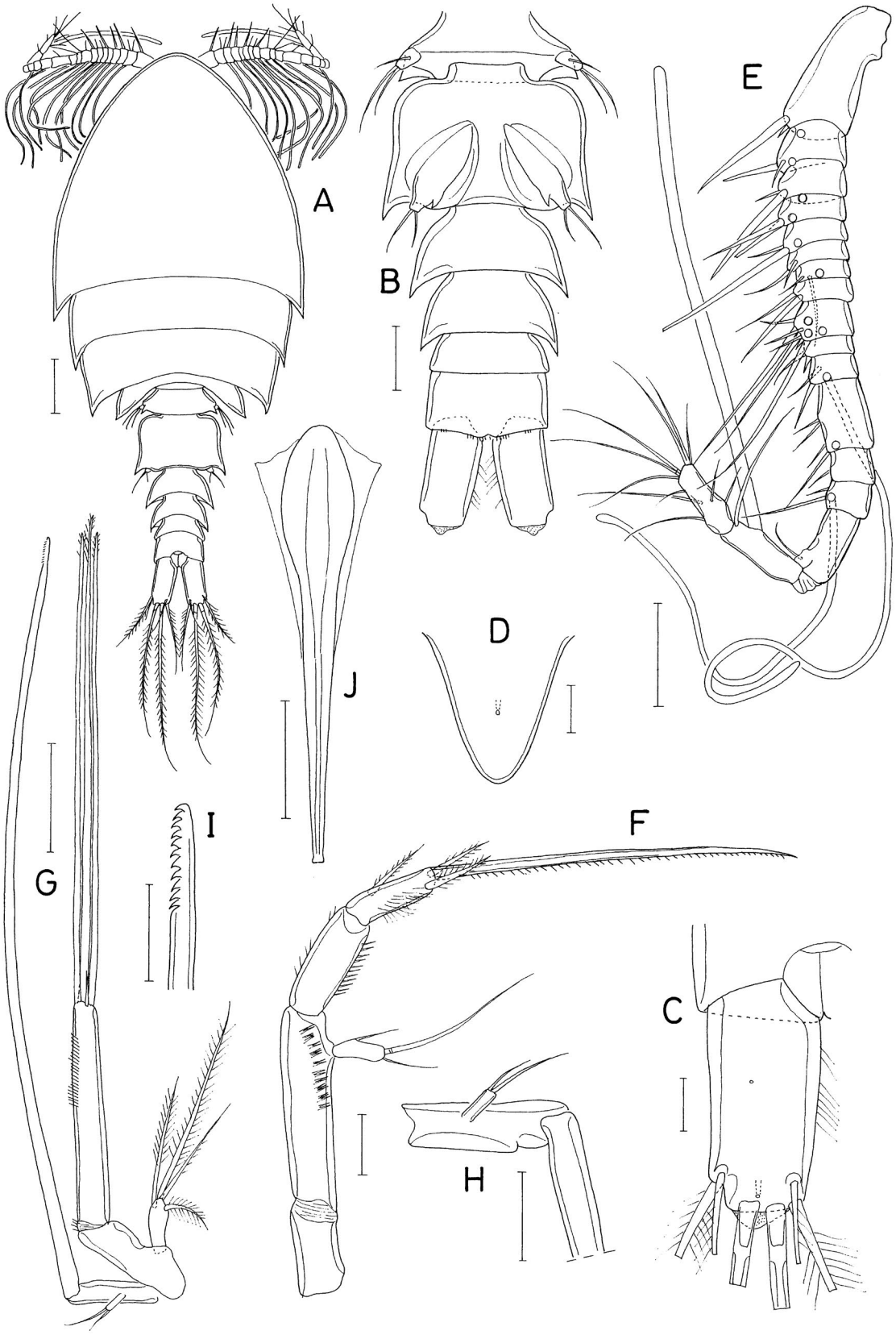


Fig. 14. *Thermocheres viridis* n. sp., male. A, habitus, dorsal; B, urosome, ventral; C, left caudal ramus, dorsal; D, rostrum; E, antennule; F, antenna; G, mandible and maxillule; H, proximal part of mandible; I, distal part of mandible; J, oral siphon. Scale bars: A, J, 0.1 mm; B, E, G, 0.05 mm; C, D, F, H, 0.02 mm; I, 0.01 mm.

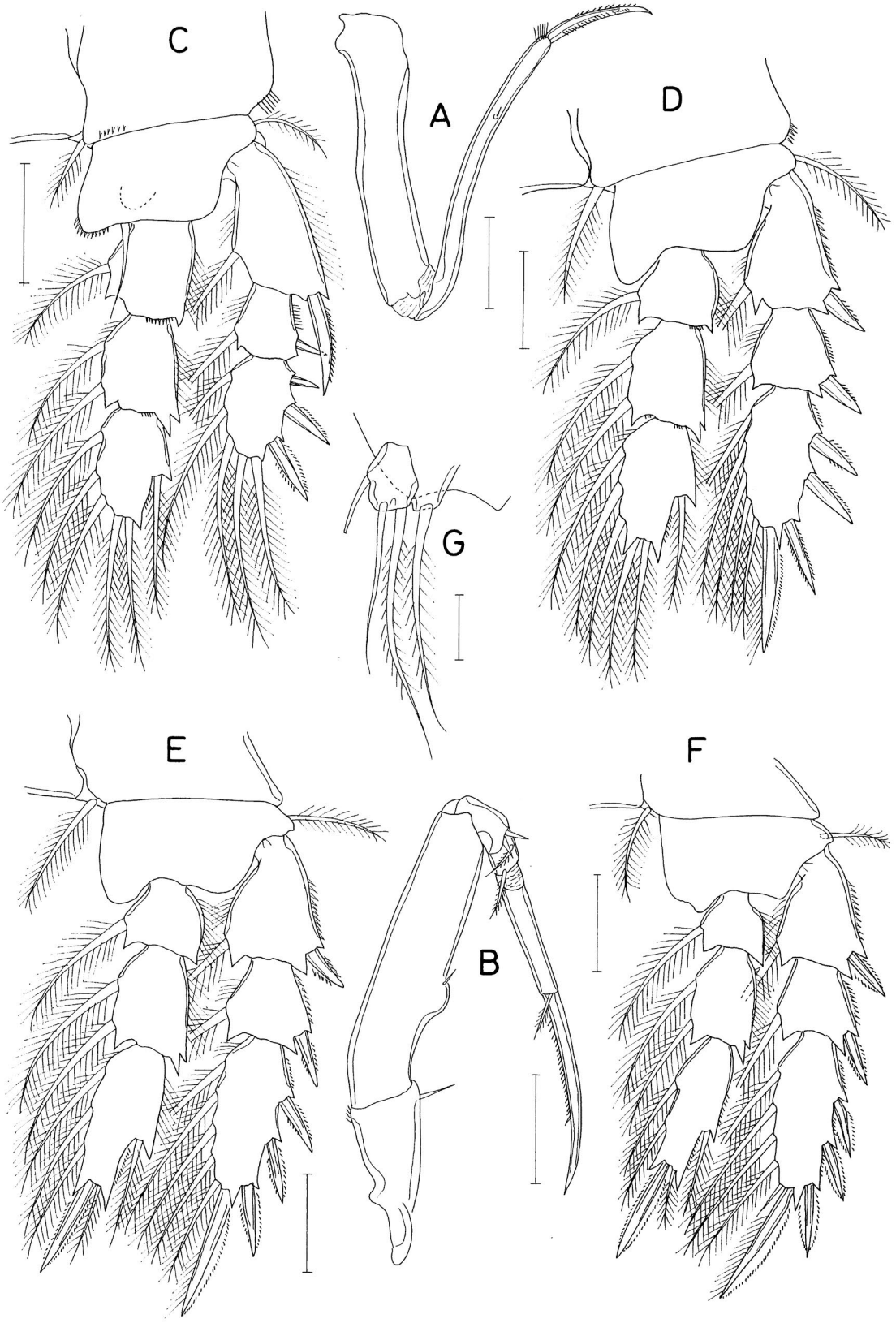


Fig. 15. *Thermocheres viridis* n. sp., male. A, maxilla; B, maxilliped; C, leg 1; D, leg 2; E, leg 3; F, leg 4; G, leg 5. Scale bars: A–F, 0.05 mm; G, 0.02 mm.

seta on third exopodal segment. Armature formula for legs 1–4 as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-1	I-1; I-1; III, 2, 3	0-1; 0-2; 1, 2, 3
Leg 2	0-1	1-0	I-1; I-1; III, I, 5	0-1; 0-2; 1, 2, 3
Leg 3	0-1	1-0	I-1; I-1; III, I, 5	0-1; 0-2; 1, 1 + I, 3
Leg 4	0-1	1-0	I-1; I-1; III, I, 5	0-1; 0-2; 1, 1 + I, 2

Leg 5 (Fig. 15G) consisting of pinnate dorsolateral seta of fifth pedigerous somite and small, free exopod; exopodal segment 1.24 times longer than wide (21 × 17 μm), bearing 3 setae of unequal lengths, longest one pinnate, others naked. Leg 6 (Fig. 14B) represented by 2 small distal setae and 1 subdistal cusp on genital operculum.

Female. Unknown.

Remarks. The genus *Thermocheres* includes two species, *T. validus* Kim, 2010, known from Madagascar, and *T. pacificus* Lee, Chang and Kim, 2022 from Korea (Kim, 2010; Lee *et al*., 2022). Although differences between species of *Thermocheres* are slight, *T. viridis* n. sp. is distinguishable from the two congeners: (1) by the one-segmented mandibular palp tipped with two setae, as opposed to the palp represented by a vestigial seta in *T. validus* or the absence of a palp in *T. pacificus*; (2) by possessing the rudimentary inner distal seta on the third endopodal segment of leg 4, as opposed to the seta well-developed seta in *T. validus* or the reduced size in *T. pacificus*; and (3) by having a rounded distal apex of the rostrum, in contrast to the acutely pointed, beak-like apex in *T. validus* or the angular apex in *T. pacificus*. Additionally, the new species features one spine plus four setae (formula 1, 1 + I, 2) on the third endopodal segment of leg 4, further distinguishing it from *T. pacificus* (formula 1, I, 2 in the latter species).

Family Dinopontiidae Murnane, 1967
Genus *Stenopontius* Murnane, 1967

***Stenopontius brevicauda* n. sp. (Figs. 16–18)**
https://zoobank.org/urn:lsid:zoobank.org:act:1B23B07E-6276-46BB-AFBE-2A09695621E2

Type material. Holotype (intact ♀; HNIBR IV7486), intact paratypes (2 ♀♀; HNIBR IV7487), and dissected paratypes (2 ♀♀, 1 ♂) from washings of mixed species of sponges, Ullung Island (37°31′31.98″N, 130°56′17.35″E), SCUBA, depth 32 m, collected by Tae Won Jung, 28 November 2022. The holotype and intact paratypes have been deposited in the Honam National Institute of Biological Resources (HNIBR), Mokpo. The dissected paratypes are retained in the collection of I.-H. Kim.

Etymology. The name, a noun, derives from Latin *brev* (= short) and *cauda* (= tail), referring to the short caudal rami of the new species.

Female. Body (Fig. 16A) narrow. Body length 536 μm in figured and described specimen. Prosome elliptical, 307 μm long. Cephalothorax 175 × 190 μm. Cephalothorax and first pedigerous somites with angular posterolateral corners; third and fourth pedigerous somites with rounded lateral corners. Fourth pedigerous somite with deeply concave posterodorsal margin. Urosome (Fig. 16B) 5-segmented. Genital double-somite 1.7 times longer than wide (95 × 56 μm), widest at proximal 36% region, followed by concave lateral margins; genital apertures indistinct, positioned dorsolaterally at widest region. Three free abdominal somites 35 × 46, 33 × 43, and 18 × 43 μm, respectively. Anal somite (Fig. 16C) characteristically short. Caudal rami (Fig. 16C) widely divergent, widely separated from each other; each ramus 0.94 times longer than wide (16 × 17 μm), quadrangular, armed with 6 setae; setae II, III, and VII naked, other 3 setae pinnate; inner and outer margins naked or ornamented with few spinules. Egg sac (Fig. 16A) 243 × 125 μm, containing single large egg.

Rostrum absent. Antennule (Fig. 16D) 132 μm long, 10-segmented; armature formula 0, 1, 6, 4, 3, 3, 1, 0, 1, and 11 + 2 aesthetascs; third segments with incomplete articulation on ventral surface; terminal segment longest, occupying 40% length of antennule, its proximal major aesthetasc extending slightly over distal end of segment, constricted at region slightly distal to middle; all setae naked. Antenna (Fig. 16E) slender, 5-segmented; coxa (first segment) unarmed and unornamented; basis (second segment) with 2 small setae subdistally representing exopod, and ornamented with minute spinules along outer margin; armature formula of endopod 0, 1, and 1 + spine; terminal spine straight, as long as sum of 3 endopodal segments; first and third endopodal segment with setules on outer margin.

Oral cone (Fig. 16F) stout, 84 μm long, with truncate distal tip. Mandible (Fig. 16G) represented by attenuated stylet of 80 μm long, lacking palp; stylet acutely pointed at tip, lacking any visible tooth. Maxillule (Fig. 16H) bilobed, each lobe with 3 setae distally; inner lobe with spinules on outer margin, its 3 distal setae pinnate, subequal in length, innermost one 45 μm long. Maxilla (Fig. 16I) with smooth proximal segment; distal segment slender, arched, bearing row of spinules in subdistal region. Maxilliped (Fig. 16J) 5-segmented; armature formula 0. 0. 2, 1, and 1 + claw; articulation between first and second segments incomplete; second segment with minute, rudimentary cusp on middle of inner margin; second endopodal segment 18 μm long, with inflated outer margin, with few spinules at apical region of outer margin; third endopodal segment 22 μm long; terminal claw straight, spiniform, 58 μm long, with few spinules on inner margin.

Legs 1–4 (Fig. 17A–D) with 2-segmented protopod and 3-segmented rami; inner coxal seta absent in leg 1 but pre-

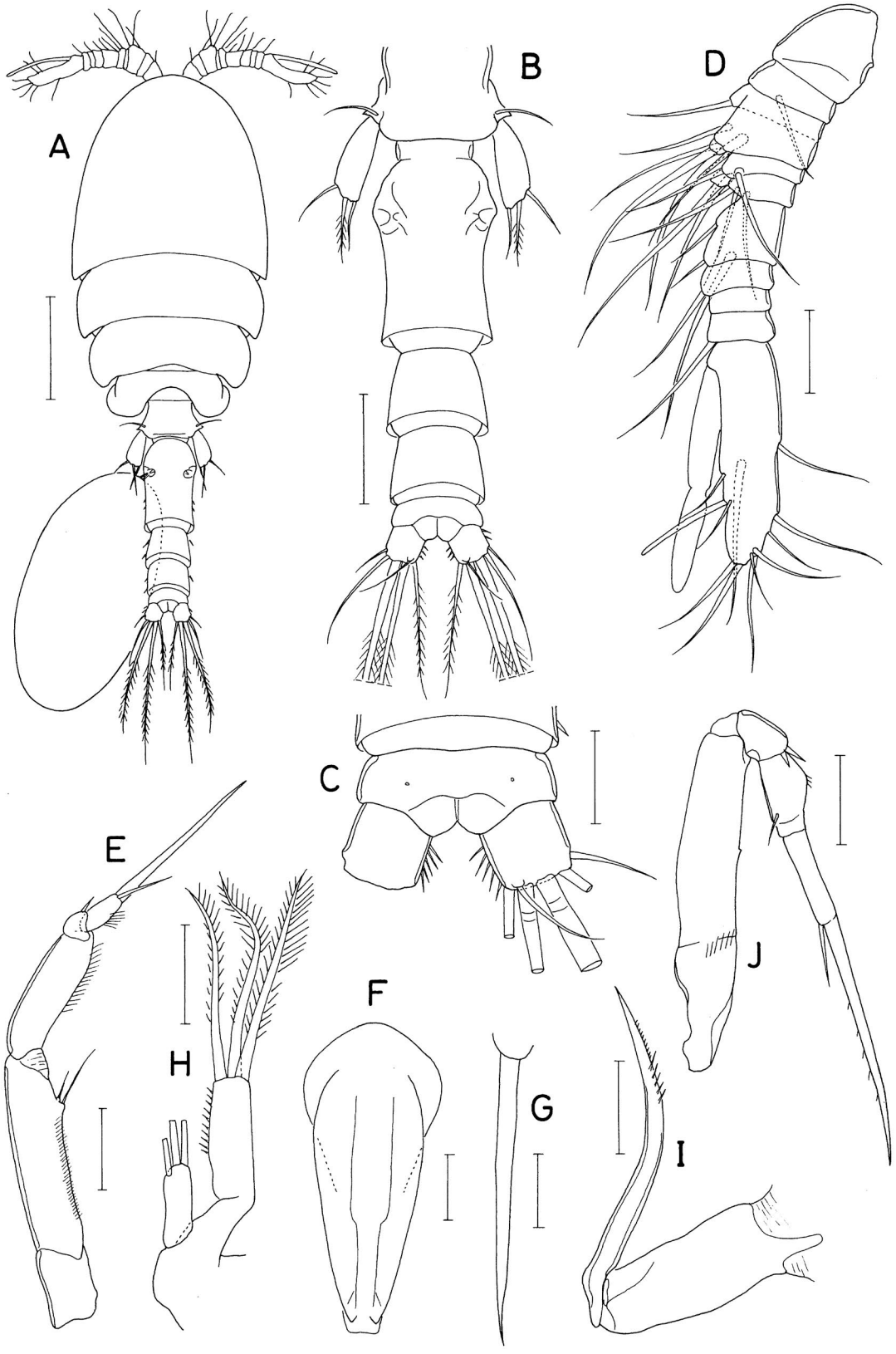


Fig. 16. *Stenopontius brevicauda* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, anal somite and caudal rami, dorsal; D, antennule; E, antenna; F, oral cone; G, mandible; H, maxillule; I, maxilla; J, maxilliped. Scale bars: A, 0.1 mm; B, 0.05 mm; C–J, 0.02 mm.

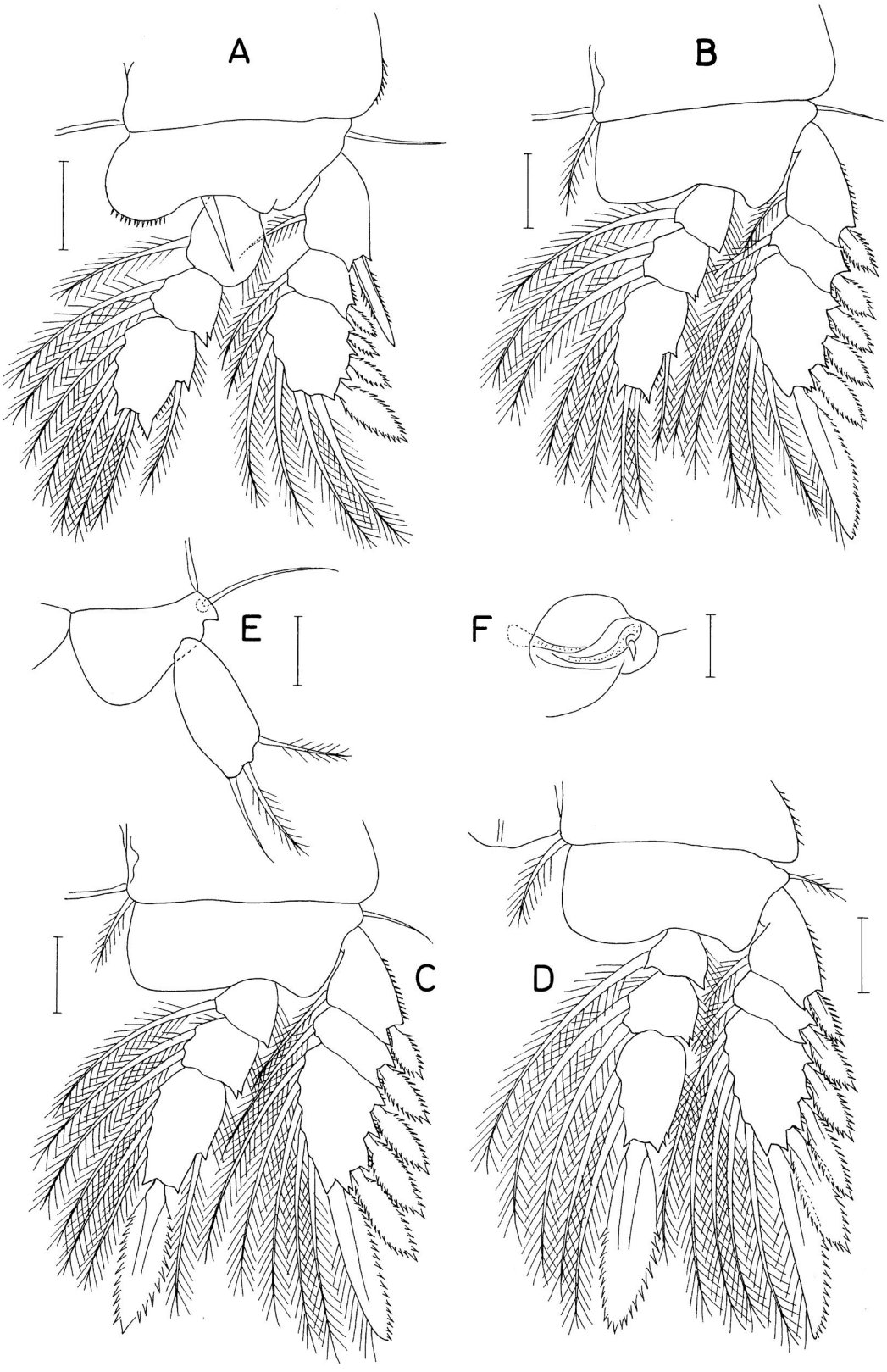


Fig. 17. *Stenopontius brevicauda* n. sp., female. A, leg 1; B, leg 2; C, leg 3; D, leg 4; E, left leg 5, ventral; F, genital aperture. Scale bars: A–E, 0.02 mm; F, 0.01 mm.

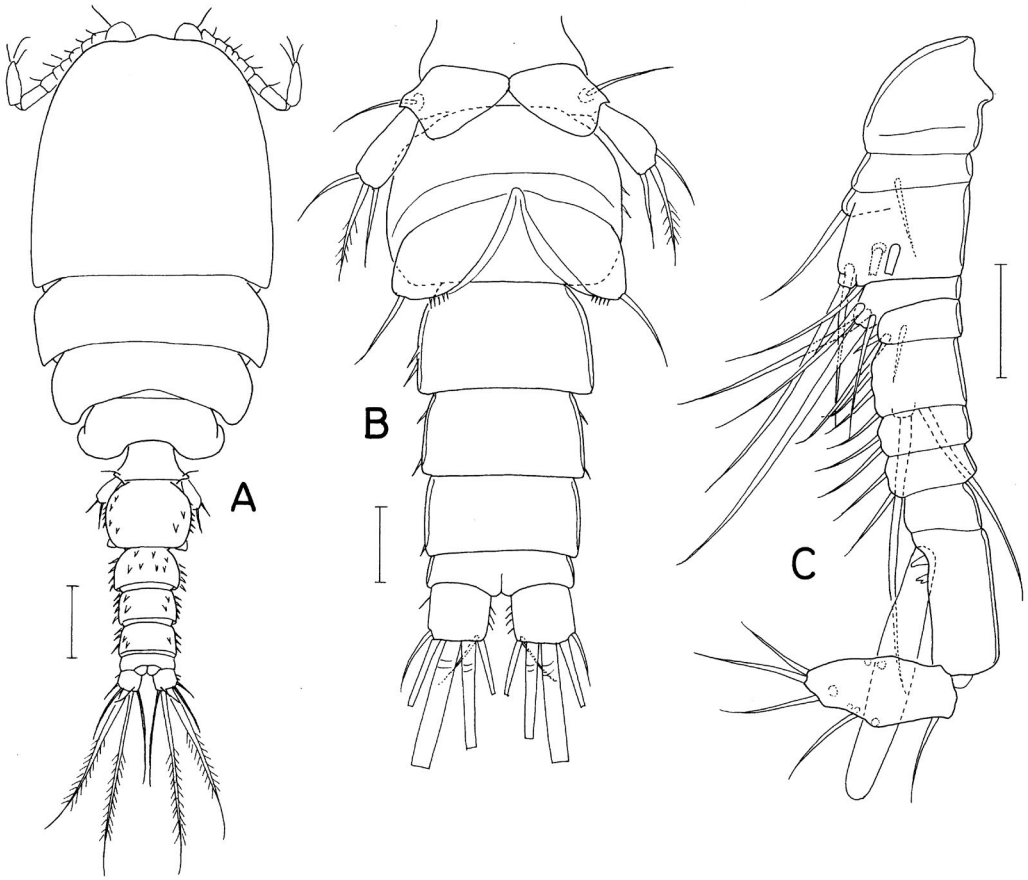


Fig. 18. *Stenopontius brevicauda* n. sp., male. A, habitus, dorsal; B, urosome, ventral; C, antennule. Scale bars: A, 0.05 mm; B, C, 0.02 mm.

sent in legs 2–4. Basis of leg 1 with spiniform inner distal seta and spinules on inner distal margin. Most of spines, especially those of legs 2–4 broad, leaf-like. Second endopodal segment of legs 1–4 with bicuspid outer distal corner. Third exopodal segment of legs 2 and 3 also with bicuspid outer distal process. Third endopodal segment of leg 4 with 2 denticles at subdistal region of outer margin. Armature formula for legs 1–4 as follows:

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

Leg 5 (Fig. 17E) 2-segmented; protopod (proximal segment) wider than long (27 × 40 μm), articulated from somite, bearing 1 naked dorsolateral seta and pointed lateral apex; exopodal segment 2.29 times longer than wide (39 × 17 μm), broadening distally, with 1 subdistal pinnate outer seta and 2 distal setae (naked inner seta and pinnate outer one). Leg 6 (Fig. 17F) probably represented by 1 small spinule on genital operculum.

Male. Body (Fig. 18A) similar in form to that of female. Body length 462 μm. Prosome 282 μm long. Cephalothorax 170 × 173 μm, with almost parallel lateral margins. Urosome (Fig. 18B) 6-segmented. Genital somite 47 × 63 μm, nearly quadrangular; genital operculum with several minute spinules on subdistal inner margin and tipped with 1 seta. Four abdominal somites 28 × 47, 23 × 44, 22 × 41, and 12 × 40 μm, respectively. Caudal ramus 15 × 16 μm, armed as in female.

Rostrum absent. Antennule (Fig. 18C) 11-segmented, geniculate between penultimate and terminal segments; armature formula for first to penultimate segments 0, 1, 6, 4, 3, 5, 2, 2, 0, 2 + aesthetasc; armature of terminal segment uncertain due to detachment of armature elements. Antenna as in female.

Oral siphon, mandible, maxillule, maxilla, maxilliped, and legs 1–4 not different from those of female. Leg 5 (Fig. 18B) armed as in female, but outer subdistal seta on exopod naked. Leg 6 (Fig. 18B) represented by 1 naked seta tipped on distal apex of genital operculum.

Remarks. The genus *Stenopontius* comprises four species associated with sponges in shallow waters: *S. boxshalli* Malt, 1991 and *S. parvus* Boxshall, 1990, both document-

ed in Hong Kong (Boxshall, 1990; Malt, 1991); *S. humesi* Murnane, 1969, from the Atlantic coast of North America (Murnane, 1967); and *S. spinulatus* Kim, 2010 from Madagascar (Kim, 2010). *Stenopontius brevicauda* n. sp. can be distinguished from its four congeners by its elongate genital double-somite, which is approximately 1.7 times longer than it is wide (in contrast, in the four congeners, the double-somite is wider than long or as long as wide), by its short caudal rami, which are wider than long (whereas the caudal rami in the four congeners are significantly longer than wide), by having three setae on the outer lobe of the maxillule (compared to two setae in the four congeners), and by having an inner seta on the coxa of legs 2−4 (unlike the absence of an inner seta in all legs 1−4 of the congeners).

***Stenopontius alatus* n. sp. (Figs. 19, 20)**

https://zoobank.org/urn:lsid:zoobank.org:act:3E665D8C-5CDF-407B-A8FE-BFAEDC3AD997

Type material. Holotype (♀, dissected and mounted on a slide; MABIK CR00257790) from washings of dredged invertebrates collected from the Korea Strait (33°13′ 39.4″N, 126°33′48.2″E), at a depth of 127 m, collected by Jimin Lee, on 17 March 2024. The holotype has been deposited in the National Marine Biological Institute of Korea (MABIK), Seocheon.

Etymology. The name is derived from the Latin *alat* (= winged), referring to the wing-like lateral expansions of the genital double-somite and first abdominal somite.

Female. Body (Fig. 19A) small, narrow, slightly dorsoventrally depressed. Body length 455 μm. Prosome with parallel lateral margins, 239 × 155 μm. Cephalothorax 134 μm long, wider than long. Fourth pedigerous somite with irregularly curved posterior margin. Urosome (Fig. 19B) 5-segmented. Fifth pedigerous somite narrow, 60 μm wide. Genital double-somite 85 × 126 μm, peculiarly consisting of narrow anterior 40% (68 μm wide at this part) and greatly broadened, wing-like posterior 60%, with produced, angular posterolateral corners; genital apertures positioned dorsolaterally at posterior region of narrow anterior part. First abdominal somite 34 × 85 μm, with wing-like, posterolateral expansions. Second abdominal somite 22 × 56 μm, posterolaterally expanded. Anal somite (Fig. 19C) 27 × 43 μm, with angular posterolateral corners and large anal operculum; anal operculum covering proximal part of caudal rami. Caudal ramus (Fig. 19C) 1.56 times longer than wide (25 × 16 μm), broadening distally, with pointed posterolateral and posteromedial corners, armed with 6 short setae; dorsal seta (seta VII) naked, other setae pinnate.

Rostrum absent. Antennule (Fig. 19D) 12-segmented; armature of proximal segments uncertain, due to damage of setae; armature of distal 3 segments 3, 1, and 10 + aesthetasc; terminal segment longest; ninth segment distinctly narrower than other segments. Antenna (Fig. 19E) consisting of 4 segments and terminal claw; coxa short, unarmed; basis 33 μm long; exopod represented by 2 subequal, naked setae located at about distal third of outer margin of basis; first endopodal segment (third segment) unarmed, 30 μm long; second endopodal segment 12 μm long, bearing 3 small setae; terminal claw smooth, strongly curved at distal 80% region, with acutely pointed distal tip.

Oral siphon (Fig. 19F) 98 × 34 μm, gradually narrowing distally, with truncate distal apex. Mandible (Fig. 19G) represented by straight, needle-like stylet of 84 μm long; palp absent. Maxillule (Fig. 19G) bilobed; small outer lobe 10 μm long, tipped with 2 equal, naked setae, both of them 38 μm long; inner lobe about 30 μm long, tipped with 3 subequal, naked setae, longest middle one of them 59 μm long. Maxilla (Fig. 19H) 2-segmented; proximal segment (syncoxa) 30 μm long, smooth; distal segment (basis) 60 μm long, bearing spinules and setules on convex margin. Maxilliped (Fig. 19I) 5-segmented; armature formula 0, 0, 0, 2, and 1 + claw; lengths of second segment (basis) to terminal claw 42, 9, 30, 24, and 32 μm, respectively.

Legs 1−4 (Fig. 20A−D) with 3-segmented rami; coxa lacking inner seta. Intercoxal plate of legs 2−4 sparsely covered with spinules. Inner margin of basis of legs 1−4 protruded, that of legs 1−3 with fine spinules. First exopodal segment of leg 1 lacking inner seta. Outer and distal setae on third endopodal segment of leg 1 naked. Inner seta on first exopodal segment of legs 2−4 short. Second endopodal segment of legs 1−4 with bicuspid outer distal corner. Armature formula for legs 1−4 as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-0	1-I	I-0; I-1; II, 2, 3	0-1; 0-2; 1, 2, 3
Leg 2	0-0	1-0	I-1; I-1; III, I, 5	0-1; 0-2; 1, 2, 3
Leg 3	0-0	1-0	I-1; I-1; III, I, 5	0-1; 0-2; 1, I, 3
Leg 4	0-0	1-0	I-1; I-1; III, I, 5	0-1; 0-2; 0, I, 2

Leg 5 (Fig. 19J) consisting of protopod and exopod; protopod distinctly articulated from somite, bearing 1 pinnate seta on outer margin; exopodal segment oval, 1.42 times longer than wide (51 × 36 μm), bearing 3 small, naked setae on distal margin; lengths of these setae 25, 9, and 14 μm, respectively, from inner to outer. Leg 6 (Fig. 19K) represented by 1 seta and 1 spinules on genital operculum.

Male. Unknown.

Remarks. The new species is clearly distinguishable from other species of *Stenopontius* by the unique shape of the female urosome, featuring wing-like lateral expansions on the genital double-somite and the first abdominal somite. These are distinctive key characteristics of the new species.

The armature condition (two spines plus five setae) on the third exopodal segment of leg 1 is also unique with-

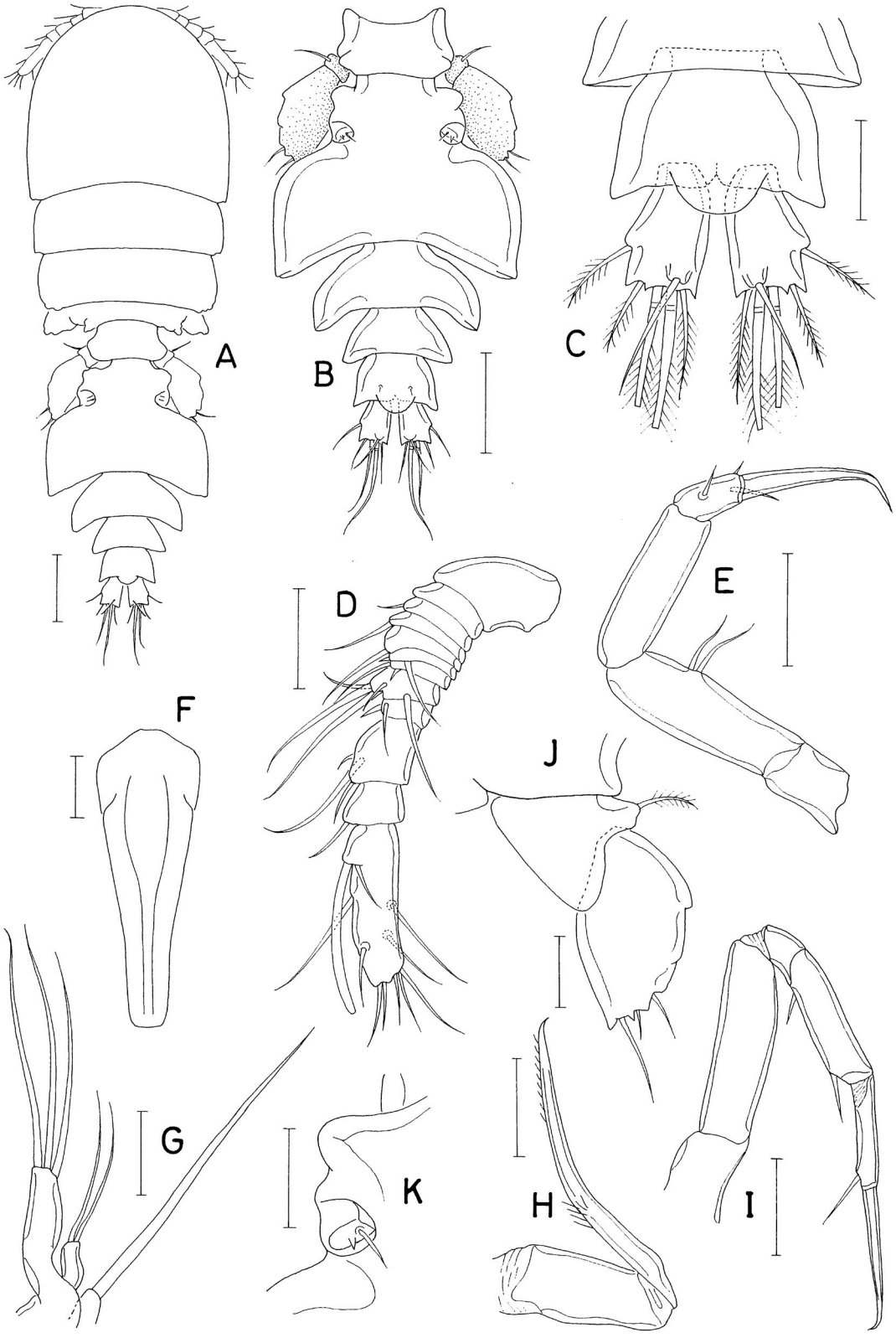


Fig. 19. *Stenopontius alatus* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, anal somite and caudal rami, dorsal; D, antennule; E, antenna; F, oral siphon; G, mandible and maxillule; H, maxilla; I, maxilliped; J, leg 5; K, left genital aperture, dorsal. Scale bars: A, B, 0.05 mm; C–K, 0.02 mm.

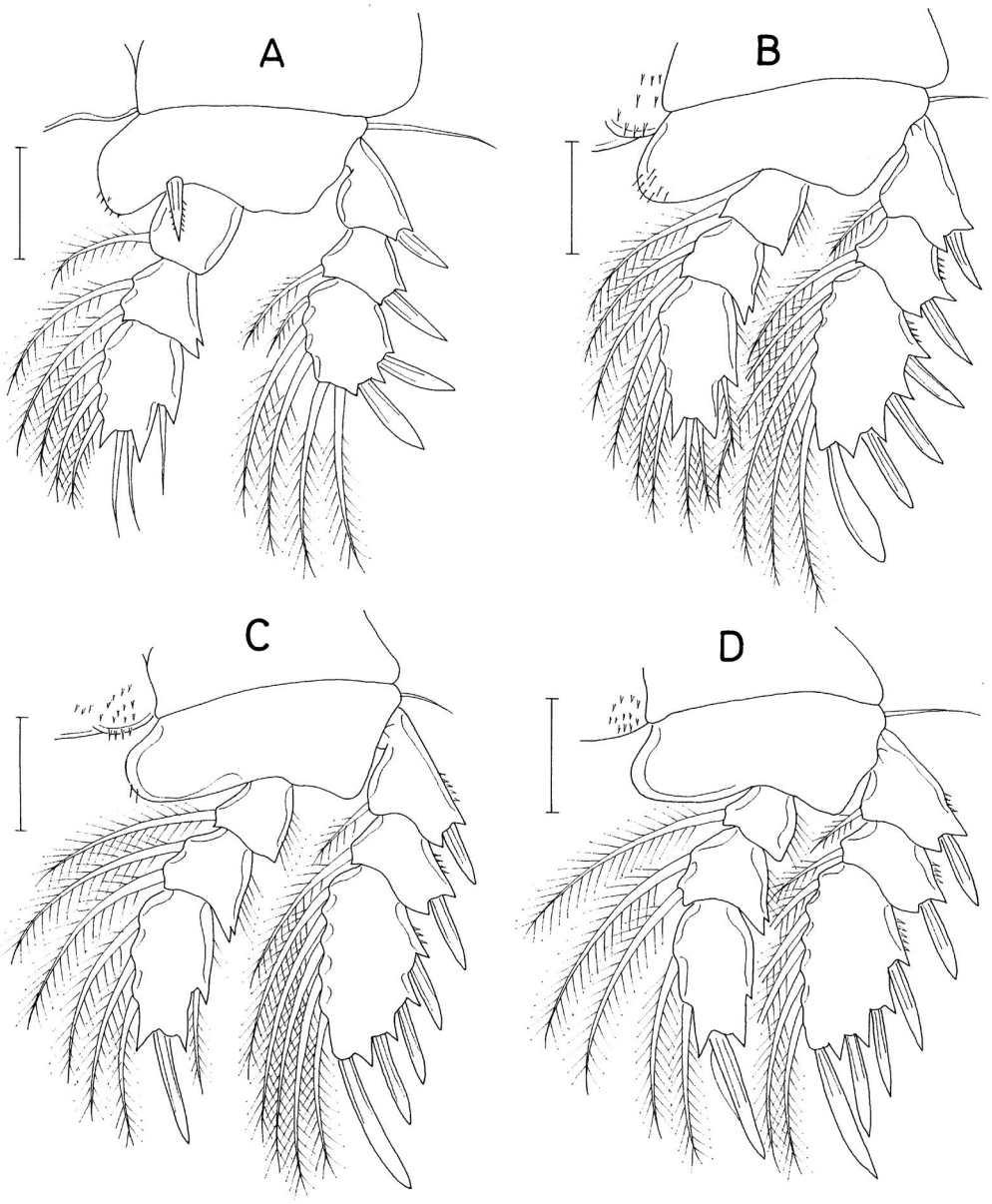


Fig. 20. *Stenopontius alatus* n. sp., female. A, leg 1; B, leg 2; C, leg 3; D, leg 4. Scale bars: 0.02 mm.

in the genus, as it differs from the condition present in *S. boxshalli* and *S. humesi* or compared to three spines plus five setae in *S. parvus* and *S. brevicauda* n. sp., or three spines plus four setae in *S. spinulatus* Kim, 2010 (Marnane, 1967; Boxshall, 1990; Malt, 1991; Kim, 2010).

Family Entomolepididae Brady, 1899
Genus *Entomopsyllus* McKinnon, 1988

***Entomopsyllus aplanatus* n. sp. (Figs. 21–23)**
https://zoobank.org/urn:lsid:zoobank.org:act:2A265E6C-B851-4207-860E-4DD6E5B96470

Type material. Holotype (intact ♀; MABIK CR00257791), intact paratypes (20 ♀♀, 2 ♂♂; MABIK CR00257792), and dissected paratypes (2 ♀♀, 2 ♂♂) from washings of mixed species of sponges, Munseom, Seogwipo, Jeju Island (33°13′39.4″N, 126°33′48.2″E), SCUBA, at a depth of 56 m, collected by Taekjun Lee, on 25 April 2023. The holotype and intact paratypes have been deposited in the National Marine Biodiversity Institute of Korea (MABIK), Seocheon. The dissected paratype is retained in the collection of I.-H. Kim.

Etymology. The specific name is derived from the Latin *aplanat* (= flattened), referring to the flattened body of the new species.

Female. Body (Fig. 21A) extremely flattened dorsoventrally, oval in dorsal view. Mean body length (prosomal length) 1.19 mm (1.13–1.26 mm), based on 10 specimens. Body of figured and described specimen 1.22 mm long. Maximum width 0.84 mm. Prosome covering whole body, except distal part of caudal rami, consisting of cephalothorax and second to fourth pedigerous somites; lengths of these 4 somites 606, 115, 76, and 436 μm, respectively, measured along midline. Urosome (Fig. 21B) 3-segmented, consisting of genital complex and 2-segmented abdomen. Fifth pedigerous somite completely fused with genital double-somite to form genital complex. Genital complex 170 × 132 μm, consisting of broader proximal 47% and narrower remaining part; genital apertures positioned dorsolaterally at about proximal third of genital complex; ventrodistal part of genital complex bearing pair of oblique rows of spinules on each side (Fig. 21C). Two free abdominal somites 80 × 75 and 90 × 75 μm, respectively. Caudal rami widely separated from each other; each ramus (Fig. 21D) 2.96 times longer than wide (77 × 26 μm), armed distally with 6 setae and ornamented with thin setules on inner margin; setae II and VII naked, tipped on digitiform process; setae III and VI unilaterally pinnate; 2 median terminal setae (setae IV and V) bipinnate.

Rostrum absent. Antennule (Fig. 21E) slender, 17-segmented; first, third, sixth, fifteenth, and terminal segments bearing 1 seta, 8 setae, 7 setae, 2 setae + aesthetasc, and 11 setae, respectively; other segments bearing 2 setae each; all setae naked; aesthetasc on fifteenth segment thin; first segment longest, and third segment second longest. Antenna (Fig. 21F) consisting of coxa, basis, 1-segmented exopod, and 2-segmented endopod; coxa short, unarmed; basis unarmed but with longitudinal row of several denticles on proximal region; exopodal segment elongate, 71 μm long, longer than first endopodal segment, with 1 distal and 1 subdistal small setae and setules on margins; first endopodal segment 64 μm long, unarmed but ornamented with minute, distally bifurcate spinules in distal half of segment; second endopodal segment 33 μm long, bearing 4 setae (1 proximal, 1 subdistal, and 2 small, distal) and setules on all surfaces and tipped with spiniform claw of 64 μm long.

Oral siphon (Fig. 21G) consisting of short, expanded proximal part and long, slender distal part, extending to posterior margin of genital double-somite. Mandible (Fig. 21H) consisting of fibril-like stylet and slender palp; palp 2-segmented, proximal segment 45 μm long, distal segment arched, 77 μm long, setulose along convex margin, tipped with 2 unequal, naked setae (85 and 38 μm long, respectively). Maxillule (Fig. 21I) bilobed; outer lobe 64 μm long, spinulose along outer margin, tipped with 4 thin, naked setae; inner lobe 26 μm long, obscurely defined at base, tipped with 3 very unequal, thin setae (750, 197, and 68 μm long, respectively). Maxilla (Fig. 22A) stout; proximal segment with protruded outer margin proximally; distal segment strongly curved at subdistal region, bearing several spinules near distal third. Maxilliped (Fig. 22B) 6-segmented; armature formula 1, 1, 1, 2, 1, and 1 + claw; syncoxa with spinules at outer subdistal region; basis 152 μm long, bearing several minute spinules on outer margin and rudimentary inner seta; terminal segment 50 μm long, bearing patch of spinules in distal half; terminal claw 65 μm long.

Legs 1–3 (Fig. 22C–E) with 3-segmented rami. Leg 4 (Fig. 22F) with 3-segmented exopod, without endopod. Rami of legs slender. Inner coxal seta present in leg 1, but absent in legs 2–4. Basis of leg 1 with filiform inner distal seta and row of thin spinules near base of seta. Endopod of leg 1 arched, extremely slender, much longer than exopod. Outer distal corner of second endopodal segment of leg 3 bicuspid, but monocuspid in legs 1 and 2. Armature formula for legs 1–4 as follows:

	Coxa	Basis	Exopod	Endopod
Leg 1	0-1	1-1	I-1; I-1; I, I, 4	0-1; 0-2; 0, 5
Leg 2	0-0	1-0	I-1; I-1; III, I, 4	0-1; 0-2; 0, 2, 2
Leg 3	0-0	1-0	I-1; I-1; III, I, 4	0-1; 0-1; 0, 1, 1
Leg 4	0-0	1-0	I-1; I-1; III, I, 4	lacking

Leg 5 (Fig. 21B) consisting of short protopod and elongated exopod; protopod not articulated from genital complex, with 1 small dorsodistal seta; exopodal segment arched, 173 × 17 μm, with 1 subdistal and 2 distal setae. Leg 6 (Fig. 21J) represented by 2 small, naked setae on genital operculum.

Male. Body (Fig. 23A) oval, 764 μm long. Prosome segmented as in female. Urosome (Fig. 23B) consisting of genital complex and 3-segmented abdomen. Genital complex roughly quadrate, wider than long. Anal somite longer than anterior abdominal somites. Caudal ramus 1.64 times longer than wide (36 × 22 μm), armed as in female.

Antennule 14-segmented, not geniculate; armature formula 1, 2, 8, 2, 2, 7, 2, 2, 4, 2, 2, 4, 4 + aesthetasc, and 11. Antenna as in female.

Oral siphon, mandible, maxillule, maxilla, maxilliped, and legs 1–4 not different from those of female. Leg 5 (Fig. 23B) exopodal segment 62 μm long, much shorter than that of female. Leg 6 (Fig. 23B) represented by 1 seta on distal apex of genital operculum.

Remarks. Currently, the genus *Entomopsyllus* includes five known species: *E. adriae* (Eiselt, 1959), *E. nicholsi* McKinnon, 1988, *E. stocki* Kim, 2004, *E. brevicaudatus* Lee & Kim, 2017, and *E. takara* Uyeno & Johnsson, 2018. Among these, *E. aplanatus* n. sp. is most closely related to *E. brevicaudatus*, as both species exhibit a 17-segmented female antennule, four setae on the third endopodal segment of leg 2, four spines plus four setae (formula III, I, 4) on the third exopodal segment of leg 3, and two setae

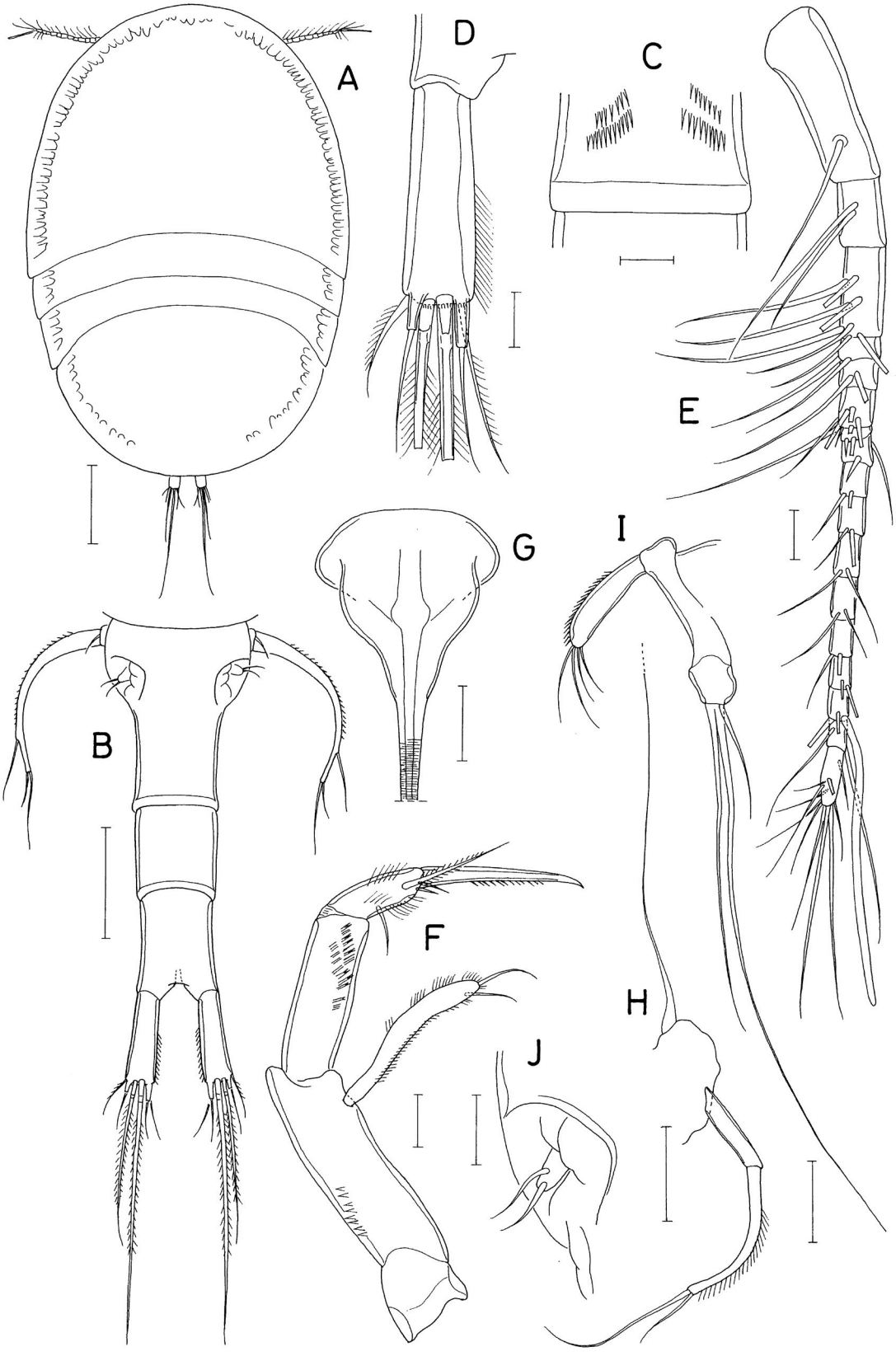


Fig. 21. *Entomopsyllus aplanatus* n. sp., female. A, habitus, dorsal; B, urosome, dorsal; C, distal part of genital complex, ventral; D, left caudal ramus, dorsal; E, antennule; F, antenna; G, proximal part of oral siphon; H, mandible; I, maxillule; J. left genital aperture, dorsal. Scale bars: A, 0.2 mm; B, 0.1 mm; C–F, J, 0.02 mm; G–I, 0.05 mm.

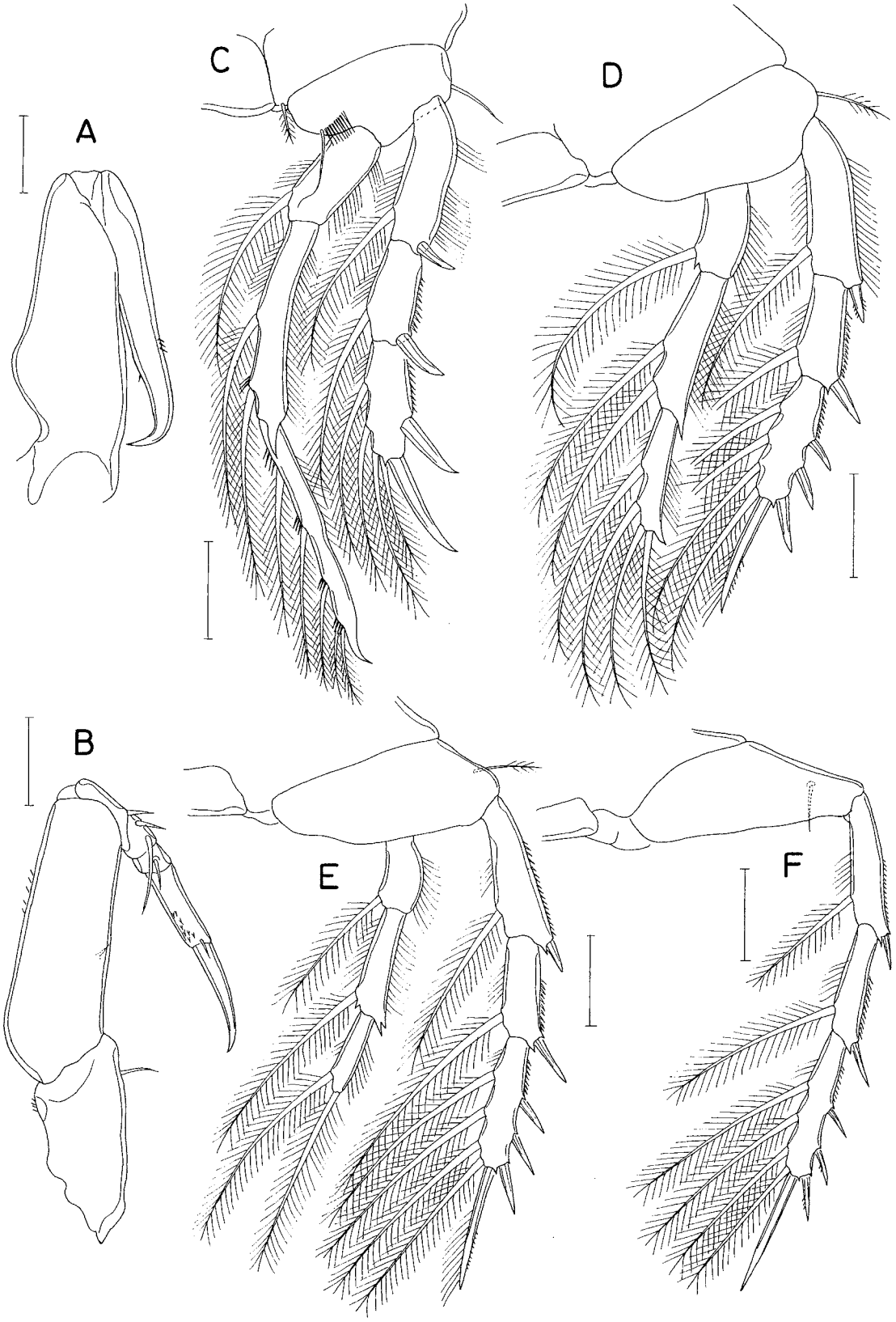


Fig. 22. *Entomopsyllus aplanatus* n. sp., female. A, maxilla; B, maxilliped; C, leg 1; D, leg 2; E, leg 3; F; leg 4. Scale bars: 0.05 mm.

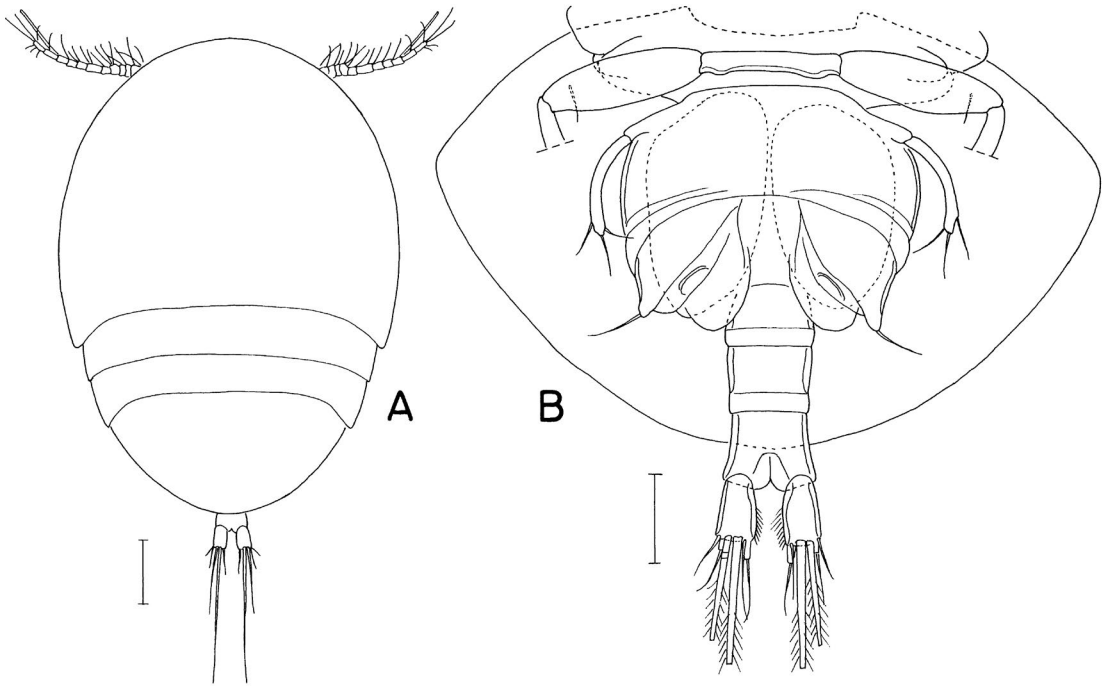


Fig. 23. *Entomopsyllus aplanatus* n. sp., male. A, habitus, dorsal; B, fourth pedigerous somites and urosome, ventral. Scale bars: A, 0.1 mm; B, 0.05 mm.

on the third endopodal segment of leg 3. However, *E. aplanatus* n. sp. differs from *E. brevicaudatus* by having four spines plus four setae (formula III, I, 4, rather than II, I, 4 as in *E. brevicaudatus* and other species) on the third exopodal segment of leg 2, and similarly on the third exopodal segment of leg 4. In the original description of *E. brevicaudatus*, Lee & Kim (2017) noted that the anal somite is unornamented. Therefore, the presence in *E. aplanatus* n. sp. of two pairs of rows of spinules on the ventral surface of the anal somite marks an additional distinctive difference from *E. brevicaudatus*.

Conflicts of Interest

The authors of this paper have no affiliations with any conflicting interests and are solely responsible for the contents of this paper.

Acknowledgements

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Three new records of bdelloid rotifers from Korea

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A taxonomic study on bdelloid rotifers collected from various terrestrial habitats such as mosses, mushrooms and tree barks at two different locations in Korea resulted in three new Korean records belonging to three genera in two families, Habrotrochidae and Philodinidae, of bdelloid rotifers: *Habrotrocha minima* De Koning, 1947, *Mniobia barbatula* Donner, 1950 and *Scepanotrocha parva* Schulte, 1954. These three new records are rare species with poorly known distributions, and this study is only the second or third report since their original descriptions. In the genus *Scepanotrocha* Bryce, 1910, 11 taxa are known to the world, and in Korea, three species including *S. parva* have been recorded so far. A taxonomic key to the *Scepanotrocha* species reported from Korea to date is provided here. In addition, partial sequences of mitochondrial cytochrome c oxidase subunit 1 gene (CO1) for *Mniobia barbatula* and *Scepanotrocha parva* are presented.

Keywords: Bdelloidea, Korea, new records, taxonomy, terrestrial habitats

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Introduction

Bdelloids sorted out from various terrestrial habitats such as mosses, mushrooms and tree barks were taxonomically investigated from May 20, 2023 to May 11, 2024. Three species belonging to three genera in two families, Habrotrochidae and Philodinidae, were identified: *Habrotrocha minima*, *Mniobia barbatula* and *Scepanotrocha parva*. These three bdelloids are rare species with poorly known distributions, and new to Asia as well. *Habrotrocha minima* has previously been reported only from Austria (Donner, 1962) and Czech Republic (Devetter *et al*., 2020) since the description from the Netherlands by De Koning (1947). *Mniobia barbatula* has been recorded from only two countries, Czech Republic (Devetter *et al*., 2020) and Italy (Melone *et al*., 1998), after the description from Austria by Donner (1950). *Scepanotrocha parva* was reported only from Czech Republic (Devetter *et al*., 2020) after description from Germany (Schulte, 1954). In the genus *Scepanotrocha*, 11 taxa have been described so far (Jersabek and Leitner, 2013), and the main diagnostic characteristic of this genus is the upper lip that is usually expanded like a hood and covers the corona fully or partially (Donner, 1965). In Korea, *Scepanotrocha corniculata* Bryce, 1910 and *S. simplex* De Koning, 1947 were reported before *S. parva* (Song and Lee, 2021). In *Scepanotrocha corniculata* and *S. parva*, the hood covers corona completely, while the hood covers slightly more than half of corona in *S. simplex* (Schulte, 1954; Song and Lee, 2021).

The result of the present study increased the number of Korean bdelloids records to 160 taxa, of which 122 at species-level and 38 at subspecies-level (Song and Lee, 2020a; 2020b; 2021; 2022; 2023; 2024). Here diagnostic characteristics, illustrations and photomicrographs are presented for each new Korean record. Partial sequences of mitochondrial cytochrome c oxidase subunit 1 gene (CO1) for *Mniobia barbatula* and *Scepanotrocha parva* are presented as well.

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Materials and Methods

The samples were collected from mosses, mushrooms and tree barks at two different locations in Korea, from May 20, 2023 to May 11, 2024. The identified species as well as detailed habitat information and sampling dates for each locality are listed in Table 1.

The bdelloid specimens were sorted out from samples conforming to a previously described method (Song, 2014). All of the living bdelloids were examined and identified under a light microscope with a magnification of ×400 to ×600. The photography and motion records of living specimens were done with an Infinity 2 digital camera (Lumenera Corporation, ON, Canada). The photographs were used for illustrations. Measurements were performed using GIMP 2.8 (the GNU Image Manipulation Program). The boiling water fixation method (Edmondson, 1959) was used to kill specimens with head, foot, and toes extended as previously described (Song and Min, 2015). The method of Stemberger (1979) was employed for the preparation of permanent mounts.

The classification scheme is accordant with those of Melone and Ricci (1995) and Segers (2002). The scheme of measurements is partially referred to Iakovenko *et al*. (2013).

DNA was extracted from one specimen of each new record using Extract-N-Amp^{TM} Tissue PCR Kit (Sigma-Aldrich, MO, USA) following the manufacturer's instructions. Partial mitochondrial cytochrome c oxidase subunit 1 gene (CO1) was amplified following the procedures described in Iakovenko *et al*. (2013) with some modifications (Song and Lee, 2020a). Sequencing was outsourced to Macrogen (Seoul, South Korea) using universal primers LCO1490 and HCO2198 (Folmer *et al*., 1994). The forward and reverse sequences were assembled using ClustalW program implemented in MEGAX (https://www.megasoftware.net/).

Results and Discussion

Phylum Rotifera Cuvier, 1817
Class Eurotatoria De Ridder, 1957
Subclass Bdelloidea Hudson, 1884
Order Philodinida Melone and Ricci, 1995
Family Habrotrochidae Bryce, 1910
Genus *Habrotrocha* Bryce, 1910

1. *Habrotrocha minima* De Koning, 1947

꼬마협관윤충 (신칭)

Habrotrocha minima De Koning, 1947, p. 191, pl. 3, figs. 12a–d.

Material examined. One specimen found from mushrooms, mosses and tree barks at Daegwallyeong Guksaseonghwangdang, Hoenggye-ri, Daegwallyeong-myeon, Pyeongchang-gun, Gangwon-do, 37°41′41.12″N, 128°45′15.27″E, 20 May, 2023.

Diagnosis. Rostrum round. Corona much narrower than cingulum; the ratio of corona width to cingulum width about 1 : 1.43. Upper lip slightly concave and with low arch medially; lower than sulcus base. Sulcus very narrow; narrower than 1/5 of pedicel width. Disc retractor shallow U-shaped, narrow and much higher than upper lip. Pedicel short. Cingulum pad rather long, round anterolaterally and cylindrical posteriorly. Foot very short and about 1/10 of total length in creeping. Spurs short and with narrow interspace; interspace much narrower than spur base width.

Measurements. Total length in feeding 142–148 μm. Total length in creeping 202 μm. Rostrum width in creeping 14 μm. Corona width 15–16 μm. Cingulum width 20–22 μm. Cingulum pad width 21–23 μm. Greatest trunk width in creeping 36 μm. Spur length 3 μm.

Remarks. *Habrotrocha minima* can be distinguished from the other *Habrotrocha* species by the narrow corona, lowly arched median lobe of upper lip and narrow sulcus.

Table 1. List of species and sampling localities.

Species	Habitat	Locality[a]	Previous world records
**Habrotrocha minima* De Koning, 1947	mushrooms, mosses and tree barks	1	Austria (Donner, 1962), Czech Republic (Devetter *et al*., 2020), the Netherlands (De Koning, 1947)
**Mniobia barbatula* Donner, 1950	mosses and tree barks	2	Austria (Donner, 1950), Czech Republic (Devetter *et al*., 2020), Italy (Melone *et al*., 1998)
**Scepanotrocha parva* Schulte, 1954	mosses and tree barks	1	Czech Republic (Devetter *et al*., 2020), Germany (Schulte, 1954)

*New to Asia.
[a]1 = Daegwallyeong Guksaseonghwangdang, Hoenggye-ri, Daegwallyeong-myeon, Pyeongchang-gun, Gangwon-do, 37°41′41.12″N/128°45′15.27″E, 20 May, 2023; 2 = Daegwallyeong Guksaseonghwangdang (near parking area), Hoenggye-ri, Daegwallyeong-myeon, Pyeongchang-gun, Gangwon-do, 37°41′46.9″N/128°45′13.5″E, 11 May, 2024.

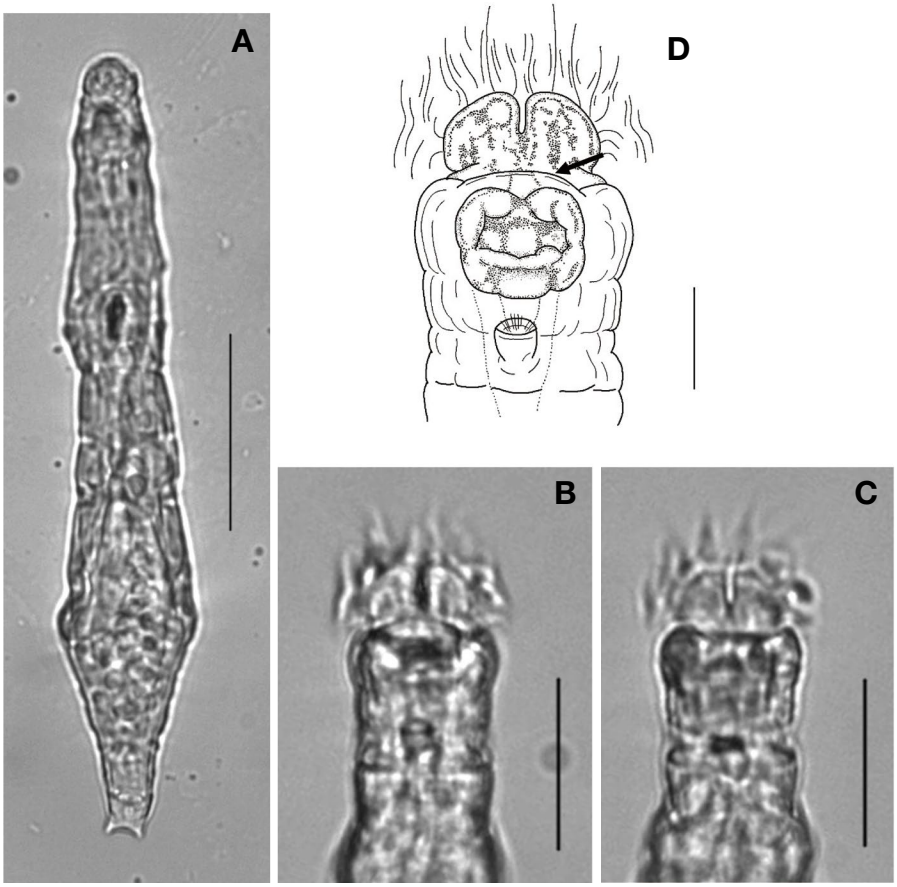


Fig. 1. *Habrotrocha minima* De Koning, 1947: A. creeping, dorsal view; B, C. feeding head and neck, dorsal view; D. feeding head, dorsal view (an arrow pointing at the upper lip) (Scales: A = 50 μm; B, C = 25 μm; D = 10 μm).

The characteristics of the Korean specimen agree well with the original description except the morphology of upper lip and the ratio of cingulum to cingulum pad. The upper lip is slightly arched in the original description, while that of the Korean specimen was rather concave and with low and arched median lobe. The cingulum of the type specimens was much wider than cingulum pad, while that of the Korean specimen was slightly narrower than cingulum pad. The shape of the feeding head of the Korean specimen was more close to that of the Austrian specimens reported by Donner (1962).

This species is rather rare and has been reported only from Austria (Donner, 1962) and Czech Republic (Devetter *et al*., 2020) since the description from the Netherlands by De Koning (1947). Devetter *et al*. (2020) reported this species only in the species list. New to Asia.

World distribution. Austria, Czech Republic, the Netherlands and Korea.

Deposition. Deposited in the collection of the National Institute of Biological Resources, Incheon, Korea (NIBR IV0000910356).

Genus *Scepanotrocha* Bryce, 1910

Key to the species of *Scepanotrocha* reported from Korea

1. Hood with a shallow median notch; without any process medially; with a bird-beak like process laterally ····· *S. corniculata**
– Hood without a median notch; with a small and low process medially; with or without a lateral expansion or process ····· 2
2. Hood covering slightly more than half of corona; much narrower than corona; without any lateral expansion ····· *S. simplex**
– Hood covering corona completely; slightly wider than corona; with a trapezoidal flap-shaped lateral expansion ····· *S. parva*

(* in Song and Lee, 2021)

2. *Scepanotrocha parva* Schulte, 1954
작은두건관윤충 (신칭)

Scepanotrocha parva Schulte, 1954, pp. 601–602, figs. 25a–d.

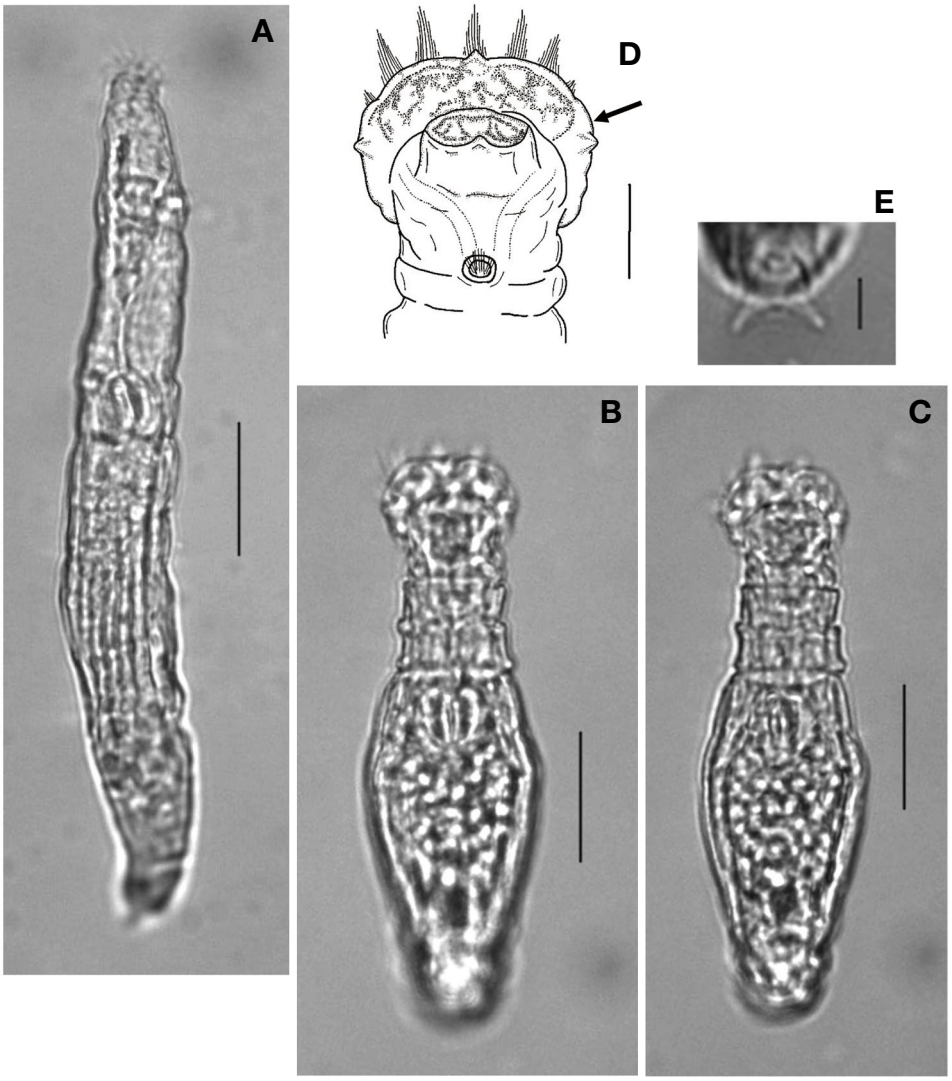


Fig. 2. *Scepanotrocha parva* Schulte, 1954: A. creeping, dorsolateral view; B, C. feeding, dorsal view; D. feeding head and neck, dorsal view (an arrow pointing at the trapezoidal flap-shaped lateral expansion of hood); E. spurs, ventral view (Scales: A–C = 25 μm; D = 10 μm; E = 5 μm).

Material examined. Three specimens sorted out from mosses and tree barks at Daegwallyeong Guksaseong-hwangdang, Hoenggye-ri, Daegwallyeong-myeon, Pyeongchang-gun, Gangwon-do, 37°41′41.12″N, 128°45′15.27″E, on 20 May, 2023.

Diagnosis. Rostrum short and round. Rostral lamella bilobed. Upper lip expanded to hood-shaped membrane with a trapezoidal flap-shaped lateral expansion; anterior margin of hood bilobed and with a low triangular process medially; hood slightly higher than trochal discs. Corona narrower than hood. Pedicel short. Pharyngeal tube as long as trophi. Foot very short; about 1/11 of total length in creeping. Spurs narrow cone-shaped and without interspace.

Measurements. Total length in feeding 97–152 μm. Total length in creeping 166–177 μm. Cingulum width 23–26 μm. Cingulum pad width 21–24 μm. Greatest trunk width in feeding 25–36 μm. Trophi length 11–12 μm. Spur length 5 μm.

Remarks. The upper lip of *Scepanotrocha parva* is expanded to a hood-shaped membrane which has a wavy anterior margin as well as a trapezoidal flap-shaped lateral expansion. Both the anterior margin and each lateral expansion of the hood have a small projection medially. The characteristics of the Korean specimens conformed to the original description except that the projection in the middle of the anterior margin of the hood was large and round in the original description, whereas it was small and pointed in the Korean specimens.

This species is very rare and the only report after description from Germany (Schulte, 1954) was Devetter *et al*. (2020), which reported it from Czech Republic in the

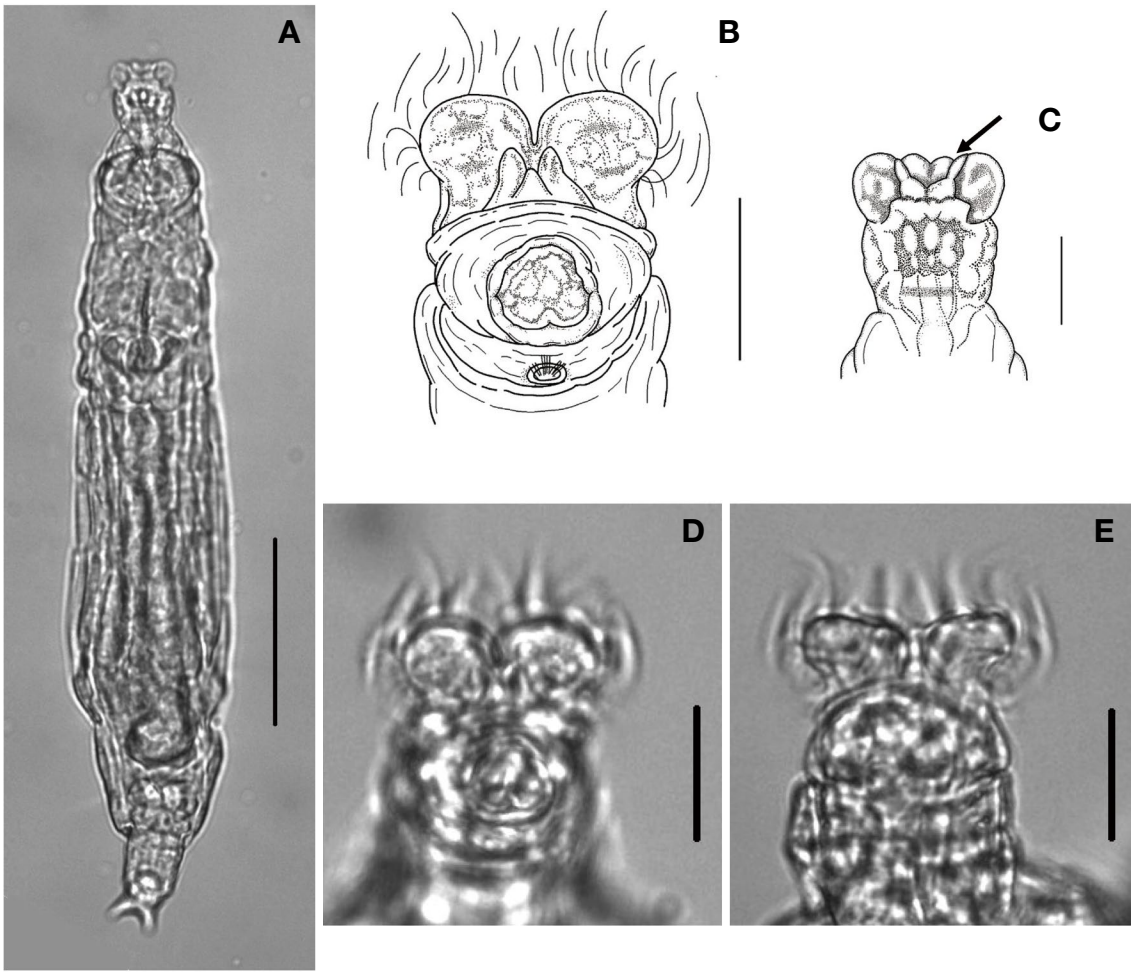


Fig. 3. *Mniobia barbatula* Donner, 1950: A. creeping, ventral view; B. feeding head, dorsal view; C. rostrum, ventral view (an arrow pointing at the tetralobed rostral lamella); D, E. feeding head and neck, dorsal view (Scales: A = 50 μm; B, D, E = 20 μm; C = 10 μm).

species list without remarks or figures. New to Asia.

Partial CO1 was amplified using DNA extracted from one specimen of *Scepanotrocha parva*. One sequence (619 bp in length) was obtained and submitted to GenBank under accession number PQ498773.

World distribution. Czech Republic, Germany and Korea.

Deposition. Deposited in the collection of the National Institute of Biological Resources, Incheon, Korea (NIBR IV0000910357).

Family Philodinidae Ehrenberg, 1838
Genus *Mniobia* Bryce, 1910

3. *Mniobia barbatula* Donner, 1950
콧수염흡반윤충 (신칭)

Mniobia barbatula Donner, 1950, p. 334, figs. 22a–f; Melone *et al*., 1998, p. 1758, fig. 8.

Material examined. Four specimens found from mosses and tree barks at Daegwallyeong Guksaseonghwangdang (near parking area), Hoenggye-ri, Daegwallyeong-myeon, Pyeongchang-gun, Gangwon-do, 37°41′46.9″N, 128°45′13.5″E, on 11 May, 2024.

Diagnosis. Rostral lamella slightly wider than rostrum width and bilobed; each lobe bilobed and inner lobe much smaller than outer lobe. Corona slightly wider than cingulum; corona as wide as cingulum pad. Upper lip arched and with a narrow and high median lobe; median lobe bilobed and with a smaller and lower lobe between them. Sulcus very narrow; much narrower than 1/2 of pedicel width. Lateral margins of cingulum pad round medially. Teeth 3/3. Trunk finely granulated. Foot very short and about 1/9 of total length in creeping. Spurs long, conical and with narrow interspace.

Measurements. Total length in creeping 209–237 μm. Rostrum width 15–16 μm. Rostral lamella width 17–18 μm. Corona width 29–33 μm. Cingulum width 28–29

μm. Cingulum pad width 31−32 μm. Greatest neck width in creeping 35−38 μm. Greatest trunk width in creeping 35−56 μm. Greatest trunk width in feeding 66 μm. Trophi length 17−19 μm. Antenna length 9−10 μm. Spur length 5−6 μm.

Remarks. *Mniobia barbatula* can be distinguished from the other *Mniobia* species by the tetralobed anterior margin of rostral lamella and the trilobed median lobe of upper lip. The characteristics of the Korean specimens agreed quite well with the original description.

This species is rather rare and has been reported from only two countries after the description from Austria by Donner (1950). In their comparative study on the trophi of bdelloids, Melone *et al*. (1998) reported this species from Italy in the species list with a scanning electron microscopy photograph of trophi. Devetter *et al*. (2020) reported it from Czech Republic in the species list without remarks or figures. New to Asia.

Partial CO1 was amplified using DNA extracted from one specimen of *Mniobia barbatula*. One sequence (614 bp in length) was obtained and submitted to GenBank under accession number PQ498739.

World distribution. Austria, Czech Republic, Italy and Korea.

Deposition. Deposited in the collection of the National Institute of Biological Resources, Incheon, Korea (OBMV IV0000000213).

Conflicts of Interest

The authors of this paper have no affiliation with any interests and are solely responsible for the paper.

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Open Access

A report of 32 unrecorded bacterial species belonging to the phylum *Bacillota* in Korea

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As a part of a comprehesive investigation project of indigenous prokaryotic species in Korea, a total of 32 bacterial strains assigned to the phyla *Bacillota* were isolated from various environmental niches, including soil, mud flat, tidal flat, wetland, and seawater. Phylogenetic analysis based on their high 16S rRNA gene sequence similarities (>98.7%) and the formation of robust phylogenetic clades with their closest related reported species, each strain was identified as belonging to a distinct and validly published bacterial species. In the present study, we report 32 species of 22 genera of three families in the phylum *Bacillota*, which have not been reported in Korea. These 32 species were assigned to *Bacillus*, *Cytobacillus*, *Exiguobacterium*, *Gottfriedia*, *Gracilibacillus*, *Halobacillus*, *Jeotgalibacillus*, *Mesobacillus*, *Metabacillus*, *Neobacillus*, *Ornithinibacillus*, *Peribacillus*, *Pontibacillus*, *Pseudoneobacillus*, *Robertmurraya*, *Siminovitchia*, *Sutcliffiella*, and *Virgibacillus* of the Family *Bacillaceae*; *Brevibacillus*, *Fontibacillus*, and *Paenibacillus* of the Family *Paenibacillacea*; *Staphylococcus* of the Family *Staphylococcaceae*. In addition, Colony and cell morphology, gram staining, basic biochemical characteristic, isolation source, and strain ID of each strain are also described in the species descriptions.

Keywords: 16S rRNA, *Bacillota*, environmental niches, taxonomy, unrecorded species

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Introduction

The phylum *Bacillota* is one of the major phyla in the domain bacteria that was renamed from "*Firmicutes*" in 2021 (Oren and Garrity, 2021) which was first described by Gibbons and Murray (1978). *Bacillota* are ubiquitous in nature, thriving in diverse environments ranging from soil and water to the gastrointestinal tracts

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of animals and humans, where they play crucial roles in nutrient cycling, fermentation, and metabolic processes. *Bacillota* species are generally Gram-staining positive, rod/cocci shaped bacteria with a low G+C content (Qin *et al*., 2010). Cells in this phylum have rigid or semi-rigid cell wall containing peptidoglycan and some species can form endospores and have flagella for motility (Gibbons and Murray, 1978). *Bacillota* includes several important bacterial groups, such as industrially valuable lactic acid-producing bacteria, and medically significant species like *Clostridium* (Dworkin *et al*., 2006). At the time of writing, based on 16S rRNA gene and genome sequences, the phylum *Bacillota* comprises seven classes: *Bacilli*, *Clostridia*, *Culicoidibacteria*, *Erysipelotrichia*, *Limnochordia*, *Negativicutes*, and *Thermolithobacteria* (Oren and Garrity, 2021). In this study, we collected several environmental samples from diverse habitats including soil, mud flat, tidal flat, wetland, and seawater in Korea and isolated various novel and unrecorded bacterial species. Consequently, we described 32 bacterial species belonging *Bacillota* phyla that have not been previously reported in Korea.

Materials and Methods

Thirty-two bacterial species belonging to *Bacillota* were isolated from various environment niches including soil, tidal flat, mud, sediment, seawater, and wetland (Table 1). Treatment of environmental samples and bacterial isolation were handled independently in several laboratories. Environmental samples were transported to the laboratory and stored at 4°C until inoculation. All environmental samples were subsequently diluted, and spread on various culture media including R2A agar (BD; USA), marine agar 2216 (MA; BD), tryptic soy agar (TSA; BD) and incubated at 25−37°C for 2−5 days (Table 1). We used appropriate media for the condition of each environmental sample. Additionally, we further used marine agar media for some wetland and agricultural soil samples to isolate salt-tolerant bacterial strains. The designated strain identifications (IDs), most closely related species, isolation sources, culture media, and incubation conditions are described in Table 1. All pure culture strains were maintained as 10−20% glycerol suspension at −80°C and as lyophilized ampoules. The 16S rRNA gene sequences of the strains were aligned with those of the related type strains of validly published bacterial species collected from the EzBioClould (https://www.ezbiocloud.net/) (Yoon *et al*., 2017). Multiple alignments of the 16S rRNA gene sequences of the strains and those of the related type strains were performed using the CLUSTALX program (Larkin *et al*., 2007) for the phylogenetic analysis. Phylogenetic trees were reconstructed by neighbour-joining (NJ), maximum-parsimony (MP), and maximum-likelihood (ML) method (Fitch, 1971; Felsenstein, 1981; Saitou and Nei, 1987) using the MEGA X program (Kumar *et al*., 2018). The Jukes-Cantor model for NJ trees, Minmini heuristic algorithm for MP trees, and Tamura-Nei model for ML trees were used to reconstruct phylogenetic trees (Jukes and Cantor, 1969; Tamura and Nei, 1993; Nei, 2000). Tree topologies were evaluated on the basis of bootstrap analysis of 1000 replicates (Felsenstein, 1985). Colony morphology of the strains was observed by eye or a magnifying glass after the isolates were cultivated to their exponential growth phases on their culture agar media. Cellular morphologies and sizes were examined by using either transmission electron or scanning electron microscopy. Gram staining was performed using a Gram-staining kit according to the manufacturer's standard procedures. Biochemical characteristics were evaluated using the API 20NE kit (bioMérieux, France), according to the manufacturer's instructions.

Results and Discussion

Thirty-two strains belonging to the phylum *Bacillota* were classified in an order; 32 strains in the *Bacillales*. In the phylum of *Bacillota*, one strain was assigned to the genus *Staphylococcus* of the family *Staphylococcaceae* and twenty one strains in the family *Bacillaceae* belonged to 18 different Genera: *Bacillus* (2 strains), *Cytobacillus* (1 strain), *Exiguobacterium* (1 strain), *Gottfriedia* (1 strain), *Gracilibacillus* (1 strain), *Halobacillus* (1 strain), *Jeotgalibacillus* (1 strain), *Mesobacillus* (1 strain), *Metabacillus* (1 strain), *Neobacillus* (1 strain), *Ornithinibacillus* (1 strain), *Peribacillus* (1 strain), *Pontibacillus* (1 strain), *Pseudoneobacillus* (1 strain), *Robertmurraya* (1 strain), *Siminovitchia* (2 strains), *Sutcliffiella* (2 strains), and *Virgibacillus* (1 strain). Ten strains in the family *Paenibacillacea* were separated into three genera: *Brevibacillus* (1 strain), *Fontibacillus* (1 strain), and *Paenibacillus* (8 strains) (Fig. 2). The 32 strains belonging to *Bacillota* were isolated from various environmental habitats, including tidal flat, mud, sediment, seawater, and wetland, which can be attributed to their diverse physiological and biochemical characteristics. All strains were gram-staining negative, and cells were rods or cocci-shaped (Fig. 1). Other biochemical and physiological characteristics are described below. In conclusion, in this study, we reported 32 unrecorded bacterial species belonging to bacterial phyla *Bacillota* which was isolated in Korea.

Description of *Bacillus pumilus* SBCC-2286

Cells are Gram-staining positive and rods with flagella. Colonies are circular to irregular, flat cream colored after

Table 1. Summary of strains isolated belonging to the phylum *Bacillota* and their taxonomic affiliations.

Phylum	Order	Family	Genus	Strain ID	NIRB ID / GenBank accession number	Most closely related species	Similarity (%)	Isolation source	Medium†	Incubation conditions
Bacillota	*Bacillales*	*Bacillaceae*	*Bacillus*	SBCC-2286	NIBRBAC000510511 / PV156053	*Bacillus pumilus*	100.00	Tidal flat	MA	25°C, 3-5d
				US27	NIBRBAC000510636 / OR251357	*Bacillus sonorensis*	99.86	Wetland	R2A	30°C, 2d
			Cytobacillus	AN479	NIBRBAC000510607 / OR527143	*Cytobacillus horneckiae*	99.86	Wetland	R2A	28°C, 3d
			Exiguobacterium	US12	NIBRBAC000510635 / OR251353	*Exiguobacterium undae*	99.60	Wetland	R2A	30°C, 3d
			Gottfriedia	GM6	NIBRBAC000510645 / OR251348	*Gottfriedia acidiceleris*	99.79	Soil	R2A	30°C, 3d
			Gracilibacillus	CAU 1725	NIBRBAC000510782 / OR708690	*Gracilibacillus halotolerans*	98.79	Mud	MA	30°C, 3d
			Halobacillus	CAU 1720	NIBRBAC000510777 / OR708684	*Halobacillus andaensis*	99.65	Mud flat	MA	30°C, 3d
			Jeotgalibacillus	MMS23-YR12	NIBRBAC000510826 / OR787593	*Jeotgalibacillus alkaliphilus*	99.86	Soil	R2A	30°C, 3d
			Mesobacillus	CAU 1751	NIBRBAC000510787 / OR770161	*Mesobacillus harenae*	100.00	Mud flat	MA	30°C, 3d
			Metabacillus	SBCC-1704	NIBRBAC000510513 / PV156054	*Metabacillus sediminilitoris*	99.86	Tidal flat	MA	25°C, 3d
			Neobacillus	DHW12	NIBRBAC000510806 / OR751824	*Neobacillus massiliamazoniensis*	98.85	Soil	R2A	28°C, 3d
			Ornithinibacillus	MBLB2969	NIBRBAC000510680 / PQ496898	*Ornithinibacillus bavariensis*	99.42	Mud flat	TSA	30°C, 3d
			Peribacillus	SBCC-2316	NIBRBAC000510512 / PV156057	*Peribacillus huizhouensis*	99.86	Tidal flat	MA	25°C, 3d
			Pontibacillus	CAU 1722	NIBRBAC000510779 / OR708687	*Pontibacillus litoralis*	99.79	Mud	MA	30°C, 3d
			Pseudoneobacillus	SBCC-1576	NIBRBAC000510517	*Pseudoneobacillus rhizosphaerae*	99.39	Tidal flat	MA	25°C, 5d
			Robertmurraya	MBLB3707	NIBRBAC000510683 / PQ510306	*Robertmurraya siralis*	99.63	Soil	MA	30°C, 3d
			Siminovitchia	CAU 1715	NIBRBAC000510774 / OR708679	*Siminovitchia acidinfaciens*	100.00	Mud flat	MA	30°C, 3d
				CAU 1724	NIBRBAC000510781 / OR708688	*Siminovitchia composti*	100.00	Mud	MA	30°C, 3d
			Sutcliffiella	CAU 1717	NIBRBAC000510775 / OR708682	*Sutcliffiella cohnii*	99.46	Mud flat	MA	30°C, 3d
				CAU 1748	NIBRBAC000510785 / OR770157	*Sutcliffiella rhizosphaerae*	99.52	Wetland	MA	30°C, 3d
			Virgibacillus	DCW25	NIBRBAC000510843 / OR751822	*Virgibacillus indicus*	99.79	Mud flat	MA	28°C, 3d
		Paenibacillacea	*Brevibacillus*	MBLB3826	NIBRBAC000510682 / PQ499573	*Brevibacillus agri*	99.55	Soil	MA	30°C, 3d
			Fontibacillus	J2W1	NIBRBAC000510848 / OR636890	*Fontibacillus aquaticus*	98.84	Soil	R2A	28°C, 3d
			Paenibacillus	SBCC-1747	NIBRBAC000510515 / PV156733	*Paenibacillus piscarius*	99.30	Tidal flat	MA	25°C, 5-7d
				16S3E7	NIBRBAC000510541 / OR717470	*Paenibacillus agaridevorans*	98.78	Sediment	R2A	30°C, 2d
				AN637	NIBRBAC000510612 / OR527148	*Paenibacillus rhizoryzae*	99.66	Wetland	R2A	28°C, 3d
				SU15	NIBRBAC000510754 / AB818664	*Paenibacillus radicis*	98.91	Soil	MA	25°C, 3d
				AHW26	NIBRBAC000510803 / OR636871	*Paenibacillus phytorum*	99.24	Soil	R2A	28°C, 3d
				MMS23-KR7	NIBRBAC000510824 / OR787591	*Paenibacillus plantarum*	99.16	Soil	R2A	30°C, 3d
				DSW28	NIBRBAC000510839 / OR751825	*Paenibacillus planticolens*	98.74	Soil	R2A	28°C, 3d
				DCW4	NIBRBAC000510845 / OR751676	*Paenibacillus aquistagni*	99.86	Mud flat	MA	28°C, 3d
		Staphylococcaceae	*Staphylococcus*	SBCC-2353	NIBRBAC000510524 / PV156056	*Staphylococcus shinii*	99.93	Tidal flat	MA	25°C, 3d

†Abbreviations: MA, marine agar; R2A, Reasoner's 2A

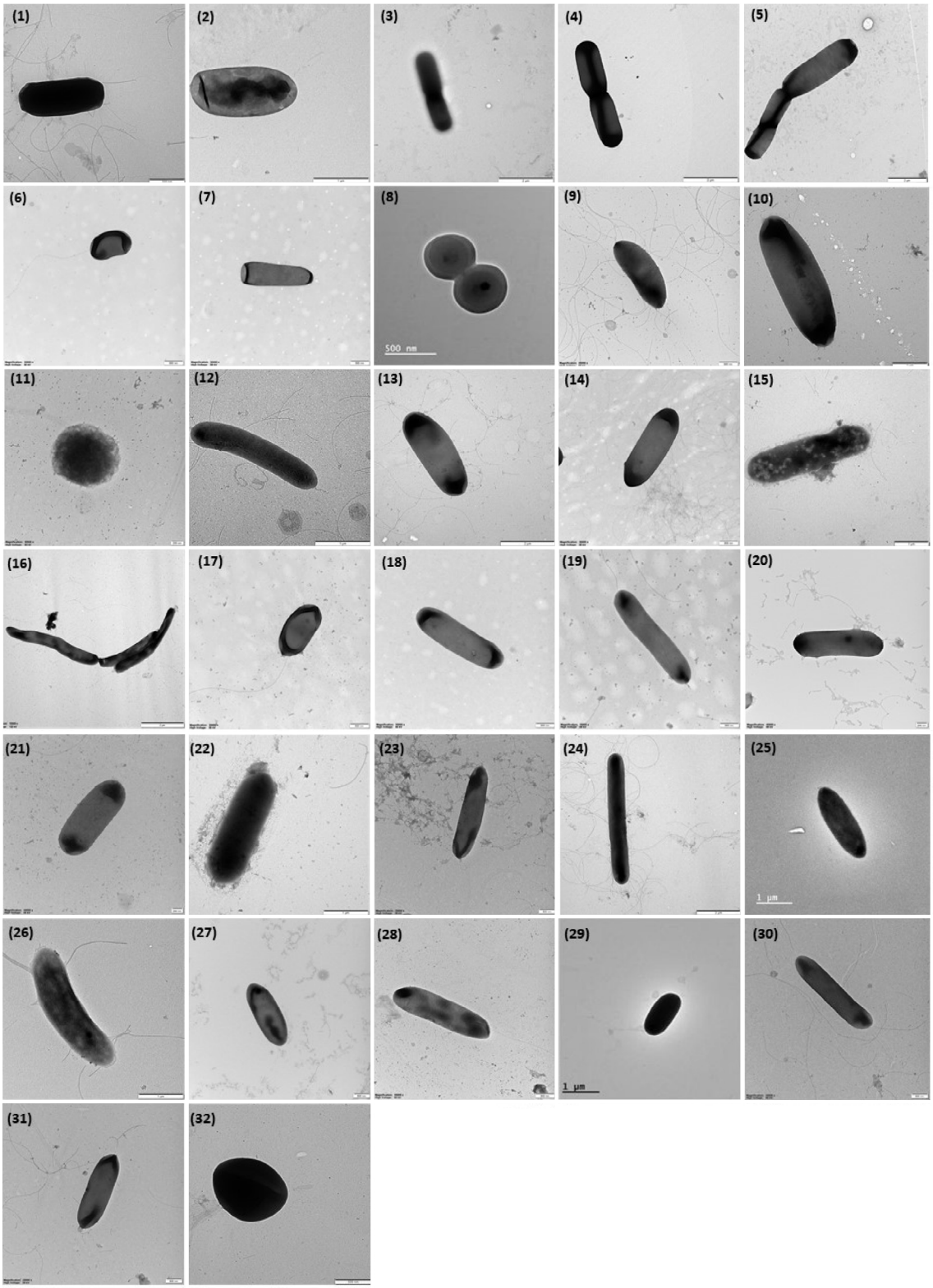


Fig. 1. Transmission electron micrographs showing the cell morphologies of bacterial strains isolated in this study. The strains were cultured at their optimal growth conditions. Strains: 1, SBCC-2286; 2, US27; 3, AN479; 4, US12; 5, GM6; 6, CAU 1725; 7, CAU 1720; 8, MMS23-YR12; 9, CAU 1751; 10, SBCC-1704; 11, DHW12; 12, MBLB2969; 13, SBCC-2316; 14, CAU 1722; 15, SBCC-1576; 16, MBLB3707; 17, CAU 1715; 18, CAU 1724; 19, CAU 1717; 20, CAU 1748; 21, DCW25; 22, MBLB3826; 23, J2W1; 24, SBCC-1747; 25, 16S3E7; 26, AN637; 27, SU15; 28, AHW26; 29, MMS23-KR7; 30, DSW28; 31, DCW4; 32, SBCC-2353.

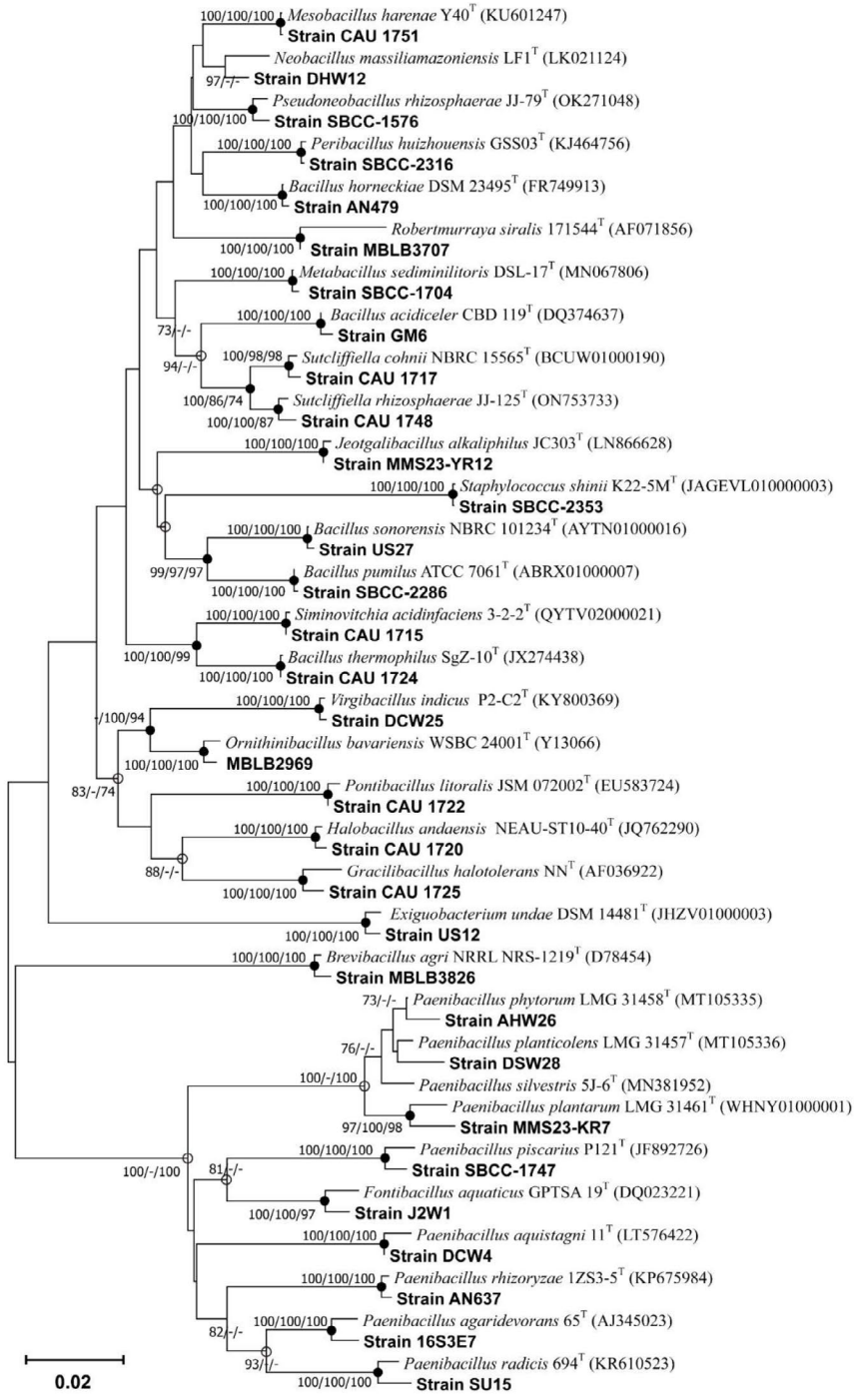


Fig. 2. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between bacterial strains isolated in this study and their relatives in the class *Bacilli* of the phylum *Bacillota*. Filled circles indicate that both maximum-likelihood and maximum-parsimony trees obtained the same node arrangement, while empty circles indicate either only maximum-likelihood or maximum-parsimony obtained the same corresponding nodes. Bootstrap values ($>70\%$) are shown on the nodes. Scale bar: 0.02 changes per nucleotide.

3–5 days of incubation at 25°C on MA. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease activities. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, malic acid, and trisodium citrate. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain SBCC-2286 (NIBRBAC000510511)

was isolated from tidal flat from Taean, Chungcheongnam-do, Korea (36°53′17″N 126°19′39″E).

Description of *Bacillus sonorensis* US27

Cells are Gram-staining positive and short-rods without flagella. Colonies are pale yellow, irregular, raised after 2 days of incubation on R2A at 30°C. Positive for nitrate reduction, esculin hydrolysis, arginine dihydrolase, esculin hydrolysis and urease, gelatinase, and β-galactosidase activities. Negative for indole production, glucose fermentation, urease activities. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate. Does not utilize capric acid, adipic acid and phenylacetic acid. Strain US27 (NIBRBAC000510636) was isolated from wetland of Paju, Gyeonggi-do, Korea (37°44′29.6″N, 126°47′35.2″E).

Description of *Cytobacillus horneckiae* AN479

Cells are Gram-staining positive and rods without flagella. Colonies are cream, Round, entire, and raised after 2 days of incubation on R2A at 28°C. Positive for nitrate reduction and gelatinase activity. Negative indole production, glucose fermentation, arginine dihydrolase, urease activities, esculin hydrolysis, and β-galactosidase activity. Utilizes D-maltose, potassium gluconate, adipic acid, malic acid, trisodium, and citrate acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, capric acid, and phenylacetic acid. Strain AN479 (NIBRBAC000510607) was isolated from wetland of Anseong, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

Description of *Exiguobacterium undae* US12

Cells are Gram-staining positive and rod-shaped with 1.1−1.2 μm in diameter. Colonies are ivory, circular and raised after 3 days of incubation on R2A at 30°C. Positive for glucose fermentation, esculin hydrolysis, gelatinase, and β-galactosidase activity. Negative for nitrate reduction, indole production, arginine dihydrolase, and urease activities. Utilizes D-maltose only and does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain US12 (NIBRBAC000510635) was isolated from wetland of Paju, Gyeonggi-do, Korea (37°44′29.6″N, 126°47′35.2″E).

Description of *Gottfriedia acidiceleris* GM6

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are yellow, circular, convex, and transparent after 3 days of incubation on R2A at 30°C. Positive for esculin hydrolysis and gelatinase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain GM6 (NIBRBAC000510645) was isolated from soil of Paju, Gyeonggi-do, Korea (37°14′29.4″N 131°52′00.9″E).

Description of *Gracilibacillus halotolerans* CAU 1725

Cells are Gram-staining positive and short-rod with flagella. Colonies are cream, circular, smooth, entire, convex and translucent after 3 days of incubation on MA at 30°C. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CAU 1725 (NIBRBAC000510782) was isolated from wetland of Ansan, Gyeonggi-do, Korea (37°17′19.8″N 126°34′57.1″E).

Description of *Halobacillus andaensis* CAU 1720

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are cream, circular, smooth, entire, convex and opaques after 3 days of incubation on MA at 30°C. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CAU 1720 (NIBRBAC000510777) was isolated from mud flat of Ansan, Gyeonggi-do, Korea (37°14′31.0″N 126°33′38.4″E).

Description of *Jeotgalibacillus alkaliphilus* MMS23-YR12

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are ivory, circular, convex and entire after 3 days of incubation on R2A at 30°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase, and β-galactosidase activities. Negative for indole production, arginine dihydrolase, and urease activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and phenylacetic acid. Does not utilize capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain MMS23-YR12 (NIBRBAC000510826) was

isolated from soil of Gangneung, Gangwon-do, Korea (37°47′53″N 128°53′49″E).

Description of *Mesobacillus harenae* CAU 1751

Cells are Gram-staining positive and short-rod shaped with flagella. Colonies are cream, circular smooth, convex, entire and opaques after 3 days of incubation on MA at 30°C. Positive for nitrate reduction. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activity, esculin hydrolysis, gelatinase, and β-galactosidase activities. Utilizes *N*-acetyl-glucosamine. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CAU 1751 (NIBRBAC000510787) was isolated from soil of Ansan, Gyeonggi-do, Korea (37° 14′31.0″N 126°33′38.4″E).

Description of *Metabacillus sediminilitoris* SBCC-1704

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are light yellow, Irregular, flat, and glistening after 3 days of incubation on MA at 25°C. Positive for esculin hydrolysis, and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain SBCC-1704 (NIBRBAC000510513) was isolated from tidal flat of Ansan, Gyeonggi-do, Korea (37°12′24″N 126°38′39″E).

Description of *Neobacillus massiliamazoniensis* DHW12

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are white, circular, entire convex, and smooth after 3 days of incubation on R2A at 28°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease activity, gelatinase, activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DHW12 (NIBRBAC000510806) was isolated from soil of Jeonbuk-do, Korea (35°41′29.4″N 126°32′48.8″E).

Description of *Ornithinibacillus bavariensis* MBLB2969

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, round, smooth, and flat after 3 days of incubation on TSA at 30°C. Positive for esculin hydrolysis, and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MBLB2969 (NIBRBAC000510680) was isolated from mud flat of Gyeongsangbuk-do, Korea (36°37′01.3″N 129°41′36.8″E).

Description of *Peribacillus huizhouensis* SBCC-2316

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circular to irregular, flat, smooth and glistening after 3 days of incubation on MA at 25°C. Positive for urease activity, esculin hydrolysis, and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and gelatinase activity. Utilize D-glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and trisodium citrate. Does not utilize L-arabinose, D-mannose, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain SBCC-2316 (NIBRBAC000510512) was isolated from tidal flat of Taean Chungchungnam-do, Korea (36°53′17″N 126°19′39″E).

Description of *Pontibacillus litoralis* CAU 1722

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, Circular, smooth, entire, convex, and opaques after 3 days of incubation on MA at 25°C. Positive for esculin hydrolysis and gelatinase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CAU 1722 (NIBRBAC000510779) was isolated from mud flat of Ansan, Gyeonggi-do, Korea (37°17′18.9″N 126°34′56.1″E).

Description of *Pseudoneobacillus rhizosphaerae* SBCC-1576

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circular, flat, smooth, and glistening after 5 days of incubation on MA at 25°C. Positive for nitrate reduction. Negative indole production, glucose fermentation, arginine dihydrolase, urease activity, esculin hydrolysis, gelatinase, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain SBCC-1576 (NI-

BRBAC000510517) was isolated from tidal flat of Taean Chungchungnam-do, Korea (36°50′29″N 126°11′24″E).

Description of *Robertmurraya siralis* MBLB3707

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circle, flat, and entire opaque after 3 days of incubation on MA at 30°C. Positive for urease activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, and β-galactosidase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MBLB 3707 (NIBRBAC000510683) was isolated from soil of Gangneung, Gangwon-do, Korea (37°48′14.9″N 128°54′ 23.4″E).

Description of *Siminovitchia acidinfaciens* CAU 1715

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, smooth, entire, convex, and translucent after 3 days of incubation on MA at 30°C. Positive for urease activity, esculin hydrolysis, and gelatinase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and β-galactosidase activities. Utilizes D-glucose, L-arabinose, D-maltose, malic acid, and phenylacetic acid. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, and trisodium citrate. Strain CAU 1715 (NIBRBAC000510774) was isolated from soil of Gangneung, Gangwon-do, Korea (37°48′14.9″N 128° 54′23.4″E).

Description of *Siminovitchia composti* CAU 1724

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, smooth, entire, convex, and translucent after 3 days of incubation on MA at 30°C. Positive for nitrate reduction, esculin hydrolysis, and gelatinase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and malic acid. Does not utilize capric acid, trisodium citrate, and phenylacetic acid. Strain CAU 1724 (NIBRBAC000510781) was isolated from mud flat of Ansan, Gyeonggi-do, Korea (37°17′19.8″N 126°34′57.1″E).

Description of *Sutcliffiella cohnii* CAU 1717

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, smooth, entire, convex, and translucent after 3 days of incubation on MA at 30°C. Positive for glucose fermentation, esculin hydrolysis, gelatinase, and β-galactosidase activities. Negative for nitrate reduction, indole production, arginine dihydrolase, urease activity. Utilizes *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, capric acid, adipic acid, malic acid, and phenylacetic acid. Strain CAU 1717 (NIBRBAC000510775) was isolated from mud flat of Ansan, Gyeonggi-do, Korea (37°14′31.3″N 126°33′38.2″E).

Description of *Sutcliffiella rhizosphaerae* CAU 1748

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, smooth, entire, convex, and translucent after 3 days of incubation on MA at 30°C. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease activity. Utilizes D-glucose, D-mannitol, D-maltose, potassium gluconate, malic acid, and trisodium citrate. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, capric acid, adipic acid, and phenylacetic acid. Strain CAU 1748 (NIBRBAC000510785) was isolated from wetland of Jeongeup, Jeollabuk-do Korea (35°31′29.2″N 126°52′37.5″E).

Description of *Virgibacillus indicus* DCW25

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, entire convex, and smooth after 3 days of incubation on MA at 28°C. Positive for esculin hydrolysis, gelatinase, and β-galactosidase activities. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DCW25 (NIBRBAC000510843) was isolated from mud flat of Ganghwa, Incheon, Korea (37°40′08.8″N 126°20′07.8″E).

Description of *Brevibacillus agri* MBLB3826

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, and flat after 3 days of incubation on MA at 30°C. Positive for nitrate reduction, and urease activity. Negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, and β-galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and malic acid. Does not utilize D-mannose, capric acid, trisodium citrate, and phenylacetic acid. Strain MBLB

3826 (NIBRBAC000510682) was isolated from soil of Gangneung, Gangwon-do Korea (37°48′14.9″N 128° 54′23.4″E).

Description of *Fontibacillus aquaticus* J2W1

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circle, entire convex, and smooth after 3 days of incubation on R2A at 28°C. Positive for esculin hydrolysis, and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase activities. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain J2W1 (NIBRBAC000510848) was isolated from soil of Yeongwol, Gangwon-do Korea (37°19′08.0″N 128°11′01.7″E).

Description of *Paenibacillus piscarius* SBCC-1747

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle, flat, smooth, and glistening after 5−7 days of incubation on MA at 25°C. Positive for esculin hydrolysis, and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase activities. Utilizes L-arabinose, D-mannose, *N*-acetyl-glucosamine, and potassium gluconate. Does not utilize D-glucose, D-mannitol, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain SBCC-1747 (NIBRBAC000510515) was isolated from tidal flat of Seocheon, Chungcheongnam-do, Korea (36°01′45″N 126°39′57″E).

Description of *Paenibacillus agaridevorans* 16S3E7

Cells are Gram-staining negative and rod-shaped without flagella. Colonies are white, Round with entire margins after 2 days of incubation on R2A at 30°C. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase activities. Utilizes D-mannitol, *N*-acetyl-glucosamine, D-maltose, adipic acid, and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, potassium gluconate, capric acid, malic acid, and phenylacetic acid. Strain 16S3E7 (NIBRBAC000510541) was isolated from soil of Yeoju, Gyeonggi-do, Korea (37°17′23.51″ N 127° 39′5.92″E).

Description of *Paenibacillus rhizoryzae* AN637

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, Round, entire and convex after 3 days of incubation on R2A at 28°C. Positive for esculin hydrolysis, and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activity. Utilizes D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain AN637 (NIBRBAC000510612) was isolated from wetland of Anseong, Gyeonggi-do, Korea (37°01′02.6″N 127°15′48.9″E).

Description of *Paenibacillus radicis* SU15

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are cream, circle slightly convex, and glistening after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, gelatinase and urease activity. Utilizes L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, and D-maltose. Does not utilize D-glucose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain SU15 (NIBRBAC000510754) was isolated from soil of Nowon, Seoul, Korea (37°38′20.4″N 127°02′53.3″E).

Description of *Paenibacillus phytorum* AHW26

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circle undulate, convex, and smooth after 3 days of incubation on R2A at 28°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase and urease activity. Utilizes D-glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and malic acid. Does not utilize L-arabinose, D-mannose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain AHW26 (NIBRBAC000510803) was isolated from soil of Pyeongchang, Gangwon-do, Korea (37°22′18.5″N 128°29′07.4″E).

Description of *Paenibacillus plantarum* MMS23-KR7

Cells are Gram-staining positive and rod-shaped without flagella. Colonies are transparent, circular, convex, and entire after 3 days of incubation on R2A at 30°C. Positive for nitrate reduction, indole production, esculin hydrolysis, and β-galactosidase activity. Negative for glucose fermentation, arginine dihydrolase, gelatinase, and urease activity. Utilizes D-glucose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain MMS23-KR7 (NIBRBAC000510824) was isolated from

soil of Gyeongju, Gyeongsangbuk-do, Korea (35°50′17″N 129°17′11″E).

Description of *Paenibacillus planticolens* DSW28

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circular, entire convex, and smooth after 3 days of incubation on R2A at 28°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase activity. Utilizes D-glucose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DSW28 (NIBRBAC000510839) was isolated from soil of Jeju-do, Korea (33°31′25.3″N 126°37′10.6″E).

Description of *Paenibacillus aquistagni* DCW4

Cells are Gram-staining positive and rod-shaped with flagella. Colonies are white, circular, entire convex, and smooth after 3 days of incubation on MA at 28°C. Positive for esculin hydrolysis and β-galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activity. Utilizes D-glucose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain DCW4 (NIBRBAC000510845) was isolated from mud flat of Ganghwa, Incheon, Korea (37°40′08.8″N 126°20′07.8″E).

Description of *Staphylococcus shinii* SBCC-2353

Cells are Gram-staining positive and coccus-shaped without flagella. Colonies are milky white, Circular to slightly irregular, slightly convex, and smooth after 3 days of incubation on MA at 25°C. Positive for nitrate reduction, urease activity, and β-galactosidase activity. Negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, and gelatinase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and trisodium citrate. Does not utilize capric acid, adipic acid, and phenylacetic acid. Strain SBCC-2353 (NIBRBAC000510524) was isolated from tidal flat of Seocheon, Chungcheongnam-do, Korea (36°01′45″N 126°39′57″E).

Conflicts of Interest

These authors of this paper have no affiliation with any interests and is solely responsible for the paper.

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A new record of the species *Caprella carinata* (Crustacea, Amphipoda, Caprellidae) from Korean waters

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A newly recorded species *Caprella carinata* Arimoto, 1934 belonging to the family Caprellidae Leach, 1814 was collected from the East Sea, Korea. This species is morphologically similar to *Caprella acantogaster* Mayer, 1890, *Caprella eximia* Mayer, 1890, and *Caprella mutica* Schurin, 1935 as all four species share dorsal projections on pereonites and possess robust (grasping) setae on pereopods 5−7. However, *C. carinata* can be distinguished from relative species by the curved projections on pereonites 1 and 2, as well as by the distinct shape of the poison tooth on gnathopod 2. In this study, *C. carinata* is illustrated based on mature specimens and its morphological characteristics are compared with those of relative species.

Keywords: amphipod, *Caprella*, Korea, new record, taxonomy

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Introduction

The genus *Caprella* Lamarck, 1801 is one of the 96 genera belonging to the family Caprellidae Leach, 1814, encompassing 187 species (Horton *et al*., 2024). This genus is defined by the following morphological characteristics: (1) antenna 2 with biarticulate flagellum; (2) mandibular palp absent; (3) gills present on pereonites 3−4; (4) pereopods 3−4 absent; and (5) abdomen with a pair of appendages and a pair of lobes in male. Species in the genus *Caprella* show various morphological differences, such as variations in body thickness, length, and protrusions (McCain, 1968; Arimoto, 1976; Heo *et al*., 2020). In Korean waters, a total of 11 genera 44 species of caprellids have been reported. Among these 11 genera, the genus *Caprella* with 34 species showed the highest diversity and abundance (Ryu and Park, 2023; National Marine Biodiversity Institute of Korea, 2023; Shin *et al*., 2023; Heo *et al*., 2024). *Caprella carinata* was originally described by Arimoto in 1934 from its type locality in Yamada Bay, Japan. Later, Arimoto (1976) conducted further taxonomic study on this species. *Caprella carinata* is morphologically similar to *C. eximia*, and some specimens of *C. carinata* have historically been misclassified as *C. eximia* (Lee and Hong, 2011). However, despite these morphological resemblances, the two species exhibit distinct differences. In this paper, we provide detailed illustrations of *C. carinata* and compare its morphology with *C. eximia*, emphasizing the distinguishing features that differentiate the two species.

Materials and Methods

The specimens were collected by SCUBA diving, port survey from subtidal and sublittoral waters of east sea, Korea (Fig. 1). Specimens were fixed in 95% ethanol and dissected in glycerol on Cobb's aluminum hole slides. The materials were examined using stereoscope (Olympus SZX 10) and compound microscopes (Olympus BX 51). Line drawings were produced using the program Clip Studio Paint (Celsys, Japan). Specimens are deposited at

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the Marine Biodiversity institute of Korea (MABIK) in Seocheon, Korea and the Department of Biological Science, Dankook University (DKU), Cheonan, Korea.

Results

Subphylum Crustacea Pennant, 1777 갑각아문
Class Malacostraca Latreille, 1806 연갑강
Order Amphipoda Latreille, 1816 단각목
Family Caprellidae Leach, 1814 바다대벌레과
Genus *Caprella* Lamarck, 1801 바다대벌레속

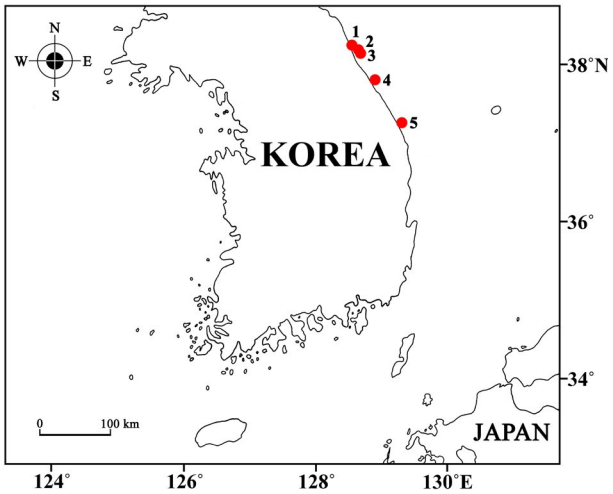


Fig. 1. Distribution of *Caprella carinata* from Korean waters (1, Chodo-port, Hyeonnae-myeon, Goseong-gun; 2, Gajin-ri, Jukwang-myeon, Goseong-gun; 3, Geojin-ri, Geojin-eup, Goseong-gun; 4, Namae-ri, Hyeonnam-myeon, Yangyang-gun; 5, Gyo-dong, Samcheok-si).

***Caprella carinata* Arimoto, 1934**
나도가시투성바다대벌레 (신칭)* (Figs. 2–4)
Caprella carinata Arimoto, 1934: 496, figs. 2, 8, 17, 21–22, 27.
Caprella (*Caprella*) *carinata*: Arimoto, 1976: 97, figs. 51–53.
Caprella eximia sensu Lee & Hong, 2011: 59, fig. 42D, E.

Material examined. 35 males, 41 females, Geojin-ri, Geojin-eup, Goseong-gun, Gangwon-do, Korea, 38°27′09″N, 128°28′24″E, S.G. Lee, by SCUBA diving, 29 August 2018; 12 males, 6 females, Namae-ri, Hyeonnam-myeon, Yangyang-gun, Gangwon-do, Korea, 37°56′39″N, 128°47′43″E, S.G. Lee, by SCUBA diving, 30 August 2018; 8 males, 9 females, Gyo-dong, Samcheok-si, Gangwon-do, Korea, 37°28′01″N, 129°10′55″E, S.G. Lee, by SCUBA diving, 31 August 2018; 2 males, 3 females, Gajin-ri, Jukwang-myeon, Goseong-gun, Gangwon-do, Korea, 38°22′44″N, 128°31′32″E, S.G. Lee, by SCUBA diving, 21 April 2019; 2 males, Chodo-port, Hyeonnae-myeon, Goseong-gun, Gangwon-do, Korea, 38°29′08″N, 128°26′19″E, Y.H. Kim, by port survey, 11 January 2023.

Description. Male (MABIK CR00257943): Body (Figs. 2A, 3A) 11.6 mm long. Head smooth without process. Eyes small and round. Pereonite 1 elongate and fused with head; pereonite 2 with a projection anterodorsally, a pair of projections laterally, two pairs of projections posterodorsally and two pairs of anteriorly curved projections middorsally and posterodorsally; pereonite 3 longest; pereonites 3, 4 with dorsal and lateral projections, elongated gill; pereonite 5 subequal to pereonites 2, 4, with dorsal and lateral projections; pereonites 6, 7 short, with dorsal projections; length ratio of pereonites 2–7 = 1.00 : 1.06 : 0.99 : 1.00 : 0.50 : 0.40.

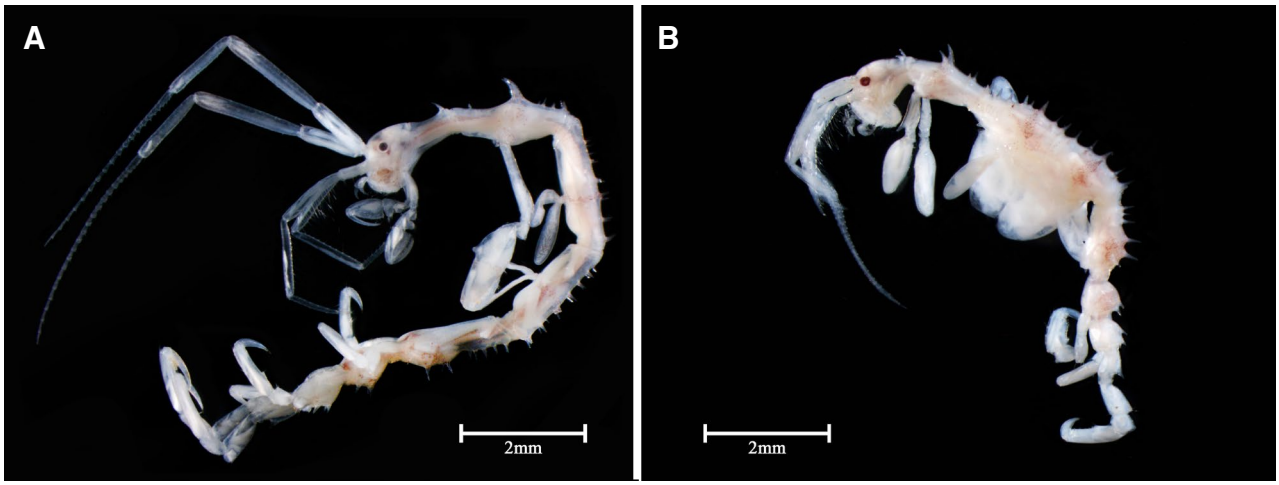


Fig. 2. *Caprella carinata*, A, adult male, 11.6 mm, habitus; B, adult female, 7.9 mm, habitus. Scale bars: A, B = 2.0 mm.

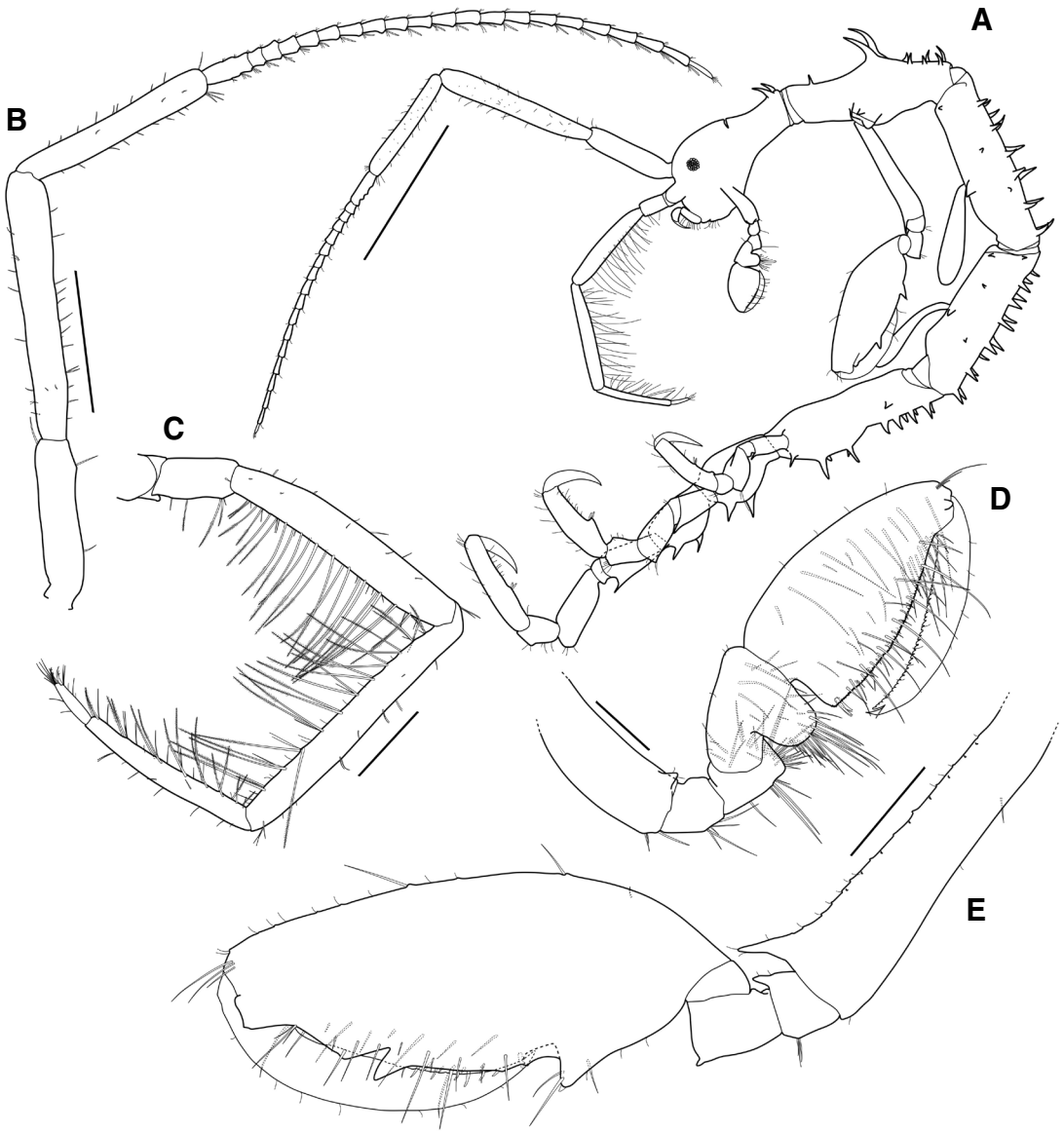


Fig. 3. *Caprella carinata*, adult male, 11.6 mm. A, habitus; B, antenna 1; C, antenna 2; D, gnathopod 1; E, gnathopod 2. Scale bars: A = 2 mm, B = 1 mm, C, E = 0.4 mm, D = 0.2 mm.

Antenna 1 (Fig. 3B) 0.67 × body length; peduncular articles with sparse short; length ratio of peduncular articles 1–3 = 1.00 : 1.58 : 1.17; flagellum slender, 21-articulate, 0.84 × peduncle, each article with 1 aesthetasc ventrodistally.

Antenna 2 (Fig. 3C) setose, shorter than antenna 1, not reaching the distal end of peduncular article 3 of antenna 1; peduncular articles 4, 5 and flagellum article 1 with many long swimming setae ventrally; length ratio of peduncular articles 3–5 = 1.00 : 3.72 : 4.02; flagellum biarticulate, 0.85 × peduncular article 5.

Gnathopod 1 (Fig. 3D) small; basis broken, with anterodistal process; merus with simple setae distally; carpus subtriangular, with expending rounded lobe and long simple setae ventrodistally; propodus narrowing distally, palm slightly convex, with unequal setae, proximal projection conspicuously provided; dactylus falcate, slightly exceeding palm; length ratio of 6 articles = 1.00 : 0.31 : 0.37 : 0.70 : 1.45 : 1.33.

Gnathopod 2 (Fig. 3E) attached midanterior margin of pereonite 2, long and slender; basis, anterior margin serrate slightly; basis, ischium, merus with process on each distal portion, propodus elongate, longer than basis, width 0.38 × length, with a small robust (grasping) seta on proximal projection, palm with triangular projection near distal margin, small poison tooth situated nearly triangular

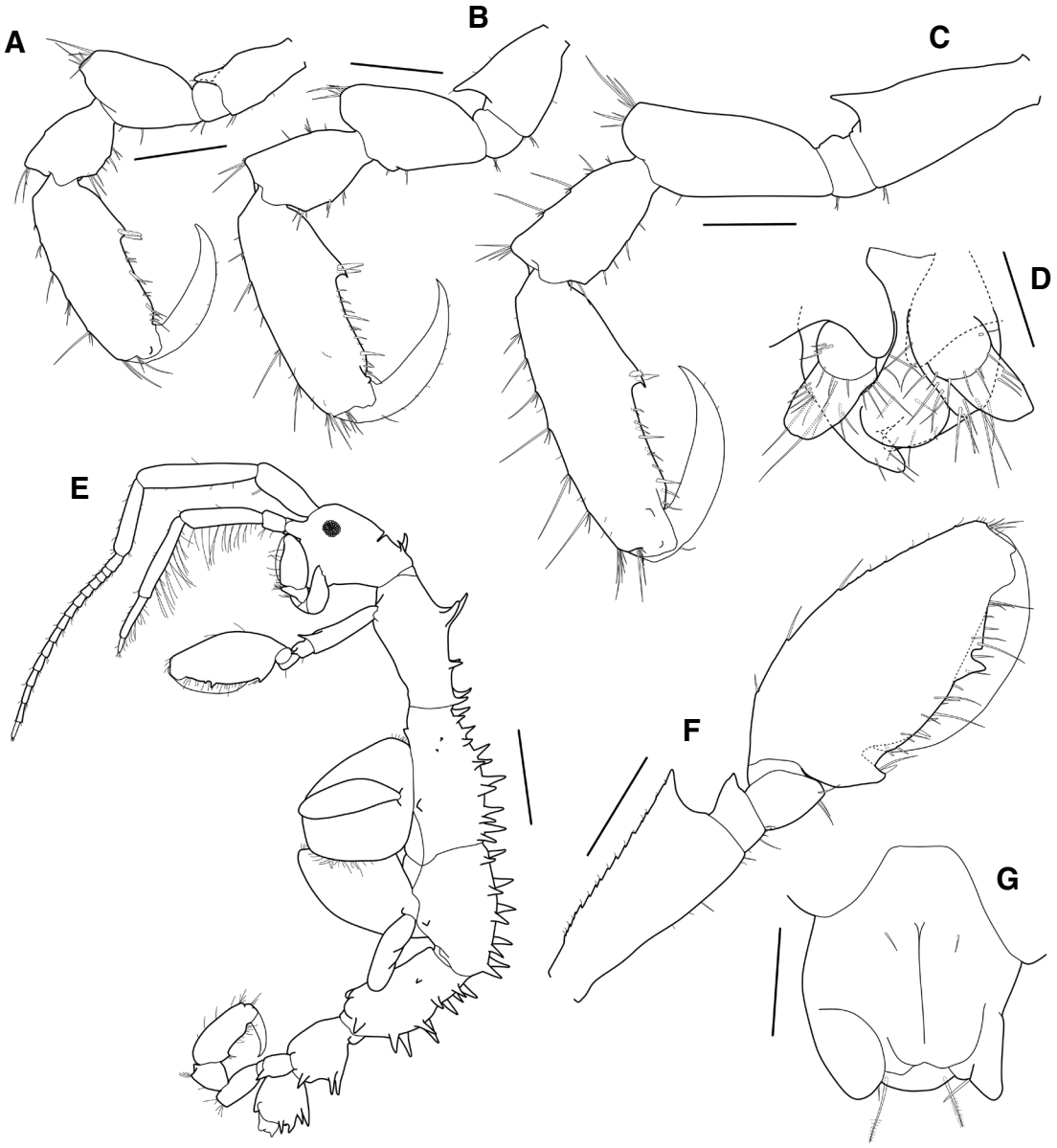


Fig. 4. *Caprella carinata*, adult male, 11.6 mm. A, pereopod 5; B, pereopod 6; C, pereopod 7; D, abdomen. Adult female, 7.9 mm. E, habitus; F, gnathopod 2; G, abdomen. Scale bars: E = 1 mm, A–C, F = 0.4 mm, D, G = 0.1 mm.

projection; dactylus falcate, slightly curved; length ratio of 6 articles = 1.00 : 0.13 : 0.24 : 0.09 : 1.33 : 0.98.

Pereopod 5 (Fig. 4A), stout; basis with large triangular process posterodistally; ischium short; merus expanded posteriorly with simple setae; propodus longest, with long simple setae posteriorly, palm defined by a pair of robust (grasping) setae midanteriorly; dactylus falcate; length ratio of 6 articles = 1.00 : 0.32 : 1.05 : 1.08 : 2.16 : 2.11.

Pereopod 6 (Fig. 4B) similar to pereopod 5, but merus more elongated than that of pereopod 5; length ratio of 6 articles = 1.00 : 0.25 : 1.14 : 1.08 : 2.16 : 2.11.

Pereopod 7 (Fig. 4C) similar to pereopod 6, but basis and merus narrower and more elongated than that of pereopod 6; length ratio of 6 articles = 1.00 : 0.18 : 0.91 : 0.77 : 1.50 : 1.16.

Abdomen (Fig. 4D) with a pair of appendages, lateral lobes and a dorsal lobe; plumose setae on dorsal lobe missing; penes medial.

Female (DKUAMP202410): Body (Figs. 2B, 4E) about 7.9 mm long; generally as in male, but stouter than male, pereonites 3, 4 with rounded brood pouches.

Gnathopod 2 (Fig. 4F) similar to that of male but each article shorter than those of male.

Abdomen (Fig. 4G) without appendage, with a pair of

Table 1. Morphological characters of *Caprella carinata* and related species.

Characters	Species (male)			
	C. acanthogaster	*C. mutica*	*C. eximia*	*C. carinata*
Body length (mm)	25.0−38.0	17.7−25.0	27.0	11.6−24.5
Antenna 1, peduncular article 3	Widening distally	Widening distally	Straight	Straight
Pereonite 1, anteriorly curved dorsal projection	×	×	×	○
Pereonite 2 and pereopod 2	Without hair	With hair	Without hair	Without hair
Gnathopod 2, poison tooth of propodus	Straight	Straight	Curved	Straight
Pereonites 2−5, lateral projection	○	○	×	○
Distribution	North-Pacific Ocean, South America	Cosmopolitan	Korea, Japan	Korea, Japan
References	Mayer, 1890, 1903; Utinomi, 1943, 1947; McCain & Steinberg, 1970; Vassilenko, 1974; Arimoto, 1976; Martin, 1977; Kim & Lee, 1978; Heo *et al*., 2020; Takeuchi *et al*., 2022	Scurin, 1935; Ashton *et al*., 2007; Peters & Robinson, 2017; Heo *et al*., 2020	Mayer, 1890, 1903; Schurin, 1937; Utinomi, 1947; Vassilenko, 1967; Arimoto, 1971, 1976; Kim & Lee, 1975	Arimoto, 1934, 1976; Present study

lateral lobes and a dorsal lobe; with a pair of plumose setae on dorsal lobe.

Remarks. *Caprella carinata* is morphologically similar to *C. acanthogaster* Mayer, 1890, *C. eximia* Mayer, 1890, and *C. mutica* Schurin, 1935, and can be characterized by the following features: (1) pereonites 2−5 with dorsal projections; (2) gnathopod 2 elongate, with propodus bearing a poison tooth and a robust (grasping) seta; (3) gill elongated; and (4) pereopods 5−7, bases with triangular process posterodistally and palms of propodi defined by a pair of midanterior robust (grasping) setae (Mayer, 1890; Arimoto, 1976; Lee and Hong, 2011; Heo *et al*., 2020). These morphological similarities often lead to misidentification among these species. However, *C. carinata* can be distinguished from these species by the following features: (1) pereonite 1 with anteriorly curved dorsal projection (vs. without dorsal projection on *C. acanthogaster*, *C. eximia*, and *C. mutica*); (2) pereonite 2 and gnathopod 2 without hair (vs. with hair on *C. mutica*); (3) pereonites 2−5 with lateral projections (vs. without lateral projection in *C. eximia*); (4) antenna 1, peduncular articles of equal thickness (vs. peduncular article 3 widening distally in *C. acanthogaster* and *C. mutica*); and (5) gnathopod 2, propodus with straight poison tooth (vs. curved poison tooth in *C. eximia*) (see Table 1).

Confusion between *Caprella acanthogaster* and *C. mutica* is common (Arimoto, 1976; Kim and Lee, 1978; Heo *et al*., 2020), and historical records have also misidentified *C. carinata* as *C. eximia* (Lee and Hong, 2011). Distinguishing these species is particularly challenging in juvenile or female specimens, underscoring the need for refined classification keys and molecular data to clarify the taxonomy of these closely related species.

Distribution. Korea (East Sea), Japan (Yamada Bay, Kuji Bay, Sunosaki to Kinkazan).

Conflicts of Interest

The authors of this paper have no affiliation with any interests and is solely responsible for the paper.

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Two new species records of the class Chromadorea Inglis, 1983 (Nematoda) from Korea

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Two known species of the free-living nematodes were collected from marine sediments in the sublittoral zone of Korea and were described and illustrated. *Actinonema pachydermatum* Cobb, 1920, which was originally described from the Key West, Florida, USA, was reported for the first time in the South Sea of Korea. It shows slight difference in body length and thickness (817−850 μm vs. 830−900 μm; 33−35 μm vs. 27−30 μm). *Sphaerolaimus gracilis* de Man, 1876, which was considered the cosmopolitan species of nematodes spread widely from the North Sea, Spitsbergen, East Sea. The present specimen differs from the redescription by Fadeeva from the East Sea in body length and body thickness (1365−1520 μm vs. 1300−1700 μm; 65−78 μm vs. 60−83 μm); the spicules length and the apophysis length of gubernaculums (55−70 μm vs. 45−55 μm, 15−20 μm vs. 13−14 μm). Studies on previously unrecorded species enhance our understanding of nematode species diversity in Korean waters.

Keywords: *Actinonema*, free-living nematodes, *Sphaerolaimus*

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Introduction

Throughout "Marine Biological Resources Survey Project", focusing on marine nematodes in Korea from 2022 to 2023, we reported more than 80 species of free-living nematodes include two unrecorded species belonging to the genus *Actinonema* and *Sphaerolaimus* from Korea. The genus *Actinonema* is distinguished by its heterogeneous cuticle with lateral differentiation, six outer labial setae, and four setaceous cephalic setae arranged in a single circle. It also features a conspicuous, transversely oval amphidial fovea with a double contour and simple, curved spicules accompanied by an L-shaped telamon. (Cobb, 1920; Tchesunov, 2014). The genus *Actinonema* was established by Cobb in 1920, with *A. pachydermatum* Cobb, 1920 designated as the type species. To date, 12 species of *Actinonema* have been described (Chu et al., 2023; Nemys eds., 2024; WoRMS Editorial Board, 2024) Currently, 39 species are recognized within the genus *Sphaerolaimus* Bastian, 1865 (Nemys eds., 2024; WoRMS Editorial Board, 2024). *Sphaerolaimus hirsutus* Bastian, 1865 was designated as the type species of the genus *Sphaerolaimus*. The genus *Sphaerolaimus* is primarily characterized by the presence of four setiform cephalic setae and eight groups of subcephalic setae, along with somatic setae that are usually present. It features a large, barrel-shaped buccal cavity, where the anterior section has longitudinal ridges, and the posterior section is heavily sclerotized and surrounded by pharyngeal muscles. Additionally, the genus has a circular amphidial fovea located at variable positions relative to the anterior end. The pharynx is cylindrical with a cuticularized inner lining. Males typi-

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cally possess two testes, curved spicules accompanied by a gubernaculum, and a tail that is generally conical or conico-cylindrical, ending with two or three terminal setae (Fonseca and Bezerra, 2014; Zograf *et al*., 2020; Tchesunov *et al*., 2024). This paper describes two known species belonging to these two genera, collected from Korean waters.

Materials and Methods

While conducting a survey of the meiofauna community along the Korean coastline, *Actinonema* specimens were collected along the coast of Samsan-myeon, Goseong-gun, Gyeongsangnam-do (34°56′2.1″N, 128°15′20.6″E, at a depth of 25 m, in the clay sediment) on 2nd May 2023 and *Sphaerolaimus* specimens were collected along the coast of Ilgwang-myeon, Gijang-gun, Busan (35°16′0.0″N, 129°16′50.0″E, at a depth of 16 m, in the clay sediment) on 30th April 2023. Sediment samples were collected using a Smith-McIntyre grab with an area of 0.1 m^2, while meiofauna samples were obtained using a hand-held corer that has a surface area of 10 cm^2 and a depth of 5 cm. All samples were promptly fixed in 4% neutral buffered formalin at room temperature. In the laboratory, meiofauna were extracted according to the protocol established by Burgess (2001) and nematodes were classified using a grid-patterned Petri dish while observing them under a stereoscopic microscope (S APO; Leica, Wetzlar, Germany). Sorted nematodes were subsequently placed in Seinhorst solution for dehydration and infiltration with glycerin, utilizing the glycerin-ethanol method (Seinhorst, 1959). Nematode specimens were then prepared on glass slides using anhydrous glycerin and glass beads (the size of the beads was chosen based on the diameter of the specimens) using a standard waxring technique (Hooper, 1986). For morphological analysis, nematode specimens were observed using a 100× oil immersion objective combined with Nomarski Differential Interference Contrast (DIC) illumination. Drawings were created using an optical microscope with a camera lucida (BX-51; Olympus, Tokyo, Japan). All morphometric measurements were acquired from the figures utilizing a digital map-measurer and Leica Application Suite Version 4.12.0 (Leica, Wetzlar, Germany). The classification follows that given in de Ley and Blaxter (2004). Abbreviations used are as follows: a - the ratio of body length to maximum body diameter; abd - anal body diameter (μm); AL - distance from anterior apex to anterior edge of amphids (μm); amp - diameter of amphidial fovea (μm); amp cbd - corresponding body diameter at the level of amphidial fovea (μm); b - body length in relation to the pharynx length; BL - buccal cavity length (μm); bcd - buccal cavity diameter (μm); c - body length divided by tail length; c′ - tail length divided by anal body diameter; car - length of cardia (μm); cbd - corresponding body diameter (μm); cs - length of cephalic setae (μm); EPL - distance of ventral excretory pore from anterior end of body (μm); gub - length of gubernacular apophyses (μm); hd - diameter at the level of cephalic setae (μm); L - total body length (μm); mbd - maximum body diameter (μm); N% - nerve ring distance from the anterior apex as percentage of pharynx length; N cbd - corresponding body diameter at nerve ring (μm); NL - nerve ring from anterior apex of body (μm); P cbd - corresponding body length at cardia (μm); (μm); s - spicule length as arc length divided by anal body width; spia - spicule length as arc (μm); TEL - length of telamon (μm); TL - tail length (μm); V% - vulva distance from anterior end as percentage of total body length.

Results and Discussion

Class Chromadorea Inglis, 1983
Subclass Chromadoria Pearse, 1942
Order Chromadorida Chitwood, 1933
Family Chromadoridae Filipjev, 1917
Genus *Actinonema* Cobb, 1920

***Actinonema pachydermatum* Cobb, 1920**

Original description (Cobb, 1920: 338–339, fig. 117a–b, Florida, USA)

Other descriptions (Wieser, 1954: 81, fig. 142a–d, Chile. Lorenzen, 1971: 290, fig. 5a–d, North Sea)

Synonymised names [*Actinonema amphidiscatum* (Schuurmans Stekhoven & Adam, 1931) Wieser, 1954; *Actinonema fragile* (Allgén, 1929) Wieser, 1954; *Adeuchromadora megamphida* Boucher & Bovée, 1972; *Euchromadora fragilis* Allgén, 1933; *Pareuchromadora amphidiscata* Allgén, 1940; *Pareuchromadora amphidiscata* Schuurmans Stekhoven & Adam, 1931; *Pareuchromadora fragilis* (Allgén, 1934); *Spiliphera fragilis* Allgén, 1929]

Locality. The coast of Samsan-myeon, Goseong-gun, Gyeongsangnam-do: sub-tidal Eastern part of South Sea, Korea (34°56′2.1″N, 128°15′20.6″E), at a depth of 25 m, in the clay sediment.

Material. Three males (MABIK NA00158042, MABIK NA00158708–MABIK NA00158709) and One female (MABIK NA00158710) collected by Jung-Ho Hong at the coast of Goseong on 2nd May 2023, from Research Vessel Haeyanghwnangkyung 2.

Measurements. Check Table 1 for detailed measurements and morphometric ratios.

Description. Males (Fig. 1, Table 1). Body cylindrical, uniform in thickness, gradually narrowing toward both ends; anterior apex blunt. Cuticle heterogeneous and com-

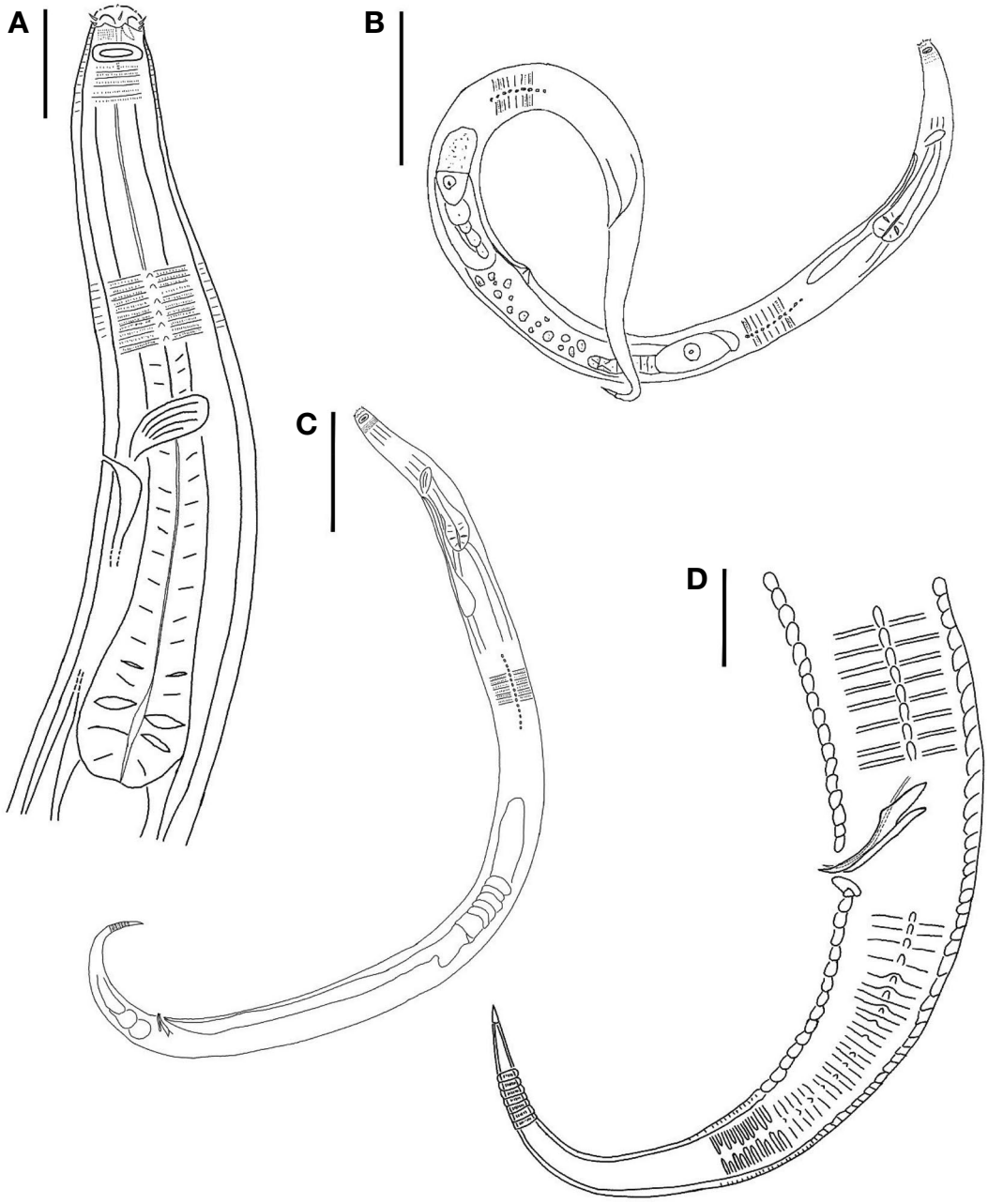


Fig. 1. *Actinonema pachydermatum* Cobb, 1920 from the South Sea, Korea: A, Male pharynx; B, Male habitus; C, Female habitus; D, Male tail. Scale bars: 20 μm (A, D) and 100 μm (B, C).

plex, with irregular minute dots beginning at the anterior border of amphids. Transverse dots present in the anterior part of the pharynx; lateral differentiation starts anterior to the nerve ring (~50–60 μm from the anterior apex) with a reversed 'V' shape. Double reversed 'V' shaped lateral differentiation present from mid-body to anus, transitioning to irregular differentiation from near the anus to the posterior tail. Lateral differentiation diminishes in the distal two-thirds of the tail. Somatic setae inconspicuous, minute, and scattered. Labial region with six inner labial papillae and six outer labial setae (~3 μm) in a single circle, accompanied by four cephalic setae (~2 μm). Six solid cones surround the labial region. Stoma conical, bearing a sharply pointed dorsal tooth. Amphideal fovea conspicuous, transversely oval with a double contour, 10–11 μm wide (75–80% of cbd), located posterior to the cephalic setae circle. Pharynx cylindrical, 130–135 μm long, with a distinct posterior bulb. Cardia inconspic-

Table 1. Morphometrics of *Actinonema pachydermatum* Cobb, 1920 from South Sea, Korea and *A. pachydermatum* Cobb, 1920 from Florida and Chile.

Characters	*A. pachydermatum* from South Sea, Korea		*A. pachydermatum* from Florida[a]	*A. pachydermatum* from Chile[b]
	♂; n = 3	♀; n = 1	♂; n = ?, ♀; n = ?	♂; n = 1, ♀; n = 3
L	820–850	817	830–900	610–790
mbd	33–35	34	27–30	27–30
hd	9–10	9	n/a	11.5
cs	2–3	2	n/a	n/a
amp	9–10	9	n/a	9–10
amp cbd	12–13	12	n/a	12–13
amp%	75–80	75	n/a	70
NL	50–60	60	n/a	n/a
N cbd	27–30	28	n/a	n/a
EPL	55–68	65	n/a	n/a
PL	130–145	155	142–147	132
P cbd	33–34	33	n/a	n/a
V%	n/a	51.5	n/a	50.6–50.7
abd	25–27	30	n/a	n/a
spia	25–30	n/a	n/a	21
s	1.0–1.2	n/a	n/a	n/a
TEL	27–31	n/a	n/a	n/a
gub	20–23	n/a	n/a	n/a
TL	135–140	145	134–156	112–124
a	24–25	24	24–34	15–29
b	5.9–6.3	5.2	6.3	5.1–5.9
c	6.0–6.3	5.6	5.3–6.7	5.9–7.0
c′	5.2–5.4	4.8	n/a	n/a

All measurements in μm unless otherwise stated, except ratio a, b, c, c′, s. n/a: not applicable. ?: unknown. Literature sources: [a]Cobb, 1920; [b]Wieser, 1954.

uous. Excretory pore located just posterior to the nerve ring, 80–85 μm from the anterior body end. Reproductive system monorchic; single testis situated on the left side of the intestine. Spicules thin, curved, measuring 25–30 μm; talamons 27–31 μm long. Gubernaculum 20–23 μm, without apophyses. Precloacal supplements absent. Tail conical, tapering to a pointed tip, with three caudal glands and a well-developed spinneret.

Females (Fig. 1, Table 1). Overall features similar to males. Reproductive system amphidelphic. Ovaries located to the left of the intestine, reflexed. Vulva slightly posterior to mid-body, positioned at 51.5% of body length.

Remarks. To date, the genus *Actinonema* Cobb, 1920 contains 12 valid species: *A. celtica* Boucher, 1976, *A. diplobulba* Chu, Hao and Huang, 2023, *A. dolichurum* Gagarin and Long, 2017, *A. falciforme* Shi, Yu and Xu, 2018, *A. fidatum* Vitiello, 1970, *A. grafi* Jensen, 1991, *A. longicaudatoides* Gagarin, 2015, *A. longicaudatum* (Steiner, 1918) Wieser, 1954, *A. pachydermatum* Cobb, 1920, *A. paraceltica* Muthumbi and Vincx, 1998, *A. parvum* Gagarin, 2015 and *A. smolae* Muthumbi and Vincx, 1998 (WoRMS Editorial Board, 2024; Nemys eds., 2024). Specimens from the eastern part of the South Sea, Korea, generally match the original description by Cobb (1920), with the exception of differences in body width (33–35 μm vs. 27–30 μm) and slight variation in body length (817–850 μm vs. 830–900 μm). These differences are considered intraspecific variations.

Class Chromadorea Inglis, 1983
Subclass Chromadoria Pearse, 1942
Order Monhysterida Filipjev, 1929
Family Sphaerolaimidae Filipjev, 1918
Genus *Sphaerolaimus* Bastian, 1865

***Sphaerolaimus gracilis* de Man, 1876**

Original description (de Man, 1876: 179–181, fig. 47a–d, North Sea)

Other descriptions (de Man, 1922: 235–236, fig. 23a–b, North Sea. Stekhoven, 1935: 148, fig. 325a–c, North Sea. Stekhoven, 1942: 228, fig. 10a–c, Mediterranean Sea. Gerlarch, 1957: 172, fig. 121l–m, Brazil. Loof,

Table 2. Morphometrics of *Sphaerolaimus gracilis* de Man, 1876 from the East Sea, Korea, and comparisons with populations from Spitsbergen, the North Sea, the Baltic Sea, and the East Sea.

Characters	*S. gracilis* from East Sea, Korea	*S. gracilis* from Spitsbergen[a]	*S. gracilis* from North Sea, Group 1[b]	*S. gracilis* from North Sea, Group 2[b]	*S. gracilis* from North Sea, Group 3[b]	*S. gracilis* from East Sea[c]	*S. gracilis* from Baltic Sea[d]
	♂; n = 3, ♀; n = 0	♂; n = 1, ♀; n = 0	♂; n = 2, ♀; n = 1	♂; n = 2, ♀; n = 1	♂; n = 1, ♀; n = 2	♂; n = 5, ♀; n = 0	♂; n = 14, ♀; n = 22
L	1365–1520	1559	725–800	1105–1360	870–940	1300–1700	972–1668
mbd	65–78	54	34	44–57	48	60–83	34–76
hd	24–26	23	17	24	20	13–25	16–24
cs	4–5	8.5	4–8	6–8	4–8	4–5	5.5–6.5
sub cep	15–20	17–21	10–12	10–15	17–21	19–20	15–16
AL	23–33	36	25	44	30	25–38	25–38
amp	8–11	10	8	8	9	8–11	6–8
amp cbd	33–39	33	25	40	33	25–38	30–36
BL	19–24	23	25	25	23	18–23	n/a
NL	140–181	n/a	n/a	n/a	n/a	138–175	n/a
N cbd	48–60	n/a	n/a	n/a	n/a	35–63	n/a
EPL	160–185	164	n/a	n/a	n/a	150–178	152–190
PL	280–335	353	150	225–330	215	261–368	192–340
P cbd	55–62	50	50	43–57	45	40–68	32–72
V%	n/a	n/a	63	68	69–70	n/a	64–72
abd	45–65	44	28	39–51	39	45–50	27–55
spia	55–70	55	29–40	56–83	47	45–55	47–64
s	1.0–1.2	1.3	1.0–1.4	1.4–1.5	1.2	0.9–1.1	1.1–1.7
gub	15–20	26	13	20	25	13–14	13–14
TL	190–215	167	100	150–155	115	145–177	120–185
a	17–23	29	21–27	17–25	18–20	17–21	18–32
b	4.5–4.9	4.4	4.5–4.8	3.2–4.9	3.9–4.0	4.4–6.3	4.2–5.1
c	7.0–8.0	9.3	7.0–7.6	7.2–8.0	7.5–8.2	7.8–10.4	7.1–9.1
c′	3.3–4.2	3.7	3.7	3.0–3.8	2.9	2.9–3.9	2.9–3.9

All measurements in μm unless otherwise stated, except ratio a, b, c, c′, s. n/a: not applicable. Literature sources: [a]Gerlach, 1965; [b]Lorengen, 1969; [c]Fadeeva, 1983; [d]Turpeenniemi, 1997.

1961: 198–199, fig. 10a–b, North Sea, reexamination of type specimen. Gerlarch, 1965: 170, fig. 44a–c. Lorenzen, 1969: 209, fig. 10a–d, North Sea. Fadeeva, 1983: 1324–1326, fig. 2a–d, East Sea. Turpeenniemi, 1997: 72–81, fig. 4a–h, Baltic Sea)

Synonymised names (*Sphaerolaimus buetschlii* Schulz, 1932; *Sphaerolaimus demani* Filipjev, 1922; *Sphaerolaimus setosus* Paramonov, 1927)

Locality. The coast of Ilgwang-myeon, Gijang-gun, Busan: sub-tidal zone Southern part of East Sea, Korea (35°16′ 0.0″N, 129°16′50.0″E), at a depth of 16 m, in the clay sediment.

Material. Three males (MABIK NA00158050, MABIK NA00158715–MABIK NA00158716) collected by Jung-Ho Hong at the coast of Gijang on 30th April 2023, from Research Vessel Haeyanghwnangkyung 3.

Measurements. See Table 2 for detailed measurements and morphometric ratios.

Description. Males (Fig. 2, Table 2). Body cylindrical, gradually tapering towards the tail end, about 1365–1520 μm long and 65–78 μm wide at the maximum body diameter. The cuticle finely striated with lateral alae measuring 10–15 μm in width, starting from the cervical region and extending to the base of the tail. Somatic setae in eight longitudinal rows, about 6–12 μm long in the cervical region, 5–7 μm long in the mid and tail part. The anterior end rounded with six small lips encircle the oral opening. Lip region rounded with six inner labial papillae. Six outer labial papillae and four cephalic setae (4–5 μm long) in one circle, anterior to buccal cavity. Subcephalic setae in eight groups situated, 2–3 setae (14–20 μm long) in each group. A series of longitudinal ridges located just in front of the buccal cavity. Buccal cavity barrel-shaped,

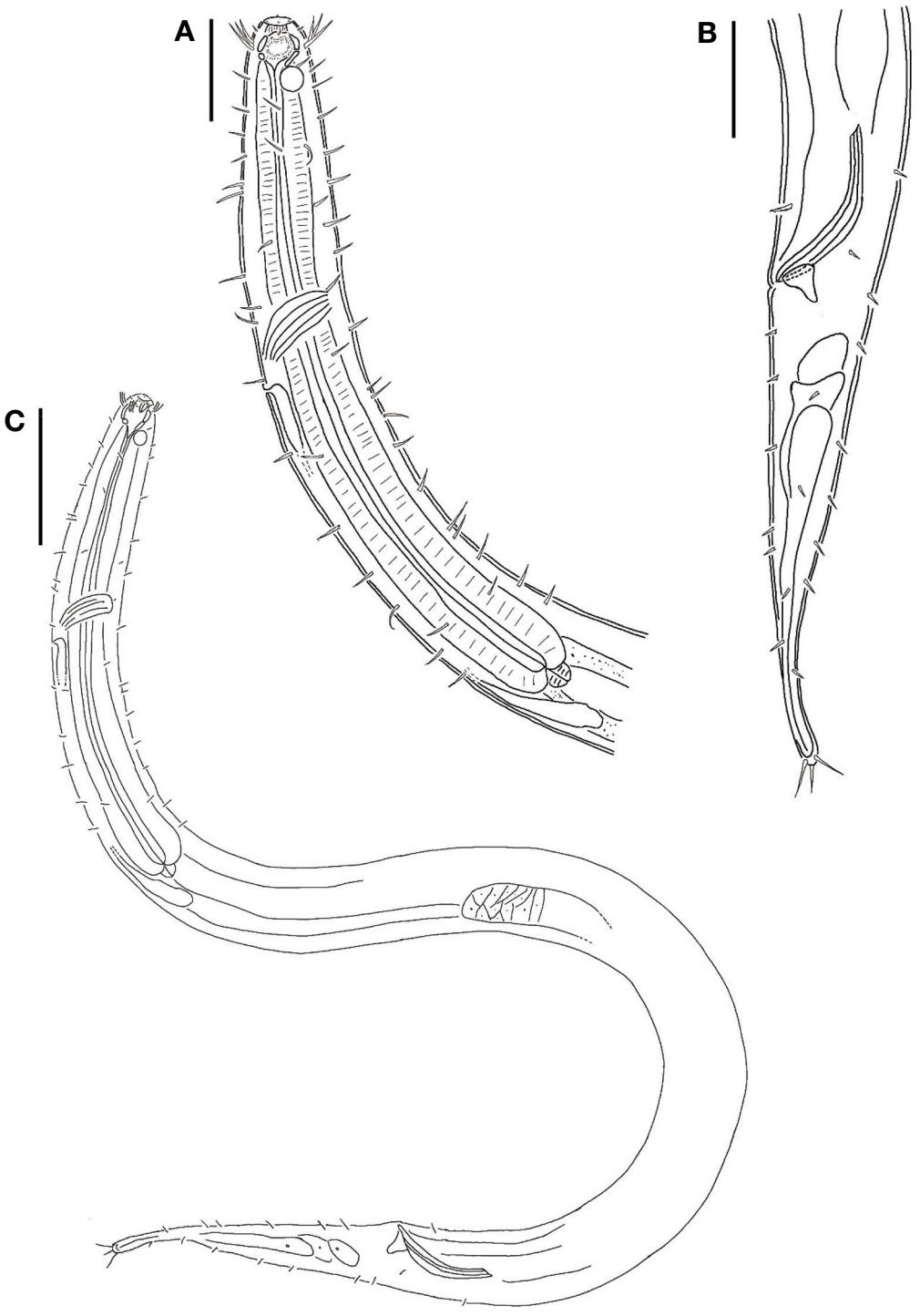


Fig. 2. *Sphaerolaimus gracilis* de Man, 1876 from the East Sea, Korea: A, Male pharynx; B, Male tail; C, Male habitus. Scale bars: 50 μm (A, B) and 100 μm (C).

23–32 μm long, 20–25 μm wide composed in two parts, thick-walled anterior part and a sclerotized, thin-walled posterior part. Amphideal fovea circular, 8–11 μm in diameter or 24–28% of corresponding body diameter, located 23–33 μm from anterior apex. Pharynx nearly cylindrical, gradually expanding towards to the cardia, strongly muscular throughout its entire length. Cardia small, rounded-conoid, surrounded by intestinal tissue. Nerve ring at 50–54% of the pharynx length (140–181 μm long). Renette cell slightly behind the base of the pharynx, small oval

shape, lateral to the intestine. Excretory pore near posterior to the nerve ring. Testes paired. Vas deferens well-developed. Spicules curved, 60−85 μm long along arch. Gubernaculum with dorsal-caudally directed apophysis 20−25 μm long. Precloacal supplements absent. Tail length 190−215 μm, conical anteriorly and cylindrical posteriorly, pointed terminal end with three terminal setae (12−15 μm long). Three caudal glands open to the spinneret.

Remarks. *Sphaerolaimus gracilis* de Man, 1876 is characterized by the position of the amphid posterior to the buccal cavity; the length of spicules shorter than twice of anal body diameter; spicules not double jointed; spicules with consistent thickness; gubernaculum present; gubernaculum with straight dorsal apophyses (Zograf *et al*., 2020). Based on the original description by de Man in 1876 from the brackish water of Walcheren Island, Netherlands, other descriptions made by many authors in the North Sea, Spitsbergen, the East Sea, and so on. Our specimens from the East Sea, Korea are similar to the description by Fadeeva in 1983 from the East Sea, but slightly smaller in body width (65−75 μm vs. 60−83 μm) and body length (1365−1520 μm vs. 1300−1700 μm). There is also a slight difference in spicules length (55−70 μm vs. 45−55 μm) and the apophysis length of the gubernaculum (15−20 μm vs. 13−14 μm).

Conflicts of Interest

The authors of this paper have no affiliation with any interests and is solely responsible for the paper.

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A new species of the genus *Synanthedon* Hübner, 1819 (Lepidoptera: Sesiidae) from Korea

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Synanthedon namdoelegans sp. nov., a new clearwing moth, is described from southern South Korea. It is closely related to *Synanthedon soffneri* Špatenka, 1983, *Synanthedon bastak* Gorbunov & Koshkin, 2023, and *Synanthedon velox* Fixsen, 1887, but can be distinguished by differences in the forewing's black band, the hindwing's discoidal vein spot, and male genitalia. The female and larval stages are unknown. DNA barcode sequences show minimal variation (0.46%) within *S. namdoelegans*, with divergence from other *Synanthedon* species ranging from 1.98% to 12.46%. Phylogenetic analysis shows separation of *S. namdoelegans* from its closest relatives, *Synanthedon fulvipes* Harris, 1839 and *Synanthedon culiciformis* Linnaeus, 1758. These differences provide clear morphological and molecular separation from other *Synanthedon* species.

Keywords: clearwing moths, Lepidoptera, new species, South Korea, *Synanthedon namdoelegans*, Synanthedonini, taxonomy

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Introduction

The genus *Synanthedon* Hübner, 1819 [1816] (Lepidoptera: Sesiidae) was designated with the type species *Sphinx oestriformis* Rottemburg, 1775 (a junior synonym of *Sphinx vespiformis* Linnaeus, 1761) and comprises 234 species and nine subspecies worldwide (Pühringer and Kallies, 2024). Sixteen and eight species of the genus *Synanthedon* are known from the Russian Far East and Korea (Arita *et al*., 2004; Gorbunov and Koshkin, 2023). The species of the genus are distinguished by a well-developed proboscis, transparent forewing, and nearly identical venation of fore- and hindwings (Gorbunov and Koshkin, 2023). Since this genus is species-rich within the family, question regarding its monophyly has been raised (Kallies, 2020) and the genus was divided into several taxa (Gorbunov and Arita, 1999; 2000; 2005; McKern and Szalanski, 2008; McKern *et al*., 2008; Taft *et al*., 2016; Lait and Hebert, 2018; Cognato *et al*., 2023; Kim *et al*., 2024).

In 2023 and 2024, field surveys were conducted to investigate *Synanthedon bicingulata* (Staudinger, 1887), a species known to cause a significant damage to urban trees, particularly cherry trees in city forests and parks (Lee *et al*., 2004; Korea Forest Service, 2023). During these surveys, adult male specimens of *Synanthedon* were

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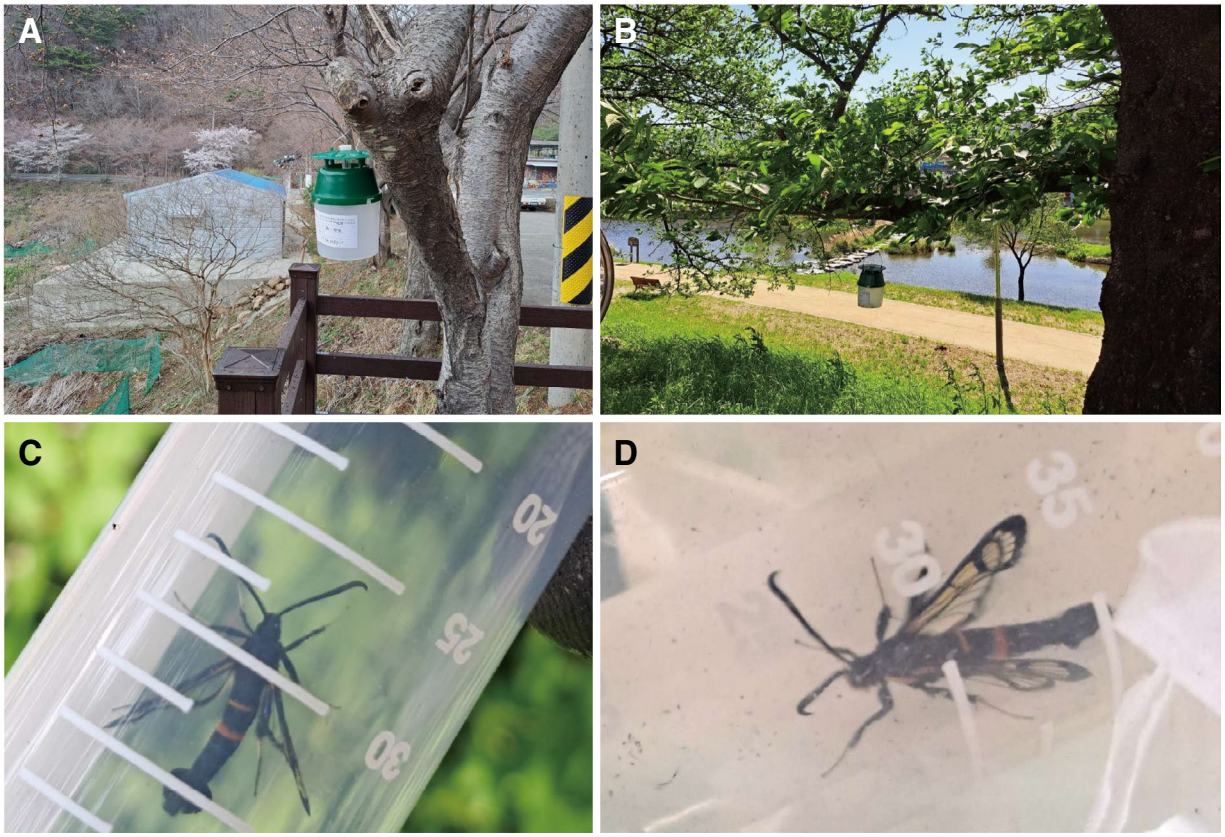


Fig. 1. *Synanthedon namdoelegans* collected in pheromone trap. (A) A pheromone trap installed on cherry tree *Prunus* × *yedoensis*. (B) A riverside, where a pheromone trap was installed. (C) and (D) An adult male transferred to a conical tube.

collected using sex pheromone traps, a method commonly employed in lepidopteran pest surveys. While their overall characteristics were similar to those of *S. velox* Fixsen, 1887 as redefined by Lee *et al.* (2011), notable morphological differences were detected. In this study, *Synanthedon namdoelegans* sp. nov. is described as new. All available information is presented, including the collection locations, and illustration of male and its genitalia. DNA barcodes are provided for precise identification.

Materials and Methods

Specimen collection and morphological analysis

Six males of *S. namdoelegans* were collected from April 2023 to May 2024 using funnel traps (170 × 250 mm) installed with the pheromone lure specific to *S. bicingulata* (Green Agrotech, Gyeongsan, South Korea). Traps were installed on the branches of the cherry tree, *Prunus* × *yedoensis*, and neighboring trees at a height of 1.2 to 1.5 meters above the ground (Fig. 1). Three non-*S. bicingulata* from Suncheon (34°58′44.4″N, 127°23′06.7″E) (MNU14991, CNU17888, and CNU17889), two from Naju (35°02′10.7″N, 126°42′22.7″E) (MNU14989 and MNU14990), and one from Jangseong (35°18′17.6″N, 126°46′40.1″E) (CNU17891) in Jeollanamdo Province, South Korea, were collected in the *S. bicingulata* traps. For adult morphological examination, one specimen of *S. namdoelegans*, which was collected from Naju (MNU14990) was used. Type specimens were deposited at Mokpo National University, Muan, Korea under the voucher specimen numbers MNU14989 and MNU14990 from Naju and MNU14991 from Suncheon. Adult images of *S. namdoelegans* (MNU14990) were taken with a Canon EOS 70D camera and a 100 mm-macro lens. For male genitalia examination, the abdomen of MNU14990, one of the individuals collected from Naju, was boiled in 10% KOH solution for approximately 20 min, removed scales and tissues, were stained with Chlorazol black, and mounted on slides in Euparal solution (Miller, 1996).

Molecular experiment

To ensure an accurate species identification, we sequenced the *COI* (DNA barcoding region) and *ND1* fragments in the mitochondrial genome and compared the genetic divergence of currant samples to those of

Synanthedon species that are publicly available. DNA sequencing was performed for four specimens, two from Suncheon, one from Naju, and one from Jangseong. The leftover tissues and DNA were deposited at Chonnam National University, Gwangju, Korea under the sample numbers CNU17888 and CNU17889 for Suncheon samples, CNU17890 for Naju sample, and CNU17891 for Jangseong sample. The specimen, MNU14990, which was used for the examination of morphological features, was also used for DNA sequencing analysis (CNU17890). Total DNA was extracted from the hind legs using the Wizard™ Genomic DNA Purification Kit (Promega, WI, USA).

The DNA barcode region is part of *COI* gene (658 bp), while *ND1* fragment (585 bp) contains a portion of the 3′ end of the *16S rRNA* gene, whole $tRNA^{Leu}$ and a portion of the 5′ end of the *ND1* gene. Primers for DNA barcode region were adapted from Hebert *et al*. (2004): LepF1 (forward primer), 5′-ATTCAACCAATCATAAAGATATTGG-3′ and LepR1 (reverse primer), 5′-TAAACTTCTGGATGTCCAAAAAATCA-3′. Primers for *ND1* fragment were adapted from Weller *et al*. (1994): forward primer, 5′-TAGAATTAGAAGATCAACCAGC-3′ and reverse primer, 5′-ACATGATCTGAGTTCAAACCGG-3′.

PCR was conducted under the following conditions using the AccuPower® PCR PreMix (Bioneer, Daejeon, South Korea): an initial denaturation step at 94°C for 5 min, 35-cycle amplification (94°C for 1 min, 48−56°C for 1 min, and 72°C for 1 min), and the final extension step continued for 7 min at 72°C. Electrophoresis was conducted to confirm successful DNA amplification using 0.5× TAE buffer on 1% agarose gel. The PCR products were purified using a PCR Purification Kit (Qiagen, Hilden, Germany). DNA sequencing for both strands was conducted using the ABI PRISM BigDye Terminator ver. 3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyzer (PE Applied Biosystems, CA, USA).

Sequence analysis

The boundary of each directional sequence was delimitated using the SeqMan software from the DNASTAR package (SeqMan NGen®, ver. 13.0, DNASTAR, WI, USA), and both directional sequences of each individual were aligned using MAFFT ver. 7 (Katoh *et al*., 2002) to have a complete individual sequence. A search of available public sequence databases, such as GenBank, through BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the BOLD system (https://v4.boldsystems.org/) was conducted to obtain the sequences belonging to the genus of *Synanthedon*. Forty-five DNA barcode sequences and six *ND1* fragment sequences were obtained from GenBank, but one sequence for DNA barcode sequence was obtained from BOLD system (*S. velox*, unpublished, BOLD system acc. no. AEZ9646). Sequence divergence was analyzed by unrooted pairwise comparison using PAUP ver. 4.0b (Swofford, 2002).

Phylogenetic analysis

Phylogenetic analysis was conducted with DNA barcoding sequences, consisted of 46 downloaded and four current data with the addition of two congeneric species (*Sophona snellingi* and *S. greenfield* (unpublished; GenBank acc. no. HQ564703 and HQ564718) as outgroups. The GTR + GAMMA + I model was selected using PartitionFinder 2 with the greedy algorithm (Lanfear *et al*., 2017), which is integrated into PhyloSuite, ver. 1.2.3 (Xiang *et al*., 2023) and applied to IQ-TREE (Nguyen *et al*., 2015). Branch supports were assessed using 1,000 replicates of Ultrafast Bootstrap (UFBoot) (Minh *et al*., 2013) and the Shimodaira-Hasegawa approximate likelihood ratio test (SH-aLRT) (Guindon *et al*., 2010). The phylogenetic tree was visualized using FigTree ver. 1.42 (http://tree.bio.ed.ac.uk/software/figtree/accessed 25 October 2024).

Results and Discussion

Systematics

Taxonomy account

Synanthedon Hübner, [1819]

Synanthedon Hübner, [1819], Verz. bek. Schmett. (9): 129. TS. *Sphinx oestriformis* Rottemburg, 1775 (= *Synanthedon vespiformis* (Linnaeus, 1761)).

Korean name: Nam-do-meot-jaeng-i-yu-ri-na-bang

Synanthedon namdoelegans Kim, Kim and Choi sp. nov.
urn:lsid:zoobank.org:pub:C425E3CC-2FB4-4CFE-8B1F-5121298651E6

Type material. Holotype. ♂ [MNU14991]. Suncheon, Jeollanamdo Province, 30. IV. 2024, Kim, Woo Jin leg. **Paratypes.** Suncheon, Jeollanamdo Province, 2♂ [CNU 17888, CNU17889], 26. IV. 2023, Kim, Woo Jin leg.; Naju, Jeollanamdo Province, 1♂ [MNU14989], 1. V. 2023; 1♂ [MNU14990], 30. IV. 2024, Kim, Woo Jin leg.; 1♂ [CNU 17891], Jangseong, Jeollanamdo Province, 1. V. 2024, Kim, Woo Jin leg.

Diagnosis. Superficially, this new species resembles *S. soffneri* Špatenka, 1983, and *S. bastak* Gorbunov & Koshkin, 2023. However, it can be distinguished by subtle differences in the thickness and shape of the black band along the forewing's outer margin and the spot on the discal vein of the hindwing. Additionally, while the male genitalia are similar to those of *S. velox*, the shapes

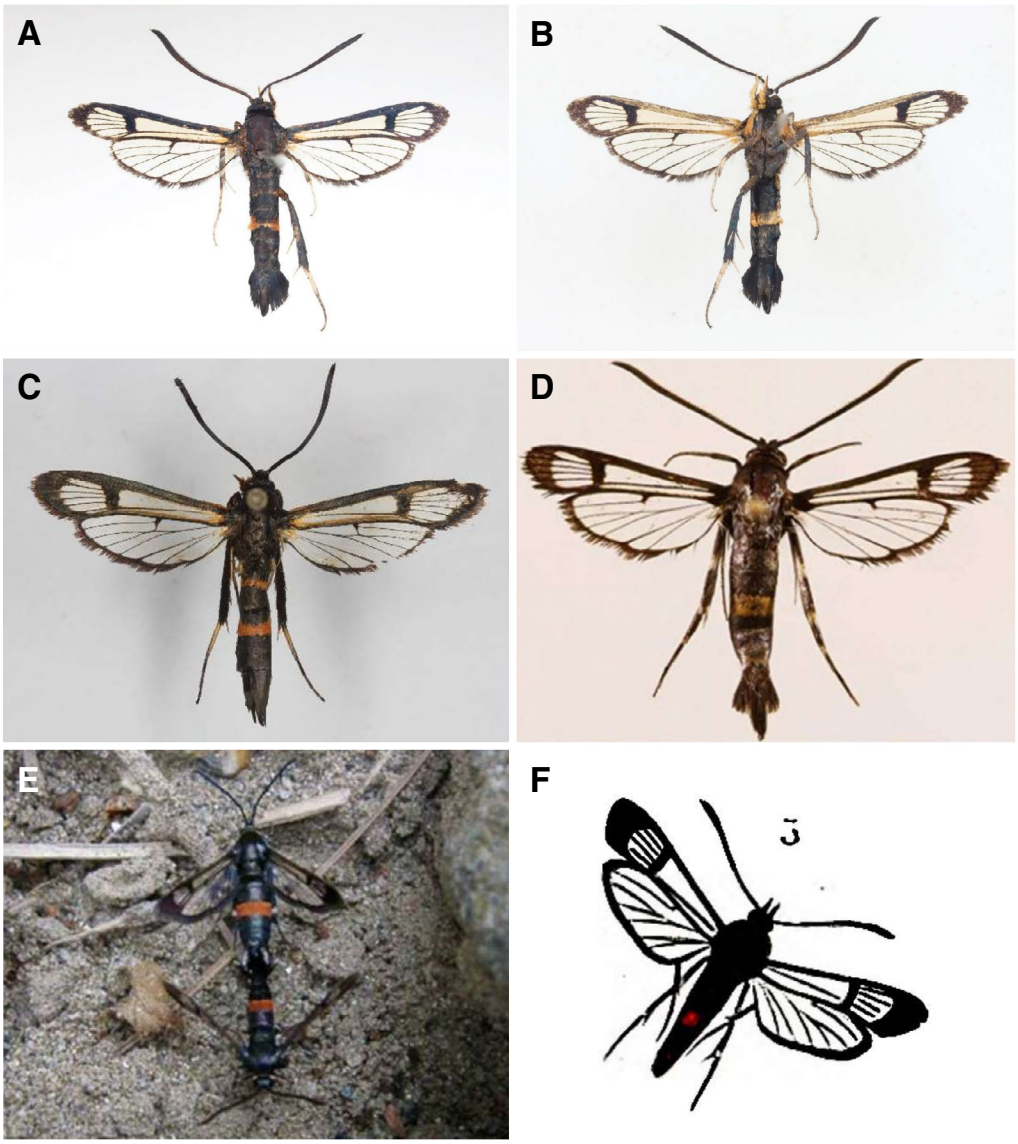


Fig. 2. *Synanthedon namdoelegans* collected in this study, along with available public data of *S. velox*. (A) and (B) The dorsal and ventral image of *S. namdoelegans* adult male, respectively (voucher specimen no. MNU14990). Photographed by one of the authors, Sung-Soo Kim. (C) The dorsal image of the proposed *S. velox* adult female collected from South Korea and registered in the BOLD system. Photographed by Sesiidae Research Group. (D) The dorsal image of *S. velox* adult male collected from South Korea and reported in Lee *et al.* (2011). (E) Copulation of *S. velox* on the ground, reported in Lee *et al.* (2011). (F) A schematic illustration of *S. velox* adult reported in Fixsen (1887).

of the crista sacculi and saccus differ.

Description. Holotype (Fig. 2A and 2B). Alar expanse 26 mm; body length 14.5 mm; forewing 11.5 mm; antenna about 9 mm. **Head**: Antennae black with a blue sheen, scapus covered in black scales; frons black, narrowly covered with white scales between frons and the compound eyes; vertex dark brown to black. Labial palpus dorsally dark brown, ventrally covered with pale orange scales, with some black scales on the sides; pericephalic scales black.

Thorax: Dorsally, laterally, and ventrally all black; patagia black; tegula and thoracic dorsum devoid of scales, laterally clearly marked with nearly triangular yellow scales, accompanied by a band of long pale yellow hairs anteriorly. Fore-, mid-, and hind-tarsi with yellow and black scales, otherwise all black, except for a few yellow scales on the ventral surface of the distal third of each femur; spurs black at the base but mostly yellow. Forewing glossy bluish with transparency; costal margin black, with a few indistinct yellow scales near the base; hind margin black, with a yellowish tinge at the basal third; discal spot black, almost as wide as the costal margin, with a

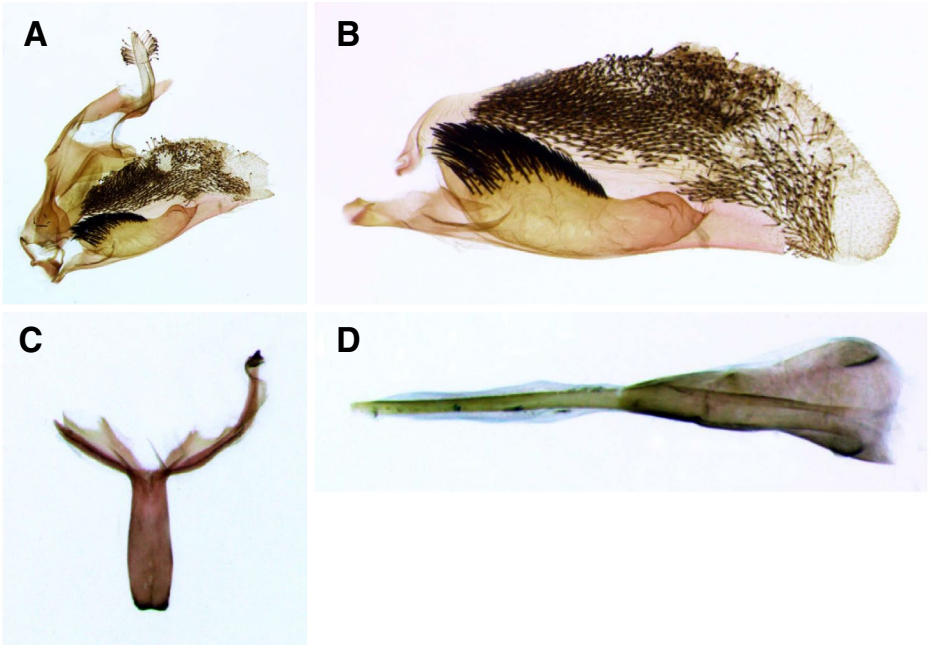


Fig. 3. Male genitalia of *Synanthedon namdoelegans*. (A) Tegumen-uncus complex. (B) Valva. (C) Saccus. (D) Aedeagus.

few yellow scales distally; veins black; outer margin dark brown; hind margin of the discoidal cell more strongly yellowish than other veins, turning yellow toward the base; cilia blackish-brown. Hindwing glossy bluish with transparency; discal spot narrow, tapering distally; veins and outer margin dark brown; cilia black, becoming longer with a few yellow hairs toward the underside. Underside of wings similar to upperside but with more abundant yellow scales at the base. Abdomen: Black with a bright blue sheen; tergite 1 yellow on the posterior third; tergites 3 and 5 each with a broad yellow stripe posteriorly; sternites black, except for sternite 5, which is orange with scattered black scales; anal tuft black with a bright blue sheen.

Male genitalia (Fig. 3): Tegumen-uncus complex not very wide; scopula androconialis less developed, measuring less than half of the tegumen-uncus complex; crista gnathi medialis short; crista gnathi lateralis slightly narrower than crista gnathi medialis; valva elongated and trapeziform-oval; crista sacculi high and pocket-shaped, not separated from the sensory field of setae proximally; saccus slightly thick, with a somewhat bifurcate basally, and slightly shorter than the vinculum. Aedeagus narrow, one-third the width of the valva; vesica without a cornutus.

Distribution. Korea (Jeollanamdo Province (Suncheon-si, Naju-si, and Jangseong-gun)].

Remarks. Most of the specimens suffered damage to their wings and bodies as a result of being trapped by the pheromone lures.

Etymology. The meaning of the name of this new species is derived from the combination of the collection site, southern part of Korea, Jeollanamdo Province, and its fine clear wings, "elegant".

Molecular data. The barcode of *Synanthedon namdoelegans* sp. nov.

Molecular analysis

DNA barcode sequences of *S. namdoelegans* specimens were either identical or had a maximum difference of only 0.46% (3 bp) (Table 1). Comparison of *S. namdoelegans* sequences to those of other *Synanthedon* species showed the sequence divergence ranging from 0.3% (2 bp; *S. velox*, unpublished, BOLD system acc. no. AEZ9646) to 12.46% (82 bp; *S. refulgens*, Lait and Hebert, 2018, MH592815). Thus, the BOLD system-registered *S. velox* sequence is well within the divergence range of *S. namdoelegans* sequences. However, after excluding *S. velox*, the minimum sequence divergence increased to 1.98% (13 bp; *Synanthedon fulvipes* Harris, 1839, unpublished, JF848560) (Table 1). Therefore, our divergence estimates strongly indicate that the proposed *S. velox* registered in BOLD system is highly likely to be *S. namdoelegans*, given that no other *S. velox* sequence is available.

For comparison of the *ND1* fragment, four sequence sequences of *S. namdoelegans* and six individual sequences in three *Synanthedon* species registered in public data were used. *S. namdoelegans* sequences were either identical or had maximum difference of only 0.52% (3 bp), while comparison of *S. namdoelegans* to other species

Table 1. Pairwise comparisons among barcode sequences of *Synanthedon* species (658 bp) obtained from current study and public data.

No.	Species	Accssion no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	*S. namdoelegans*	This study	–	0	0	0.46	0.30	7.90	7.90	9.42	10.18	10.03	7.30	8.83	9.27	8.82	9.27	2.13	7.60	10.33	8.82	9.42
2	*S. namdoelegans*	This study	0	–	0	0.46	0.30	7.90	7.90	9.42	10.18	10.03	7.30	8.83	9.27	8.82	9.27	2.13	7.60	10.33	8.82	9.42
3	*S. namdoelegans*	This study	0	0	–	0.46	0.30	7.90	7.90	9.42	10.18	10.03	7.30	8.83	9.27	8.82	9.27	2.13	7.60	10.33	8.82	9.42
4	*S. namdoelegans*	This study	3	3	3	–	0.76	8.06	8.21	9.27	10.03	9.88	7.60	8.98	9.57	8.97	9.27	1.98	7.60	10.33	9.27	9.27
5	*S. velox*	AEZ9646	2	2	2	5	–	7.90	8.21	9.42	10.18	10.03	7.30	9.13	9.27	8.82	9.27	2.13	7.90	10.03	8.82	9.42
6	*S. soffneri*	KT782535	52	52	52	53	52	–	10.49	11.09	11.09	10.94	10.18	10.35	11.55	11.09	12.01	7.90	10.33	11.40	10.18	10.64
7	*S. formicaeformis*	HM873903	52	52	52	54	54	69	–	10.94	11.85	11.40	9.73	11.42	10.49	10.79	10.79	8.66	8.21	11.09	9.57	9.27
8	*S. scoliaeformis*	HM873899	62	62	62	61	62	73	72	–	11.40	11.55	10.33	10.96	12.01	10.94	10.79	9.42	8.97	11.55	11.25	11.70
9	*S. cruciate*	GU731637	67	67	67	66	67	73	78	75	–	0.46	11.40	6.09	12.01	12.61	12.92	9.57	9.27	13.98	12.61	12.61
10	*S. loranthi*	GU731629	66	66	66	65	66	72	75	76	3	–	11.25	5.94	11.85	12.46	12.77	9.42	9.12	13.83	12.61	12.46
11	*S. polaris*	MZ610629	48	48	48	50	48	67	64	68	75	74	–	10.81	10.64	10.94	10.79	8.36	8.66	11.85	9.42	11.85
12	*S. tipuliformis*	MZ609921	58	58	58	59	60	68	75	72	40	39	71	–	12.18	11.72	12.48	8.98	9.59	12.18	11.72	10.96
13	*S. vespiformis*	OU906976	61	61	61	63	61	76	69	79	79	78	70	80	–	11.55	11.25	8.97	9.12	12.01	10.79	13.22
14	*S. novaroensis*	GU661886	58	58	58	59	58	73	71	72	83	82	72	77	76	–	4.56	8.36	9.27	12.16	10.03	12.16
15	*S. pini*	JN279256	61	61	61	61	61	79	71	71	85	84	71	82	74	30	–	8.97	8.97	11.85	10.49	12.16
16	*S. fulvipes*	JF848560	14	14	14	13	14	52	57	62	63	62	55	59	59	55	59	–	7.60	9.88	9.12	9.42
17	*S. acerrubri*	JF848555	50	50	50	50	52	68	54	59	61	60	57	63	60	61	59	50	–	10.94	8.66	10.64
18	*S. scitula*	HQ977589	68	68	68	68	66	75	73	76	92	91	78	80	79	80	78	65	72	–	11.25	12.77
19	*S. sapygaeformis*	HQ977588	58	58	58	61	58	67	63	74	83	83	62	77	71	66	69	60	57	74	–	11.85
20	*S. rubrofascia*	HQ977587	62	62	62	61	62	70	61	77	83	82	78	72	87	80	80	62	70	84	78	–
21	*S. kathyae*	HQ977584	58	58	58	57	57	72	65	61	73	72	56	75	64	63	58	57	54	68	63	77
22	*S. exitiosa*	HQ977582	49	49	49	51	49	67	60	60	69	68	65	66	65	59	60	52	46	69	63	71
23	*S. decipiens*	HQ977579	57	57	57	59	57	74	58	74	72	72	67	76	72	75	72	54	55	79	55	70
24	*S. acerni*	HQ977575	53	53	53	53	53	63	59	67	66	65	62	68	72	61	58	56	50	66	69	73
25	*S. albicornis*	HQ977574	65	65	65	67	63	79	67	79	78	77	70	83	74	82	79	63	68	78	68	78
26	*S. rhododendri*	HQ957068	64	64	64	65	64	71	74	71	74	73	70	76	77	69	65	65	61	81	70	77
27	*S. mellinipennis*	HM902931	72	72	72	73	72	73	77	84	88	88	74	85	78	78	81	73	71	89	70	77
28	*S. bibionipennis*	HM902929	75	75	75	76	75	74	74	74	80	80	78	77	79	77	81	75	63	83	74	82
29	*S. saxifragae*	HM902927	50	50	50	52	50	62	61	75	75	72	58	68	66	68	67	49	56	72	63	70
30	*S. bolteri*	HM902924	55	55	55	57	57	67	29	69	72	69	64	67	65	67	65	55	52	68	65	62
31	*S. fatifera*	HQ564768	58	58	58	60	56	71	58	59	68	65	63	62	62	65	58	56	47	64	60	72
32	*S. polygoni*	GU685617	71	71	71	70	73	77	74	80	78	78	77	78	90	70	75	72	64	77	75	81
33	*S. flaviventris*	JF854645	71	71	71	70	73	77	74	80	78	78	77	78	90	70	75	72	64	77	75	81
34	*S. spheciformis*	JF854620	67	67	67	67	65	71	75	69	73	74	66	70	77	79	79	71	72	80	90	94
35	*S. mesiaeformis*	JF853916	54	54	54	53	52	67	65	51	74	73	66	74	66	65	69	49	56	76	70	69
36	*S. culiciformis*	HM871045	31	31	31	32	31	61	57	74	72	71	62	68	62	64	62	29	57	67	64	68
37	*S. alleri*	MH592905	53	53	53	52	54	71	64	61	72	71	58	72	64	62	57	50	54	65	63	75
38	*S. pictipes*	MH592903	49	49	49	48	49	63	64	50	63	62	61	65	72	53	46	49	48	76	63	64
39	*S. myopaeformis*	MH592898	64	64	64	65	64	74	75	67	78	77	72	76	73	70	61	63	62	74	62	79
40	*S. sequoiae*	MH592890	59	59	59	59	59	67	66	71	74	73	73	73	72	48	38	56	53	77	61	76
41	*S. sigmoidea*	MH592865	48	48	48	51	46	61	62	70	68	67	42	68	67	68	67	51	58	75	60	62
42	*S. rileyana*	MH592838	72	72	72	71	72	94	89	84	88	87	89	88	87	86	85	76	86	92	94	102
43	*S. canadensis*	MH592819	55	55	55	54	57	70	62	65	86	85	72	74	71	72	73	56	60	74	64	65
44	*S. refulgens*	MH592815	82	82	82	81	84	84	85	80	95	95	79	92	79	87	85	77	74	97	80	94
45	*S. geliformis*	MH592790	67	67	67	69	69	72	68	74	83	84	81	84	79	81	77	67	65	40	80	87
46	*S. arkansasensis*	MH592782	79	79	79	80	79	91	84	79	84	82	87	85	85	88	94	76	80	91	78	89
47	*S. arctica*	GU097106	59	59	59	57	57	71	70	71	72	71	44	76	72	74	71	57	63	86	73	73
48	*S. andrenaeformis*	GU706630	53	53	53	54	53	58	64	69	69	68	67	70	67	64	67	56	54	74	64	72
49	*S. cephiformis*	KX040880	68	68	68	69	68	79	84	84	48	48	80	49	88	81	85	70	77	88	83	84
50	*S. stomoxiformis*	AJ864355	66	66	66	69	66	68	77	71	77	74	71	69	73	75	80	69	68	87	81	78

Table 1. Continued.

No.	Species	Accession no.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	*S. namdoelegans*	This study	8.82	7.45	8.66	8.06	9.88	9.73	10.94	11.40	7.60	8.36	8.82	10.79	10.79	10.18	8.21	4.71	8.06	7.45	9.73	8.97
2	*S. namdoelegans*	This study	8.82	7.45	8.66	8.06	9.88	9.73	10.94	11.40	7.60	8.36	8.82	10.79	10.79	10.18	8.21	4.71	8.06	7.45	9.73	8.97
3	*S. namdoelegans*	This study	8.82	7.45	8.66	8.06	9.88	9.73	10.94	11.40	7.60	8.36	8.82	10.79	10.79	10.18	8.21	4.71	8.06	7.45	9.73	8.97
4	*S. namdoelegans*	This study	8.66	7.75	8.97	8.06	10.18	9.88	11.09	11.55	7.90	8.66	9.12	10.64	10.64	10.18	8.06	4.86	7.90	7.30	9.88	8.97
5	*S. velox*	AEZ9646	8.66	7.45	8.66	8.06	9.57	9.73	10.94	11.40	7.60	8.66	8.51	11.09	11.09	9.88	7.90	4.71	8.21	7.45	9.73	8.97
6	*S. soffneri*	KT782535	10.94	10.18	11.25	9.57	12.01	10.79	11.09	11.25	9.42	10.18	10.79	11.70	11.70	10.79	10.18	9.27	10.79	9.57	11.25	10.18
7	*S. formicaeformis*	HM873903	9.88	9.12	8.82	8.97	10.18	11.25	11.70	11.25	9.27	4.41	8.82	11.25	11.25	11.40	9.88	8.66	9.73	9.73	11.40	10.03
8	*S. scoliaeformis*	HM873899	9.27	9.12	11.25	10.18	12.01	10.79	12.77	11.25	11.40	10.49	8.97	12.16	12.16	10.49	7.75	11.25	9.27	7.60	10.18	10.79
9	*S. cruciate*	GU731637	11.09	10.49	10.94	10.03	11.85	11.25	13.37	12.16	11.40	10.94	10.33	11.85	11.85	11.09	11.25	10.94	10.94	9.57	11.85	11.25
10	*S. loranthi*	GU731629	10.94	10.33	10.94	9.88	11.70	11.09	13.37	12.16	10.94	10.49	9.88	11.85	11.85	11.25	11.09	10.79	10.79	9.42	11.70	11.09
11	*S. polaris*	MZ610629	8.51	9.88	10.18	9.42	10.64	10.64	11.25	11.85	8.82	9.73	9.57	11.70	11.70	10.03	10.03	9.42	8.82	9.27	10.94	11.09
12	*S. tipuliformis*	MZ609921	11.42	10.05	11.57	10.35	12.63	11.57	12.94	11.72	10.35	10.20	9.44	11.87	11.87	10.65	11.26	10.35	10.96	9.89	11.57	11.11
13	*S. vespiformis*	OU906976	9.73	9.88	10.94	10.94	11.25	11.70	11.85	12.01	10.03	9.88	9.42	13.68	13.68	11.70	10.03	9.42	9.73	10.94	11.09	10.94
14	*S. novaroensis*	GU661886	9.57	8.97	11.40	9.27	12.46	10.49	11.85	11.70	10.33	10.18	9.88	10.64	10.64	12.01	9.88	9.73	9.42	8.06	10.64	7.30
15	*S. pini*	JN279256	8.82	9.12	10.94	8.82	12.01	9.88	12.31	12.31	10.18	9.88	8.82	11.40	11.40	12.01	10.49	9.42	8.66	6.99	9.27	5.78
16	*S. fulvipes*	JF848560	8.66	7.90	8.21	8.51	9.57	9.88	11.09	11.40	7.45	8.36	8.51	10.94	10.94	10.79	7.45	4.41	7.60	7.45	9.57	8.51
17	*S. acerrubri*	JF848555	8.21	6.99	8.36	7.60	10.33	9.27	10.79	9.57	8.51	7.90	7.14	9.73	9.73	10.94	8.51	8.66	8.21	7.30	9.42	8.06
18	*S. scitula*	HQ977589	10.33	10.49	12.01	10.03	11.85	12.31	13.53	12.61	10.94	10.33	9.73	11.70	11.70	12.16	11.55	10.18	9.88	11.55	11.25	11.70
19	*S. sapygaeformis*	HQ977588	9.57	9.57	8.36	10.49	10.33	10.64	10.64	11.25	9.57	9.88	9.12	11.40	11.40	13.68	10.64	9.73	9.57	9.57	9.42	9.27
20	*S. rubrofascia*	HQ977587	11.70	10.79	10.64	11.09	11.85	11.70	11.70	12.46	10.64	9.42	10.94	12.31	12.31	14.29	10.49	10.33	11.40	9.73	12.01	11.55
21	*S. kathyae*	HQ977584	–	8.82	10.03	8.36	10.49	9.88	11.09	10.79	8.97	9.12	8.97	10.49	10.49	10.18	9.42	8.82	1.82	8.06	8.82	8.51
22	*S. exitiosa*	HQ977582	58	–	10.33	9.73	9.88	8.82	11.40	9.73	8.51	8.51	8.06	9.88	9.88	11.09	6.99	8.06	8.97	6.84	8.82	8.66
23	*S. decipiens*	HQ977579	66	68	–	10.49	10.03	10.79	11.55	11.25	8.36	8.97	10.03	11.70	11.70	12.46	10.33	8.51	9.57	9.42	10.33	9.42
24	*S. acerni*	HQ977575	55	64	69	–	9.27	10.33	10.79	10.18	9.73	8.66	7.90	10.03	10.03	10.49	10.33	8.51	7.60	8.82	9.73	8.82
25	*S. albicornis*	HQ977574	69	65	66	61	–	12.16	11.85	12.46	9.27	9.27	9.42	12.61	12.61	10.49	10.94	10.49	10.49	9.88	11.09	10.18
26	*S. rhododendri*	HQ957068	65	58	71	68	80	–	12.77	10.64	10.79	11.25	10.49	11.40	11.40	11.70	10.33	9.42	10.49	8.66	10.94	9.73
27	*S. mellinipennis*	HM902931	73	75	76	71	78	84	–	9.73	11.09	11.40	10.79	9.88	9.88	13.83	11.70	11.70	10.79	11.09	12.46	12.01
28	*S. bibionipennis*	HM902929	71	64	74	67	82	70	64	–	10.18	10.33	9.57	9.27	9.27	12.16	9.88	11.55	11.70	9.42	11.25	11.09
29	*S. saxifragae*	HM902927	59	56	55	64	61	71	73	67	–	8.36	8.97	11.55	11.55	10.94	8.51	9.12	9.12	8.36	10.03	9.73
30	*S. bolteri*	HM902924	60	56	59	57	61	74	75	68	55	–	7.75	10.94	10.94	10.79	10.49	8.36	8.97	8.66	10.79	9.88
31	*S. fatifera*	HQ564768	59	53	66	52	62	69	71	63	59	51	–	10.94	10.94	10.18	8.97	9.73	8.51	8.21	8.06	9.27
32	*S. polygoni*	GU685617	69	65	77	66	83	75	65	61	76	72	72	–	0	12.61	10.79	11.40	10.03	9.73	11.55	11.09
33	*S. flaviventris*	JF854645	69	65	77	66	83	75	65	61	76	72	72	0	–	12.61	10.79	11.40	10.03	9.73	11.55	11.09
34	*S. spheciformis*	JF854620	67	73	82	69	69	77	91	80	72	71	67	83	83	–	11.40	11.40	10.64	10.33	12.01	12.16
35	*S. mesiaeformis*	JF853916	62	46	68	68	72	68	77	65	56	69	59	71	71	75	–	9.12	9.73	7.45	10.33	9.57
36	*S. culiciformis*	HM871045	58	53	56	56	69	62	77	76	60	55	64	75	75	75	60	–	8.66	8.82	9.88	8.36
37	*S. alleri*	MH592905	12	59	63	50	69	69	71	77	60	59	56	66	66	70	64	57	–	8.51	8.82	8.66
38	*S. pictipes*	MH592903	53	45	62	58	65	57	73	62	55	57	54	64	64	68	49	58	56	–	9.73	7.60
39	*S. myopaeformis*	MH592898	58	58	68	64	73	72	82	74	66	71	53	76	76	79	68	65	58	64	–	10.03
40	*S. sequoiae*	MH592890	56	57	62	58	67	64	79	73	64	65	61	73	73	80	63	55	57	50	66	–
41	*S. sigmoidea*	MH592865	58	54	62	62	66	69	70	69	50	50	57	76	76	67	58	55	62	52	67	69
42	*S. rileyana*	MH592838	79	81	87	75	91	86	94	99	74	86	88	91	91	95	77	82	74	77	86	82
43	*S. canadensis*	MH592819	67	61	69	66	76	64	78	71	60	65	63	75	75	89	66	61	65	62	67	71
44	*S. refulgens*	MH592815	79	82	82	86	93	82	91	85	81	87	89	88	88	94	79	83	81	81	89	84
45	*S. geliformis*	MH592790	74	71	72	68	81	79	85	75	70	67	67	77	77	75	74	67	75	75	82	74
46	*S. arkansasensis*	MH592782	74	76	81	91	83	79	86	87	75	75	72	93	93	89	84	85	79	74	81	93
47	*S. arctica*	GU097106	64	64	75	67	69	77	82	83	59	62	65	83	83	69	68	67	70	59	69	77
48	*S. andrenaeformis*	GU706630	62	60	62	58	72	60	78	67	58	65	53	76	76	78	68	57	61	58	62	62
49	*S. cephiformis*	KX040880	78	73	71	80	91	75	94	82	75	82	78	80	80	87	77	73	79	67	72	75
50	*S. stomoxiformis*	AJ864355	67	63	72	74	72	73	79	78	69	66	66	83	83	68	70	71	72	65	85	77

Table 1. Continued.

No.	Species	Accession no.	41	42	43	44	45	46	47	48	49	50
1	*S. namdoelegans*	This study	7.30	10.94	8.36	12.46	10.18	12.01	8.97	8.06	10.33	10.03
2	*S. namdoelegans*	This study	7.30	10.94	8.36	12.46	10.18	12.01	8.97	8.06	10.33	10.03
3	*S. namdoelegans*	This study	7.30	10.94	8.36	12.46	10.18	12.01	8.97	8.06	10.33	10.03
4	*S. namdoelegans*	This study	7.75	10.79	8.21	12.31	10.49	12.16	8.66	8.21	10.49	10.49
5	*S. velox*	AEZ9646	6.99	10.94	8.66	12.77	10.49	12.01	8.66	8.06	10.33	10.03
6	*S. soffneri*	KT782535	9.27	14.29	10.64	12.77	10.94	13.83	10.79	8.82	12.01	10.33
7	*S. formicaeformis*	HM873903	9.42	13.53	9.42	12.92	10.33	12.77	10.64	9.73	12.77	11.70
8	*S. scoliaeformis*	HM873899	10.64	12.77	9.88	12.16	11.25	12.01	10.79	10.49	12.77	10.79
9	*S. cruciate*	GU731637	10.33	13.37	13.07	14.44	12.61	12.77	10.94	10.49	7.30	11.70
10	*S. loranthi*	GU731629	10.18	13.22	12.92	14.44	12.77	12.46	10.79	10.33	7.30	11.25
11	*S. polaris*	MZ610629	6.38	13.53	10.94	12.01	12.31	13.22	6.69	10.18	12.16	10.79
12	*S. tipuliformis*	MZ609921	10.35	13.39	11.26	14.00	12.79	12.94	11.57	10.65	7.46	10.50
13	*S. vespiformis*	OU906976	10.18	13.22	10.79	12.01	12.01	12.92	10.94	10.18	13.37	11.09
14	*S. novaroensis*	GU661886	10.33	13.07	10.94	13.22	12.31	13.37	11.25	9.73	12.31	11.40
15	*S. pini*	JN279256	10.18	12.92	11.09	12.92	11.70	14.29	10.79	10.18	12.92	12.16
16	*S. fulvipes*	JF848560	7.75	11.55	8.51	11.70	10.18	11.55	8.66	8.51	10.64	10.49
17	*S. acerrubri*	JF848555	8.82	13.07	9.12	11.25	9.88	12.16	9.57	8.21	11.70	10.33
18	*S. scitula*	HQ977589	11.40	13.98	11.25	14.74	6.08	13.83	13.07	11.25	13.37	13.22
19	*S. sapygaeformis*	HQ977588	9.12	14.29	9.73	12.16	12.16	11.85	11.09	9.73	12.61	12.31
20	*S. rubrofascia*	HQ977587	9.42	15.50	9.88	14.29	13.22	13.53	11.09	10.94	12.77	11.85
21	*S. kathyae*	HQ977584	8.82	12.01	10.18	12.01	11.25	11.25	9.73	9.42	11.85	10.18
22	*S. exitiosa*	HQ977582	8.21	12.31	9.27	12.46	10.79	11.55	9.73	9.12	11.09	9.57
23	*S. decipiens*	HQ977579	9.42	13.22	10.49	12.46	10.94	12.31	11.40	9.42	10.79	10.94
24	*S. acerni*	HQ977575	9.42	11.40	10.03	13.07	10.33	13.83	10.18	8.82	12.16	11.25
25	*S. albicornis*	HQ977574	10.03	13.83	11.55	14.13	12.31	12.61	10.49	10.94	13.83	10.94
26	*S. rhododendri*	HQ957068	10.49	13.07	9.73	12.46	12.01	12.01	11.70	9.12	11.40	11.09
27	*S. mellinipennis*	HM902931	10.64	14.29	11.85	13.83	12.92	13.07	12.46	11.85	14.29	12.01
28	*S. bibionipennis*	HM902929	10.49	15.05	10.79	12.92	11.40	13.22	12.61	10.18	12.46	11.85
29	*S. saxifragae*	HM902927	7.60	11.25	9.12	12.31	10.64	11.40	8.97	8.82	11.40	10.49
30	*S. bolteri*	HM902924	7.60	13.07	9.88	13.22	10.18	11.40	9.42	9.88	12.46	10.03
31	*S. fatifera*	HQ564768	8.66	13.37	9.57	13.53	10.18	10.94	9.88	8.06	11.85	10.03
32	*S. polygoni*	GU685617	11.55	13.83	11.40	13.37	11.70	14.13	12.61	11.55	12.16	12.61
33	*S. flaviventris*	JF854645	11.55	13.83	11.40	13.37	11.70	14.13	12.61	11.55	12.16	12.61
34	*S. spheciformis*	JF854620	10.18	14.44	13.53	14.29	11.40	13.53	10.49	11.85	13.22	10.33
35	*S. mesiaeformis*	JF853916	8.82	11.70	10.03	12.01	11.25	12.77	10.33	10.33	11.70	10.64
36	*S. culiciformis*	HM871045	8.36	12.46	9.27	12.61	10.18	12.92	10.18	8.66	11.09	10.79
37	*S. alleri*	MH592905	9.42	11.25	9.88	12.31	11.40	12.01	10.64	9.27	12.01	10.94
38	*S. pictipes*	MH592903	7.90	11.70	9.42	12.31	11.40	11.25	8.97	8.82	10.18	9.88
39	*S. myopaeformis*	MH592898	10.18	13.07	10.18	13.53	12.46	12.31	10.49	9.42	10.94	12.92
40	*S. sequoiae*	MH592890	10.49	12.46	10.79	12.77	11.25	14.13	11.70	9.42	11.40	11.70
41	*S. sigmoidea*	MH592865	–	13.98	10.64	12.01	11.09	11.25	4.26	8.66	10.79	8.21
42	*S. rileyana*	MH592838	92	–	13.83	15.65	14.29	15.05	13.98	13.83	13.83	14.89
43	*S. canadensis*	MH592819	70	91	–	12.31	10.79	12.92	12.01	9.88	12.31	11.09
44	*S. refulgens*	MH592815	79	103	81	–	13.53	15.65	12.77	12.77	13.83	13.83
45	*S. geliformis*	MH592790	73	94	71	89	–	14.29	12.31	10.49	13.68	12.61
46	*S. arkansasensis*	MH592782	74	99	85	103	94	–	12.01	12.46	14.89	12.92
47	*S. arctica*	GU097106	28	92	79	84	81	79	–	10.18	12.61	10.79
48	*S. andrenaeformis*	GU706630	57	91	65	84	69	82	67	–	11.25	10.49
49	*S. cephiformis*	KX040880	71	91	81	91	90	98	83	74	–	12.31
50	*S. stomoxiformis*	AJ864355	54	98	73	91	83	85	71	69	81	–

The numbers above the diagonal are the mean distance values; the numbers below the diagonal are the absolute distance values.

Table 2. Pairwise comparisons among ND1 sequences of *Synanthedon* species (585 bp) obtained from current study and public data.

No.	Species	Genbank no.	1	2	3	4	5	6	7	8	9	10
1	*S. namdoelegans*	This study	–	0.17	0.17	0.35	2.79	3.14	2.96	2.79	2.97	11.01
2	*S. namdoelegans*	This study	1	–	0.00	0.52	2.61	2.96	2.79	2.62	2.79	10.84
3	*S. namdoelegans*	This study	1	0	–	0.52	2.61	2.96	2.79	2.62	2.79	10.84
4	*S. namdoelegans*	This study	2	3	3	–	2.79	3.48	2.96	3.14	3.31	11.34
5	*S. culiciformis*	AY304162	16	15	15	16	–	1.04	0.17	1.22	1.05	9.97
6	*S. culiciformis*	AY304163	18	17	17	20	6	–	1.22	0.87	0.70	10.14
7	*S. culiciformis*	AY304166	17	16	16	17	1	7	–	1.39	1.22	10.12
8	*S. pamphyla*	AY304164	16	15	15	18	7	5	8	–	0.17	9.79
9	*S. pamphyla*	AY304165	17	16	16	19	6	4	7	1	–	9.62
10	*S. spheciformis*	AY304167	63	62	62	65	57	58	58	56	55	–

The numbers above the diagonal are the mean distance values; the numbers below the diagonal are the absolute distance values.

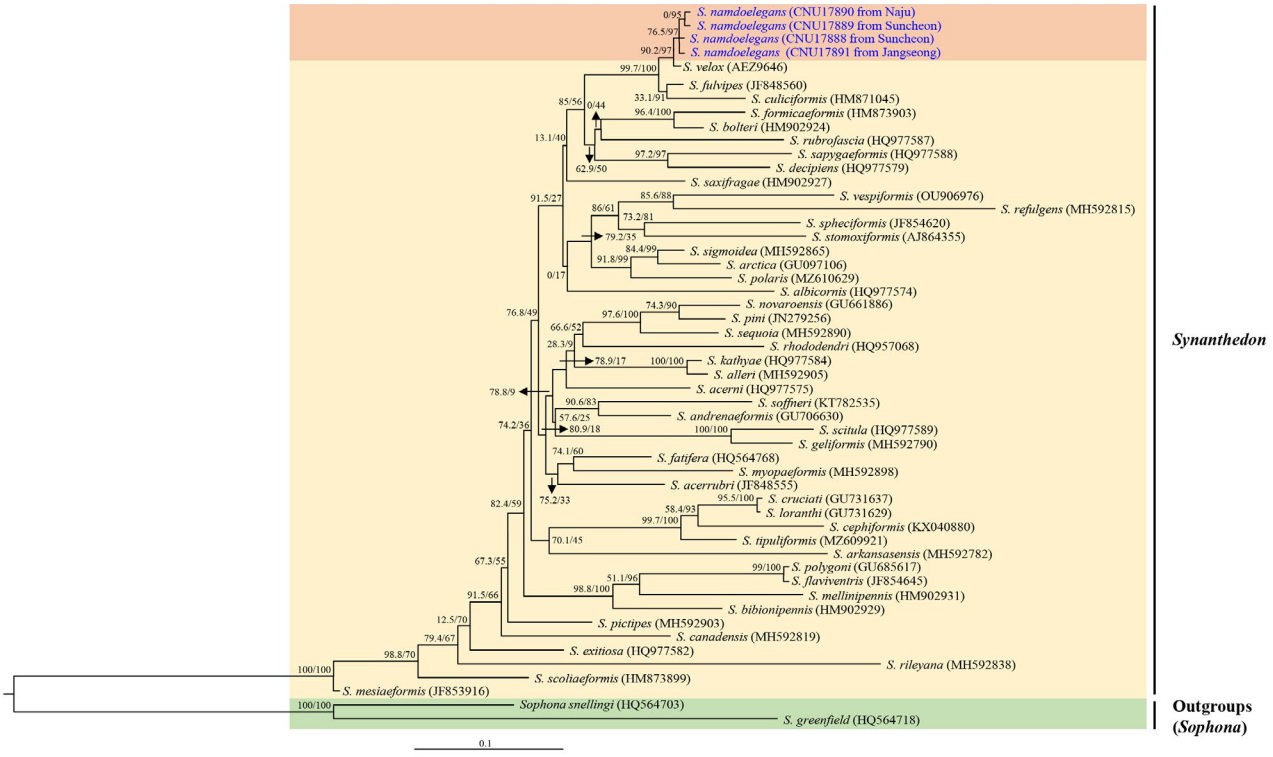


Fig. 4. Maximum-likelihood tree for 50 DNA barcode sequences of *Synanthedon* genus, including *Synanthdon namdoelegans*. The numbers at each node specifies SH-aLRT/UFBoot values. The scale bar indicates the number of substitutions per site. *Sophona snellingi* (HQ564703, unpublished), and *Sophona greenfield*, (HQ564718, unpublished) were used as outgroups. *S. namdoelegans* sequenced in this study are provided for the specimen number and collected locality within parenthesis. GenBank or BOLD system accession numbers for public data are provided in parentheses after the scientific names.

revealed the sequence divergence ranging from 2.61% (15 bp) to 11.34% (65 bp), indicating a distinct difference of *S. namdoelegans* to other species (Table 2).

Phylogenetic analysis showed that the current four samples of *S. namdoelegans* formed a well-supported inclusive group with higher nodal supports (SH-aLRT = 76.5, UFBoot = 97), but *S. namdoelegans* and the proposed *S. velox* formed the sister group with the higher nodal supports (SH-aLRT = 90.2, UFBoot = 97) (Fig. 4). As mentioned in pairwise comparison of barcode region, however, the proposed *S. velox* may have been misidentified as *S. velox*, instead of *S. namdoelegans*. Excluding the

proposed *S. velox* sequence, *S. namdoelegans* formed the sister group with *S. fulvipes* and *S. culiciformis* Linnaeus, 1758 (Mutanen *et al*., 2016, HM871045) with the higher nodal supports (SH-aLRT = 99.7, UFBoot = 100) (Fig. 4).

Our morphological and molecular analysis indicate that *S. namdoelegans* is a previously unknown species in *Synanthedon*. Its morphological features resemble those of *S. velox* (Lee *et al*., 2011), but a substantial difference was detected. The male genitalia are superficially similar to *S. velox* (Lee *et al*., 2011), but the shapes of the crista sacculi and saccus are different.

In the BOLD system, however, one dorsal image of an adult, which is designated as *S. velox* collected from South Korea (without further detailed information) is uploaded (Fig. 2C), along with the DNA barcode sequence (acc. no. AEZ9646; Table 1 and Fig. 4). The proposed *S. velox* has yellow stripes that are located in the 3rd and 5th tergites separately, rather than broadly at 4th and 5th tergites (Fig. 2C), indicating that the proposed *S. velox* is more similar to *S. namdoelegans* (Fig. 2A and 2B), rather than *S. velox* (Fig. 2D, 2E, and 2F). Moreover, *S. velox* adults are known to emerge and captured from July in South Korea (Lee *et al*., 2011), but the proposed *S. velox* is recorded for the collection date at May 10 in South Korea. Similarly, we collected *S. namdoelegans* adults from April to May in South Korea. Therefore, we believe that the proposed *S. velox* is more likely to be current *S. namdoelegans*, rather than *S. velox*. Considering all these factors, we conclude that *S. namdoelegans* is a distinct species within the genus of *Synanthedon*.

Conflicts of Interest

The author of this paper have no affiliation with any interests and is solely responsible for the paper.

Acknowledgements

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A new species of *Alteutha* Baird, 1846 (Copepoda: Harpacaticoida: Peltidiidae) from Korean waters, and a short note of *Eupelte dokdoensis* Song & Lee, 2024

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A new peltidiid species belonging to the genus *Alteutha* Baird, 1846 is described based on the material from Songjihobrackish lake on eastern coast of Korea. The new species, *Alteutha wanae* **sp. nov.** is closely related to *A. potter* Veit-Köhler & Fuentes, 2007, a species originally described from King George Island, Antarctica, in sharing the setation of P5 rami of both sexes, the shape of maxillipedal basis, setal formulae of swimming legs, P2−P4, and the setation of caudal ramus. *Alteutha wanae* **sp. nov.**, however, can be differentiated from *A. potter* by the characters, the number of antennular segment, anterior seta of antennal basis, the length to width ratios of P5 baseoendopod and exopod of both sexes, and male P6. Among benthic harpacticoid copepods collected by benthic surveys in the brackish lakes along the eastern coast of Korea in 1994, a new representative of the genus *Alteutha* was described and illustrated. In addition, we provide the registration information of Zoobank of *Eupelte dokdoensis* Song & Lee, 2024, recently collected from the algal bed bottom in Dokdo Island, Korea.

Keywords: *Alteutha*, brackish lake, Dokdo Island, East Sea, Harpacticoida, Korea

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Introduction

Most members of the family Peltidiidae Claus, 1860 are typically marine algal dwellers, with dorsoventrally flattened bodies adapted to strong water flow over flat algal surfaces (Hicks, 1982; Boxshall and Halsey, 2004). As far, eight peltidiid species were reported from Koren waters: three species belonging to Clytemnestrinae Scott A., 1909 (*Clytemnestra scutellata* Dana, 1849, *Goniopsyllus rostratus* Brady, 1883, *Goniopsyllus dokdoensis* Cho, Kim & Lee, 2010) as planktonic species (Shim and Ro, 1982; Cho *et al*., 2010; Song *et al*., 2017) and five species in Peltidiinae Claus, 1860: *Alteutha depressa* (Baird, 1837), *Alteuthoides affinis* Kim & Kim, 1998, *Eupelte dokdoensis* Song & Lee, 2024, *Peltidium quinquesetosum* Song & Yun, 1999, and *Peltidium byungwooi* Song, Dahms, Kangtia, Chullasorn, Ryu & Khim, 2015 mainly collected from algal beds (Song and Chang, 1995; Song and Yun, 1999; Song *et al*., 2015; Song and Lee, 2024), except for *A. affinis* obtained from a sponge, *Callyspongia elegans* (Thiele, 1899) by Kim and Kim (1998).

To date, 24 species are currently recognized in the genus *Alteutha* Baird, 1846 (Walter and Boxshall, 2025). The new species, named *Alteutha wanae* **sp. nov.**, is described and figured in detail. The new species was recorded from Lake Songjiho (brackish lake), Yeongdeok of eastern coast of Korea (algal beds), and Dokdo Island (using light trap near algal beds).

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Materials and Methods

The materials were obtained from the brackish lake, Songjiho (depth 1−3 m) at Gangwondo province, Korea using plankton net. All samples were fixed in 95% ethanol, immediately after collection. Harpacticoid copepods were sorted under a stereomicroscope (Olympus Model SZ11, Tokyo, Japan) at 40× to 200× magnification. Specimens were dissected, drawn and measured in glycerol. Dissected parts were mounted on slides in glycerin as mounting medium. All mounted slides were sealed with transparent nail varnish. Figures were made with the aid of a drawing tube attached to a DIC microscope (Olympus model BX-53, Japan) equipped with Nomarski optics.

The descriptive terminology for the appendages follows Huys & Boxshall (1991) and Huys *et al*. (1996). All abbreviations used in the text, figures and table are as follows: Caudal ramus (CR); antennule (A1); antenna (A2); aesthetasc (ae); exopod (exp); endopod (enp); first exopodal segment (exp-1) and first endopodal segment (enp-1); first to sixth thoracic legs (P1−P6); pinnate and bare seta (pin, bare). Acrothek is composed of 2 setae fused to an asthetasc on the distal segment of A1. Scale bars are in micrometers (μm). Measurements were made with a micrometer (slide glass type). All types of materials were deposited in the Nakdonggang National Institute of Biological Resources (NNIBR), Korea.

Systematics

Order Harpacticoida G. O. Sars, 1903
Family Peltidiidae Claus, 1860
Genus *Alteutha* Baird, 1846

***Alteutha wanae* sp. nov. (Figs. 1−5)**
부인고갑옷장수노벌레
urn:lsid:zoobank.org:act:71C19903-F874-4BD1-BEE0-F4C607F0AE79

Type locality. Lake Songjiho, Jukwang-myeon, Goseong-gun, Gangwon-do, July 10, 1994.
Material examined. Holotype: adult female (NNIBR: NNIBRIV136397) dissected on 12 slides; adult male allotype (NNIBR: NNIBRIV136396) dissected on 12 slides, 1 female and 1 male paratype (NNIBR: NNIBRIV136394−5) preserved in 70% ethanol.
Etymology. The specific name of *Alteutha wanae* **sp. nov.** is dedicated to first author's wife Kyung Wan Kang.
Additional materials. 3우우, Daejin, Yeongdeok-gun,

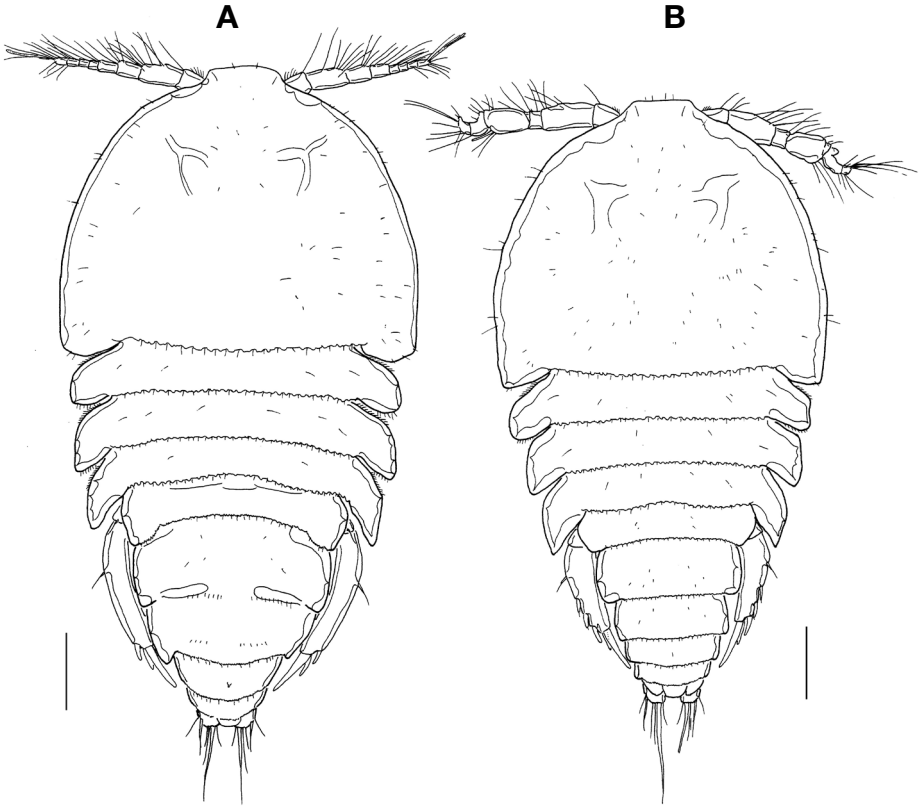


Fig. 1. *Alteutha wanae* **sp. nov.**, (A) female habitus, dorsal; (B) male habitus, dorsal. Scales: 100 mm.

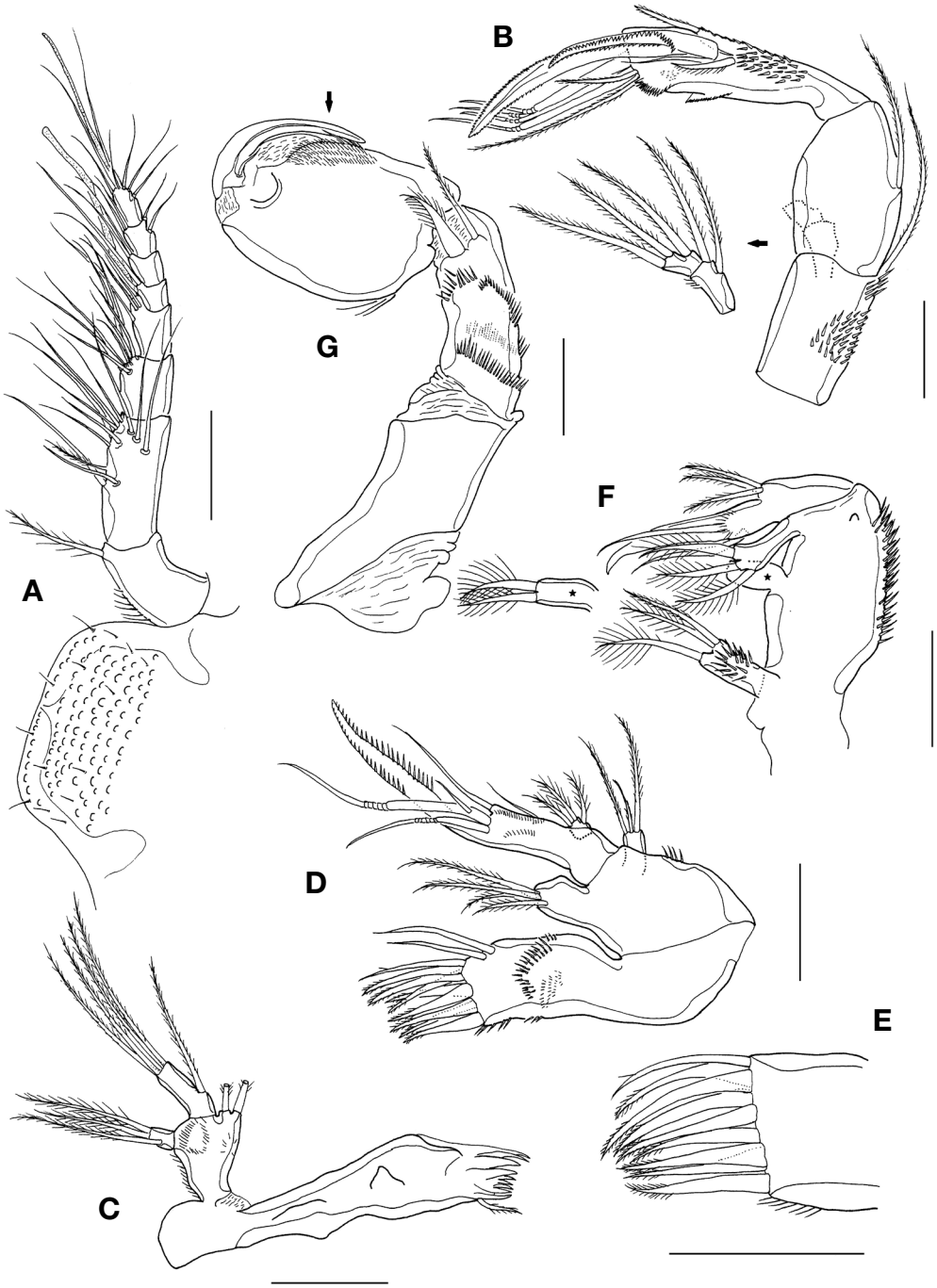


Fig. 2. *Alteutha wanae* **sp. nov.**, female: (A) rostrum and antennule; (B) antenna; (C) mandible; (D) maxillule, right; (E) arthrite of praecoxa, left maxillule; (F) maxilla; (G) maxilliped. Scales: A = 50 mm, others = 30 mm.

Gyeongsangbuk-do, July 24, 1992; 1♀ (dissected), Geumjin-ri, Yeongdeok-gun, Gyeongsangbuk-do, Sep. 25, 1998. 3♀♀, 2♂♂, Dongdo, Dokdo Island, Ulleung-gun, Gyeongsangbuk-do, using light trap, July 14, 2021.

Description of female. Habitus (Fig. 1A). Body length ranging from 0.86–0.92 mm (n = 5), measured from tip of rostrum to posterior margin of caudal ramus. Cephalothorax with scattered many sensilla on its surface as figured. Widest at posterior margin of cephalothorax. All somites of prosome ornamented with sensilla on surface and serrated distal margin.

Urosome (Fig. 1A) tapering posteriorly, 5-segmented, consisting of P5-bearing somite, genital double-somite, 2 free urosomites, and anal somite. Genital double-somite

(Figs. 1A, 5B) wider than long, with transverse surface ridges dorsally and laterally, and ornamented with several sensilla on surface. P6 (Fig. 5B) represented by 2 bare setae on single plate over gonopores.

Caudal ramus (Fig. 5A) about 1.2 times as long as wide. Each ramus with 7 setae: seta I bare, arising from subdistal lateral surface; seta II strong and bipinnate, with normal base, inserted next to seta I; seta III slender and bare; seta IV and seta V well-developed, former bipinnate and latter multipennate; seta VI bare and shortest; seta VII bare, as long as seta VI, arising from subdistal dorsal surface between setae V and VI.

Rostrum (Fig. 2A) wide and rounded rectangular, covered with numerous dents, with several setules on surface.

Antennule (Fig. 2A) 8-segmented; segment 1 ornamented with setules on anterior margin, with apinnate seta distally; segment 2 longest; segment 4 with aesthetasc fused to a seta, arising from pedestal; segment 5 shortest; distal 3 segments subequal in length. Setal formula as follows: 1-[1 pin], 2-[1 pin + 11 bare], 3-[8], 4-[3 + (1 + ae)], 5-[2], 6-[2], 7-[4], 8-[2], 9-[5 + acrothek].

Antenna (Fig. 2B) consisting of basis, two-segmented exopod and endopod. Basis longer than wide, ornamented with numerous small spinules anteriorly, with one bipinnate seta. Exopod two-segmented; first segment longer than second one, with several setules on outer margin and two bipinnate setae; second segment with 2 distal and 1 lateral bipinnate setae. Endopod 2-segmented; first segment with one bipinnate abexopodal seta on middle; second segment with many small spinules anterior surface and several spinuls on posterior surface distally, with hyaline frills on middle and subdistally. Subdistal armature consisting of 1 bare and 2 pinnate setae, and 1 serrate spine; distal armature consisting of 2 pinnate, 4 geniculate setae, and 1 serrate spine.

Mandible (Fig. 2C) gnathobase long well-developed with 9 distal cuspidate teeth and 1 bipinnate seta on dorsal corner as depicted. Palp consisting of basis, 1-segmented exopod, and 1-segmented endopod. Basis armed with many setules on surface, and with 2 setae. Exopod smaller than endopod, with 1 lateral and 2 distal bipinnate setae. Endopod with 1 lateral and 4 distal bipinnate setae.

Maxillule (Fig. 2D, E) arthrite of praecoxa well-developed, with 9 distal spines/setae (all pinnate distally) and

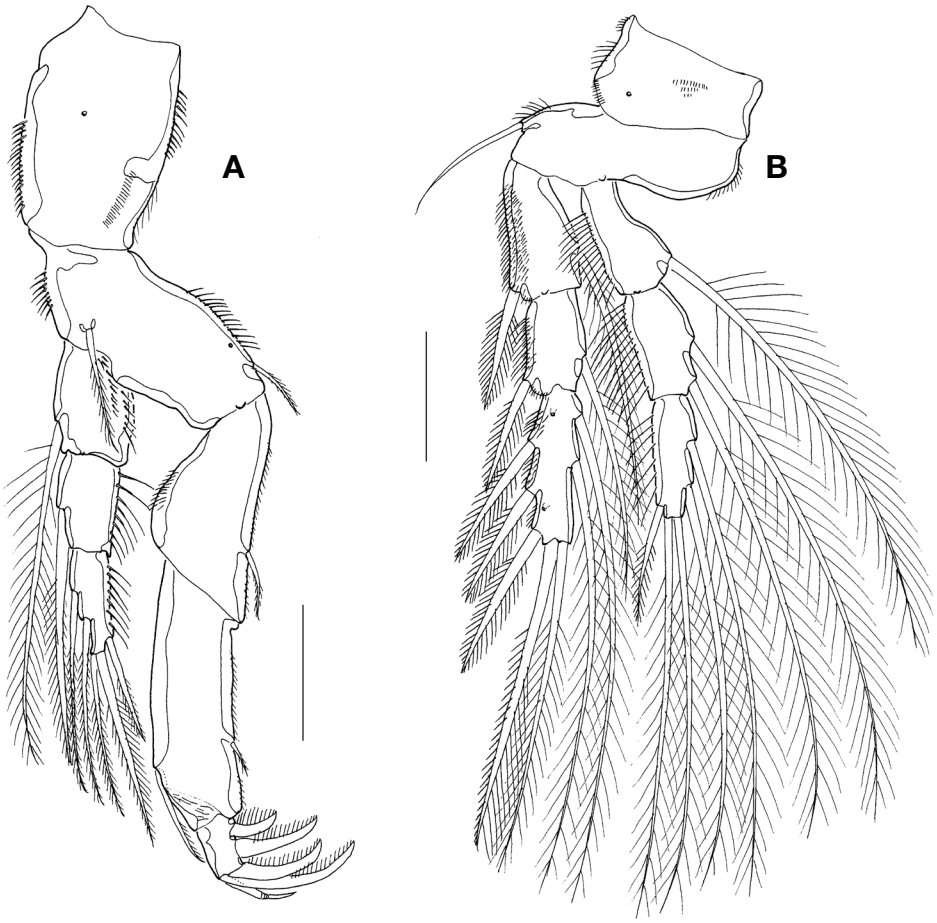


Fig. 3. *Alteutha wanae* **sp. nov.**, female: (A) P1; (B) P2. Scales: A = 30 mm; B = 50 mm.

2 setae on surface. Coxa with several setules on outer margin. Coxal endite with 4 bipinnate setae; epipodite with 3 bipinnate setae. Basis elongate with 2 setular rows on surface, and with 6 elements (2 geniculate, 3 bare, and 1 strong bipinnate seta). Exopod one-segmented with 3 short bipinnate setae.

Maxilla (Fig. 2F) syncoxa with many setules along outer surface, and with 3 endites fused to syncoxa; proximal endite somewhat apart from others, covered with setules on surface and 1 bipinnate and 2 plumose setae distally; middle endite with 2 plumose setae, and distal endite with 3 plumose setae. Basis drawn into strong claw with 2 accessory setae; Endopod represented by small protrusion bearing 3 pinnate setae.

Table 1. Setal formula of swimming legs of *Alteutha wanae* **sp. nov.**

Thoracopod	Exopod	Endopod
P2	1 1 223	1 2 221
P3	1 1 323	1 2 321
P4	1 1 323	1 2 221

Maxilliped (Fig. 2G) subchelate, well-developed, prehensile. Praecoxa elongated and unarmed. Syncoxa armed with setular rows as depicted, and with 2 bipinnate subdistal setae. Basis with several setular rows and 1 small seta on palmar surface (arrow). Endopod shorter than basis and drawn out into strong, curved claw, accessory armature consisting of 1 bare seta.

P1 (Fig. 3A). Coxa longitudinally elongate, ornamented with spinular rows along both margins and on distal surface, and with a pore as figured. Basis transversally elongate, with setules on inner and outer margins and with a pore on subdistal outer surface; with inner and outer bipinnate seta. Exopod 3-segmented, much longer than endopod; exp-1 widened distally, with inner and outer spinules, and 1 outer bipinnate seta; exp-2 longer than exp-1, with setules along outer margin, 1 small outer bipinnate seta, and 1 pinnate inner seta; exp-3 small, with 1 geniculated seta and 4 strong, outwardly curved pinnate claws. Endopod 3-segmented and gradually narrowed; ornamented with setules on outer anterior surface, with 1 plumose

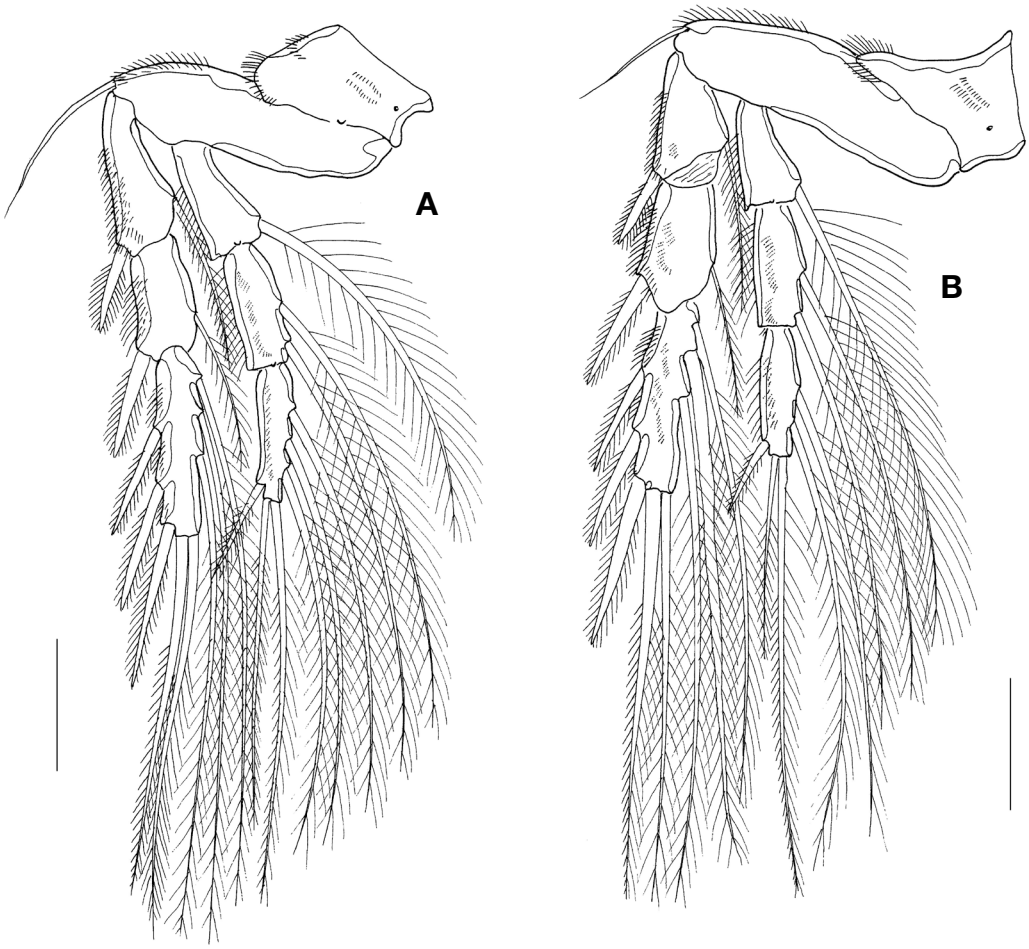


Fig. 4. *Alteutha wanae* **sp. nov.**, female: (A) P3; (B) P4. Scales: 50 mm.

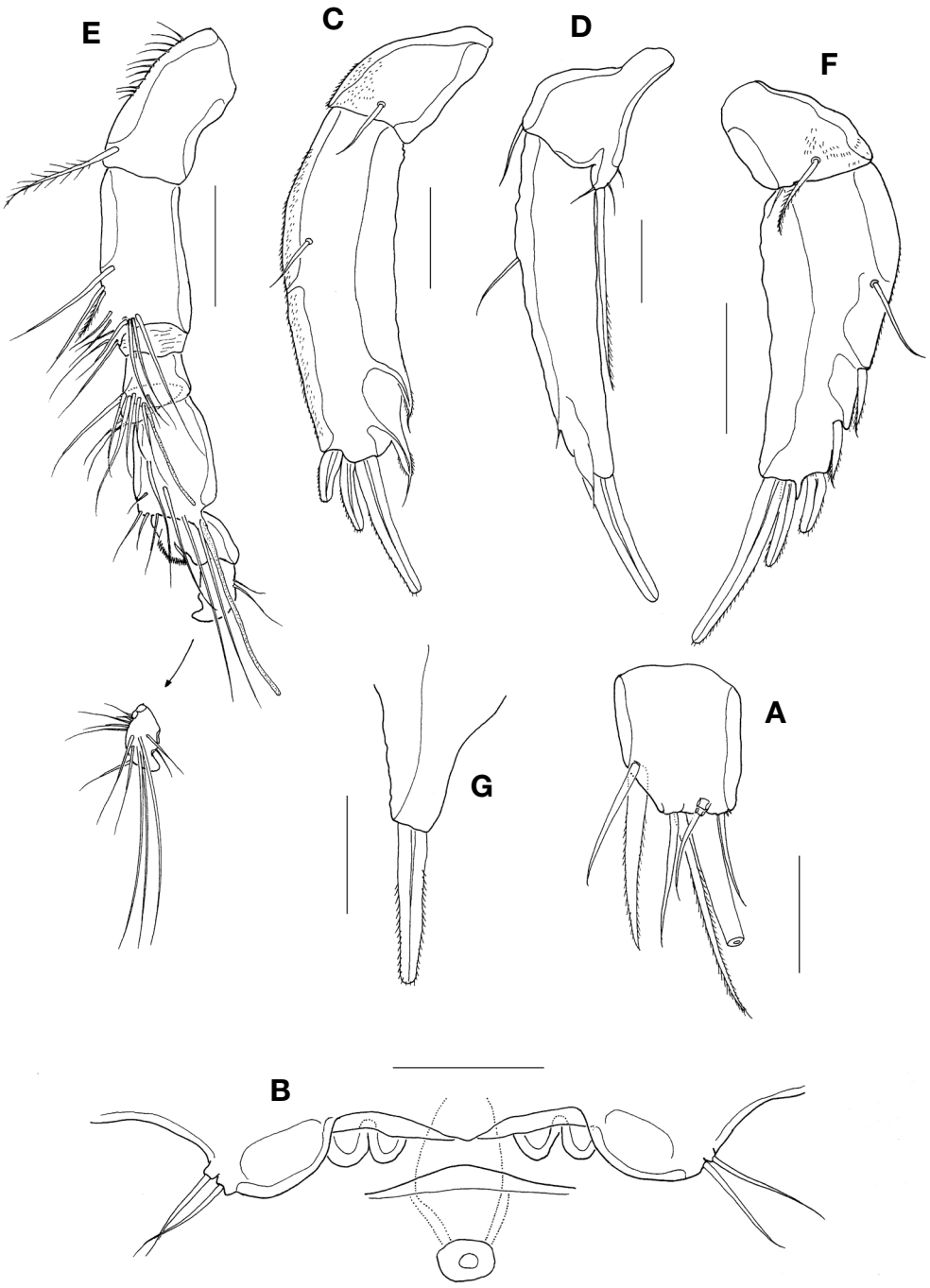


Fig. 5. *Alteutha wanae* **sp. nov.**, female: (A) caudal ramus; (B) genital field; (C) P5, anterior; (D) P5, posterior. male. (E) antennule; (F) P5; (G) P6. Scales: A, B, F = 30 mm, others = 50 mm.

inner seta; enp-2 with long setules on outer margin and with 1 bipinnate inner seta; enp-3 with long setules on outer margin, 1 outer, 2 distal, and 2 inner setae, all of which bipinnate.

P2 (Fig. 3B, Table 1). Coxa wider than long, ornamented with long and short setules on outer margin and on anterior surface. Basis much wider than long, with several setules on outerodistal and on half proximal inner margin, and with 1 bare outer seta. Exopod with 3 segments, a little longer than endopod; exp-1 with setules on outer margin and anterior surface, with 1 bipinnate outer spine and 1 plumose inner seta; exp-2 with outer setules as in exp-1, with 1 bipinnate outer spine and 1 plumose inner seta; exp-3 with spinules and two pores as figured, 3 bipinnate

outer spines, 2 terminal setae, and 2 plumose inner setae. Endopod with 3 subequal segments; enp-1 with outer long setules and 1 plumose inner seta; enp-2 with outer long setules and with 2 plumose inner setae; enp-3 with outer setules, and with 1 outer bipinnate seta, 2 distal bipinnate setae, and 2 inner plumose setae.

P3 (Fig. 4A). Coxa wider than long, with outer setules and one pore as figured. Basis much wider than long, with setules on outer margin, with 1 bare outer seta. Exopod with 3 segments, a little longer than endopod; exp-1 with setules on outer margin and anterior surface, with 1 bipinnate outer spine and 1 plumose inner seta; exp-2 with outer setules as in exp-1, with 1 bipinnate outer spine and 1 plumose inner seta; exp-3 with outer setules, 3 bipinnate outer spines, 2 terminal setae, and 3 plumose inner setae. Endopod with 3 subequal segments; enp-1 with outer long setules and 1 plumose inner seta; enp-2 with outer long setules and short setules on posterior surface and with 2 plumose inner setae; enp-3 with outer long setules and short setules on posterior surface, and with 1 outer bipinnate seta, 2 distal bipinnate setae, and 3 inner plumose setae.

P4 (Fig. 4B). Coxa wider than long, with setules and one pore as figured. Basis much wider than long, with setules on outer margin, with 1 bare outer seta. Exopod with 3 segments, a little longer than endopod; exp-1 with setules on outer margin, anterior and posterior surfaces, with 1 bipinnate outer spine and 1 plumose inner seta; exp-2 with setules as in exp-1, with 1 bipinnate outer spine and 1 plumose inner seta; exp-3 with setules as in exp-2, 3 bipinnate outer spines, 2 terminal setae, and 3 plumose inner setae (middle inner seta thicker than others). Endopod with 3 subequal segments; enp-1 with outer long setules and 1 plumose inner seta; enp-2 with outer long setules and short setules on posterior surface and with 2 plumose inner setae; enp-3 with outer long setules and short setules on posterior surface, and with 1 outer bipinnate seta, 2 distal bipinnate setae, and 2 inner plumose setae.

P5 (Fig. 5C, D). Both rami completely separated. Baseoendopod small, longer than wide, ornamented with tiny setules on outer distal half surface, with 1 long pinnate seta and 4 short setae. Exopod elongated as long as about 3 times, with many setules along outer surface, with 1 bare seta on middle (about 40% proximally), 3 apical strong spines with blunt tip and 1 spine bearing 1 setule and distally fused, and 1 sharp inner process bearing 1 setule.

Description of male. Total body length ranging from 0.77−0.85 mm (n = 6), measured as in the female specimen (Fig. 1B). Urosome tapering posteriorly. Cephalothorax a little wider than long, with lots of sensilla on surface as depicted.

Urosome (Fig. 1B) 6-segmented, comprising P5-bearing somite, genital somite, third to fifth urosomites, and anal somite. Each somite bearing setules along distal margin except for last two somites.

P6 (Fig. 5G) represented by 1 strong bipinnate spine with blunt tip.

Antennule (Fig. 5E) with 7 segments; segment 1 with long anterior setules and 1 bipinnate seta; segment 2 longest; segment 5 much swollen, with aesthetasc and seta fused basally. Setal formula as follows: 1-[1 bipinnate], 2-[1 pinnate + 11 bare], 3-[6 bare + (1 + ae)], 4-[2 bare], 5-[7 bare + (1 + ae)], 6-[1 bare + 1 serrate projection], 7-[2], 8-[11 + acrothek].

Antenna, mouth appendages, and P1, P2, P3, P4 as in female.

P5 (Fig. 5F). Both rami distinctly separated. Baseoendopod elongate, with setules on distal half as depicted, armed with 1 naked seta and 1 bipinnate seta. Exopod about 2 times as long as wide, with many setules along outer margin, and with 3 strong bipinnate distal spines with blunt tip, 2 bipinnate outer setae, and 1 bare seta on anterior surface (about 40% proximally).

Table 2. Comparison based on the selected characters between *A. potter* and *A. wanae* **sp. nov.**

Characters	*A. potter*	*A. wanae* **sp. nov.**
length to width ratio of caudal ramus	as long as	1.2 times longer
setae I and III on caudal ramus	seta I < III	as long as
number of A1 segment	9	8
A2 basal seta vs basis	short	long
P1 exp-2 outer seta	reaching end of segment	not reaching end of segment
P4 exp-3	with normal seta	with big middle inner seta
length to width ratio of female P5 benp.	1.7	1.4
length to width ratio of female P5 exp.	2.4	3.1
length to width ratio of male P5 benp.	1.8	1.2
length to width ratio of male P5 exp.	2.6	2.2
male P6 elements	1 long and 2 short setae	1 strong blunt spine

Discussion

The genus *Alteutha* Baird, 1846 is characterized by dorsoventrally compressed body shape, distinct urosomites, antennule with 8 or 9 setae, P1 with 3 segments, P1 exp-3 with 3 or 4 claws, antenna with 2 or 3 segments, P2−P4 exp-3 with 7, 8, 8, P2−P4 enp-3 with 5, 6, 5 setae, and p2−p4 exp-1 with each 1 seta (Wells, 2007). The genus *Alteutha* is the second speciose genus of the family Peltidiidae, next to *Peltidium* Philippi, 1839, currently comprising 24 species (Walter & Boxshall, 2025). Among the species, only *A. depressa* was recorded with detailed figures from Jindo Island, Korea (Song and Chang, 1995). *Alteutha depressa* has known a worldwide distribution and showed some large morphological variations (Hicks, 1982). It is assumed that the species is a polymorphic species with several cryptic species under the same species name (Lee *et al*., 2012).

Alteutha wanae **sp. nov.** is most closely related to *A. potter* from the Potter cove of King George Island, Antarctica (in front of the Argentinian Jubany Station and the German Dallmann Laboratory). These two species share 1) the setation of P5 baseoendopod and exopod of both sexes, 2) the width to length ratio of basis in maxilliped, 3) the setation and its length of caudal ramus, and 4) P2−P4 exp-1 with 1 inner seta, respectively. However, the new species differs from *A. potter* in 1) caudal seta I longer than seta III (vs. seta I as long as III in *A. potter*), 2) 8-segmented antennule (vs. 9 in *A. potter*), 3) A2 basal seta longer than segment (vs. shorter than basis in *A. potter*), 4) P1 exp-2 outer seta not reaching end of segment (vs. reaching end of segment in *A. potter*), 5) P4 exp-3 with big middle inner seta (vs. with normal seta in *A. potter*), 6) length to width ratio of baseoendopod and exopod in both sexes (Table 2), and 7) male P6 with 1 strong blunt spine (vs. 1 long and 2 short setae in *A. potter*).

In addition, *Eupelte dokdoensis* Song & Lee, 2024 was newly described from Dokdo Island, Korea. The species was mainly collected from algal bed of shallow water, using light trap. However, according to Walter and Boxshall (2024), *E. dokdoensis* is unaccepted in the genus as a valid species due to not comply with ICZN art 8.5.3 (no indication of registration in ZooBank). Here, we added the registration in ZooBank of *E. dokdoensis* Song & Lee, 2024 as follow: urn:lsid:zoobank.org:act:BEBCD3AB-7193-495D-8DEA-3AC3812BE722.

Conflicts of Interest

The authors of this paper have no affiliation with any interests and is solely responsible for the paper.

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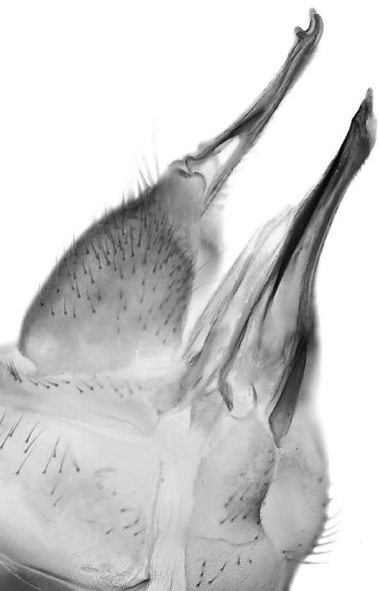
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