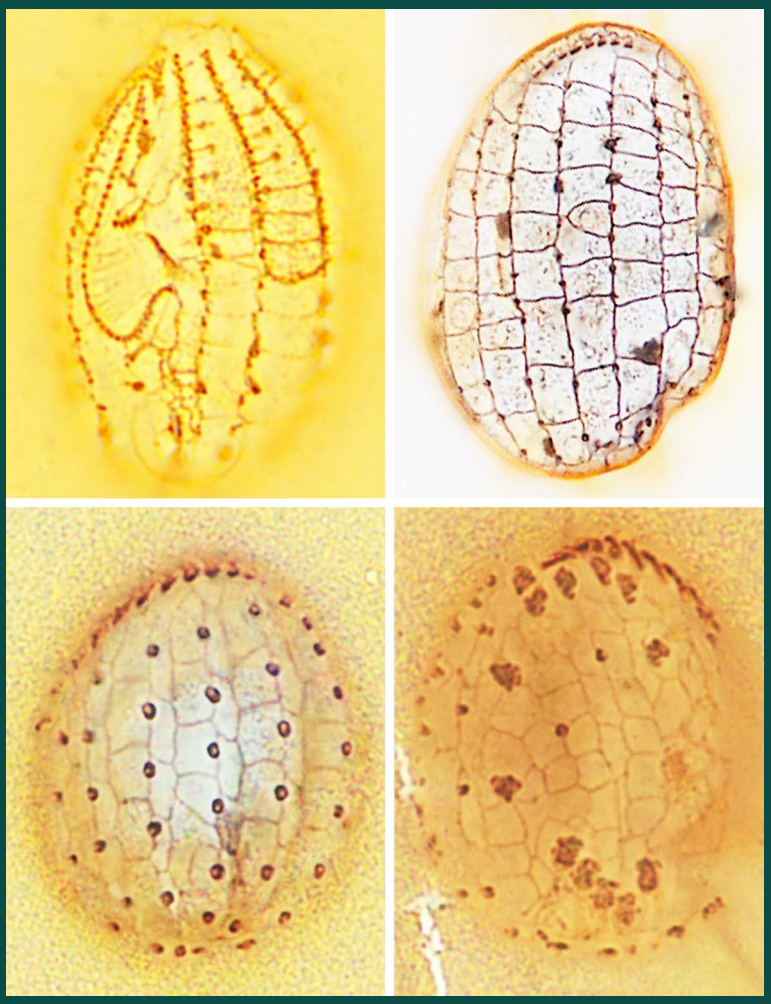
eISSN 2713-8615

JOURNAL OF SPECIES RESEARCH

Volume 13 Number 4 November 30, 2024



Ministry of Environment
National Institute of Biological Resources

JSR https://www.nibr.go.kr
JOURNAL OF SPECIES RESEARCH

eISSN 2713-8615
GPRN 11-1480592-001647-08

Volume 13 Number 4

Published on November 30, 2024
Published by National Institute of Biological Resources (NIBR)

Aims & Scope

The Journal of Species Research (JSR) an open access, international peer-reviewed journal that is published Quarterly (February, May, August and November) by the NIBR. The JSR publishes original contributions of all aspects of taxonomy and biological diversity of all kinds of organisms. The subjects covered by this journal include, but are not limited to, topics related to new and unrecorded species, taxonomic review and revision, flora/fauna of the local habitats, and species list in domestic and overseas surveys.

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Publisher

National Institute of Biological Resources (NIBR), Korea
(Environmental Research Complex), Hwangyeong-ro 42, Seo-gu, Incheon 22689, Korea
Homepage: https://www.nibr.go.kr

Printing

Junghaengsa, Inc.
E-mail : jhpub3213@naver.com, **Phone :** +82-2-2232-3281~2, **Fax :** +82-2-3298-6984
Homepage : https://blog.naver.com/jhpub3213

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Journal of Species Research 13(4):353-366, 2024

Open Access

Description of 41 unrecorded bacterial species in Korea, isolated from freshwater in 2021

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Here we describe indigenous prokaryotic species in Korea, a total of 41 bacterial strains were isolated from freshwater 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 41 species belonged to 5 phyla, 10 classes, 18 orders, 23 families, and 29 genera, which were assigned to *Streptomyces*, *Mycolicibacterium*, *Smaragdicoccus*, *Nocardiopsis*, and *Nocardia* of the phylum *Actinobacteria*; *Runella*, *Flavobacterium*, *Algoriphagus*, *Sphingobacterium*, and *Aequorivita* of the phylum *Bacteroidota*; *Paenibacillus*, *Bacillus*, *Metabacillus*, and *Fredinandcohnia* of the phylum *Firmicutes*; *Sphingobium*, *Erythrobacter*, *Duganella*, *Methylopila*, *Novosphingobium*, *Azospirillum*, *Simplicispira*, *Corallococcus*, *Pseudomonas*, *Devosia*, *Pseudorhodoferax*, *Pseudomonas*, *Prolinoborus*, *Pectobacterium*, and *Aquabacterium* of the phylum *Proteobacteria*; *Proshecobacter* of the phylum *Verrucomicrobia*. Gram reaction, colony and cell morphology, basic biochemical characteristics, isolation source, and strain IDs are also described in the species description section.

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

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DOI:10.12651/JSR.2024.13.4.353

Introduction

Fresh water accounts for 2.5% of the water-based environment, and seawater accounts for 97.5%. Fresh water is slightly different from seawater containing 3.5% salt, and includes various types of ecosystems, including rivers and river soils, where water flows depending on the appeal and altitude difference where the flow is stopped. And it exists in the form of rivers, lakes, and groundwater, and accounts for only about 0.6% of the total water system present on earth except for glaciers, but it is an essential resource for human survival (Wetzel, 2001). In a freshwater environment, most dominant prokaryotes are not cultured except for some groups. Microorganisms are more common in downstream water than in upstream water, and there are many low-nutritive microorganisms in upstream water, and many high-nutritive microorganisms multiply in downstream water. In general, most groups of bacteria form biofilm, but fungi and birds are often found. Microorganisms living in poor and eutrophobic lakes are also different. In eutrophobic lakes, photosynthetic bacteria such as *Cyanobacteria* multiply in summer. So far, bacterial clusters in the freshwater environment have been known as about 20 phyla, and among them, *Actinomycetota*, *Bacteroidota*, *Pseudomonadota*, *Cyanobacteriota*, and *Verrucomicrobiota* were dominant (Newton *et al*., 2011). According to GOLD statistics, environmental bacteria account for about 10% of all genetic projects, and most of environmental ecological research is biased toward digestive organs, oceans, and soil, accounting for 9.2% of fresh water (Mukherjee *et al*., 2022). Like the marine and land environment, freshwater-derived prokaryotes basically serve as decomposers to convert organic matter into inorganic substances by transferring organic matter to higher nutritional levels through microfed networks, and are well-known for their function in the circulation of greenhouse gases. Therefore, research on the analysis of microbial diversity derived from freshwater, which has a close impact on human life, but relatively insignificant excavation research, can contribute to the role of freshwater-

specific prokaryotes and thus the expansion of domestic microbial species discovery research.

In 2021, 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*, *Verrucomicrobiae*, *Shpingobacteriia*, *Cytophagia*, and *Flavobacteriia*. Here we shortly describe 41 unrecorded bacterial species in the 10 classes belonging to 18 orders.

Materials and Methods

A total of 41 bacterial strains affiliated to the classse *Bacilli*, *Actinomycetia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Verrucomicrobiae*, *Shpingobacteriia*, *Cytophagia*, and *Flavobacteriia* were isolated from freshwater samples. Freshwater sample was processed separately, spread onto diverse culture media including R2A, 1/5 R2A, nutrient, 1/5 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 41 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 et al., 1987) methods with the MEGA X Program (Kumar *et al*., 2018) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

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	Medium	Incubation conditions (°C, days)
Bacilli	*Bacillales*	*Bacillaceae*	*Bacillus*	MMS21-DH2R7	NNIBR2021642BA1	*Bacillus cytotoxicus*	98.91	Freshwater	MA	30°C, 2d
	Bacillales	*Bacillaceae*	*Fredinandcohnia*	MMS21-DH2MA11	NNIBR2021642BA49	*Fredinandcohnia onubensis*	99.18	Freshwater	MA	30°C, 3d
	Bacillales	*Bacillaceae*	*Metabacillus*	MMS21-DH3MA7	NNIBR2021642BA3	*Metabacillus mangrovi*	99.93	Freshwater	MA	30°C, 2d
	Bacillales	*Paenibacillaceae*	*Paenibacillus*	AS127	NNIBR2021642BA40	*Paenibacillus ihuae*	99.35	Freshwater	R2A	25°C, 3d
	Bacillales	*Paenibacillaceae*	*Paenibacillus*	AS51	NNIBR2021642BA18	*Paenibacillus massiliensis* subsp. *massiliensis*	99.73	Freshwater	R2A	25°C, 3d
	Bacillales	*Paenibacillaceae*	*Paenibacillus*	K11	NNIBR2021642BA23	*Paenibacillus ottowii*	99.12	Freshwater	R2A	25°C, 3d
Actinomycetia	*Corynebacteriales*	*Mycobacteriaceae*	*Mycolicibacterium*	CG42	NNIBR2021642BA32	*Mycolicibacterium aichiense*	98.74	Freshwater	R2A	25°C, 3d
	Corynebacteriales	*Nocardiaceae*	*Nocardia*	MMS21-JYBA42	NNIBR2021642BA8	*Nocardia africana*	99.29	Freshwater	R2A	30°C, 3d
	Streptosporangiales	*Nocardiopsaceae*	*Nocardiopsis*	K69	NNIBR2021642BA35	*Nocardiopsis dassonvillei* subsp. *dassonvillei*	99.93	Freshwater	R2A	25°C, 3d
	Corynebacteriales	*Nocardiaceae*	*Smaragdicoccus*	K21	NNIBR2021642BA34	*Smaragdicoccus niigatensis*	100	Freshwater	R2A	25°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	WG8	NNIBR2021642BA26	*Streptomyces bryophytorum*	98.75	Freshwater	R2A	25°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	AS52	NNIBR2021642BA19	*Streptomyces bullii*	98.96	Freshwater	R2A	25°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	CG18	NNIBR2021642BA20	*Streptomyces cellostaticus*	99.17	Freshwater	R2A	25°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS21-OR1	NNIBR2021642BA9	*Streptomyces shenzhenensis*	99.31	Freshwater	R2A	30°C, 3d

Table 1. Continued.

Class	Order	Family	Genus	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions (°C, days)
Actinomycetia	*Streptomycetales*	*Streptomycetaceae*	*Streptomyces*	MMS21-DH1MA22	NNIBR2021642BA48	*Streptomyces speibonae*	98.97	Freshwater	R2A	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS21-305	NNIBR2021642BA46	*Streptomyces violarus*	99.59	Freshwater	R2A	30°C, 3d
	Streptomycetales	*Streptomycetaceae*	*Streptomyces*	MMS21-MKJW7	NNIBR2021642BA45	*Streptomyces wuyuanensis*	100	Freshwater	R2A	30°C, 3d
Alphaproteobacteria	*Rhodospirillales*	*Azospirillaceae*	*Azospirillum*	AS37	NNIBR2021642BA30	*Azospirillum oryzae*	99.41	Freshwater	R2A	25°C, 3d
	Hyphomicrobiales	*Devosiaceae*	*Devosia*	AS105	NNIBR2021642BA38	*Devosia elaeis*	99.93	Freshwater	R2A	25°C, 3d
	Sphingomonadales	*Erythrobacteraceae*	*Erythrobacter*	K14	NNIBR2021642BA24	*Erythrobacter tepidarius*	99.64	Freshwater	R2A	25°C, 3d
	Hyphomicrobiales	*Methylocystaceae*	*Methylopila*	CG59	NNIBR2021642BA28	*Methylopila oligotropha*	99.64	Freshwater	R2A	25°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Novosphingobium*	WG87	NNIBR2021642BA29	*Novosphingobium arabidopsis*	99	Freshwater	R2A	25°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Novosphingobium*	AS117	NNIBR2021642BA39	*Novosphingobium sediminis*	99.78	Freshwater	R2A	25°C, 3d
	Sphingomonadales	*Sphingomonadaceae*	*Sphingobium*	K3	NNIBR2021642BA21	*Sphingobium sufflavum*	98.85	Freshwater	R2A	25°C, 3d
Betaproteobacteria	*Burkholderiales*	*Comamonadaceae*	*Aquabacterium*	MMS21-SJ6-8	NNIBR2021642BA47	*Aquabacterium lacunae*	99.59	Freshwater	MA	30°C, 3d
	Burkholderiales	*Oxalobacteraceae*	*Duganella*	CG34	NNIBR2021642BA27	*Duganella rivi*	99.24	Freshwater	R2A	25°C, 3d
	Neisseriales	*Neisseriaceae*	*Prolinoborus*	MMS21-Sa21	NNIBR2021642BA7	*Prolinoborus fasciculus*	98.83	Freshwater	R2A	30°C, 3d
	Burkholderiales	*Comamonadaceae*	*Pseudorhodoferax*	AS150	NNIBR2021642BA42	*Pseudorhodoferax aquiterrae*	99.72	Freshwater	R2A	25°C, 3d
	Burkholderiales	*Comamonadaceae*	*Simplicispira*	CG15	NNIBR2021642BA31	*Simplicispira lacusdiani*	99.31	Freshwater	R2A	25°C, 3d
Gammaproteobacteria	*Enterobacterales*	*Pectobacteriaceae*	*Pectobacterium*	MMS21-Er7	NNIBR2021642BA10	*Pectobacterium aroidearum*	99.52	Freshwater	R2A	30°C, 3d
	Enterobacterales	*Pectobacteriaceae*	*Pseudomonas*	AS210	NNIBR2021642BA44	*Pseudomonas batumici*	99.04	Freshwater	R2A	25°C, 3d
	Enterobacterales	*Pectobacteriaceae*	*Pseudomonas*	AS199	NNIBR2021642BA43	*Pseudomonas huaxiensis*	99.86	Freshwater	R2A	25°C, 3d
	Enterobacterales	*Pectobacteriaceae*	*Pseudomonas*	CG31	NNIBR2021642BA36	*Pseudomonas neuropathica*	98.86	Freshwater	R2A	25°C, 3d
Deltaproteobacteria	*Myxococcales*	*Myxococcaceae*	*Corallococcus*	CG50	NNIBR2021642BA33	*Corallococcus interemptor*	100	Freshwater	R2A	25°C, 3d
Verrucomicrobiae	*Verrucomicrobiales*	*Verrucomicrobiaceae*	*Prosthecobacter*	CG69	NNIBR2021642BA37	*Prosthecobacter dejongeii*	99.39	Freshwater	R2A	25°C, 3d
Shpingobacteriia	*Sphingobacteriales*	*Sphingobacteriaceae*	*Sphingobacterium*	MMS21-DH2R2	NNIBR2021642BA2	*Sphingobacterium canadense*	98.97	Freshwater	R2A	30°C, 2d
Cytophagia	*Cytophagales*	*Cyclobacteriaceae*	*Algoriphagus*	AS131	NNIBR2021642BA41	*Algoriphagus aquaeductus*	98.92	Freshwater	R2A	25°C, 3d
	Cytophagales	*Spirosomaceae*	*Runella*	K7	NNIBR2021642BA22	*Runella aurantiaca*	98.74	Freshwater	R2A	25°C, 3d
Flavobacteriia	*Flavobacteriales*	*Flavobacteriaceae*	*Aequorivita*	MMS21-MC1	NNIBR2021642BA11	*Aequorivita sinensis*	99.9	Freshwater	MA	30°C, 2d
	Flavobacteriales	*Flavobacteriaceae*	*Flavobacterium*	MMS21-SJ5-12	NNIBR2021642BA51	*Flavobacterium difficile*	97.77	Freshwater	R2A	30°C, 3d
	Flavobacteriales	*Flavobacteriaceae*	*Flavobacterium*	WG6	NNIBR2021642BA25	*Flavobacterium squillarum*	99.86	Freshwater	R2A	25°C, 3d

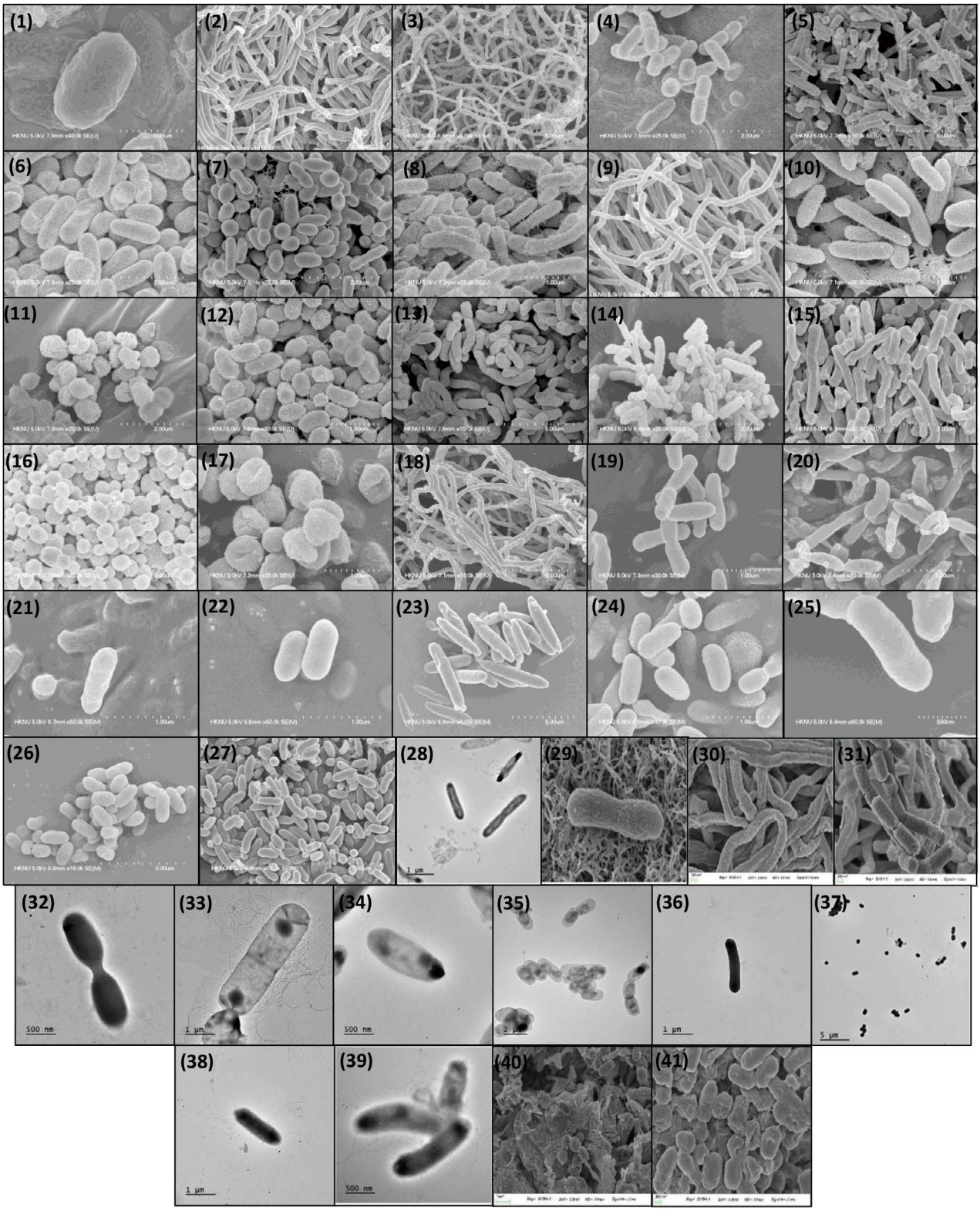


Fig. 1. Transmission and scanning electron micrographs of cells of the strains isolated in this study. Strains: 1, AS51; 2, AS52; 3, CG18; 4, K3; 5, K7; 6, K11; 7, K14; 8, WG6; 9, WG8; 10, CG34; 11, CG59; 12, WG87; 13, AS37; 14, CG15; 15, CG42; 16, CG50; 17, K21; 18, K69; 19, CG31; 20, CG69; 21, AS105; 22, AS117; 23, AS127; 24, AS131; 25, AS150; 26, AS199; 27, AS210; 28, MMS21-SJ5-12; 29, MMS21-305; 30, MMS21-MKJW7; 31, MMS21-DH1MA22; 32, MMS21-Sa21; 33, MMS21-DH2R7; 34, MMS21-DH2R2; 35, MMS21-DH3MA7; 36, MMS21-DH2MA11; 37, MMS21-MC1; 38, MMS21-Er7; 39, MMS21-SJ6-8; 40, MMS21-OR1; 41, MMS21-JYBA42.

Results and Discussion

The 41 strains were distributed in 10 classes of the *Bacilli*, *Actinomycetia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Verrucomicrobiae*, *Shpingobacteriia*, *Cytophagia*, and *Flavobacteriia*; 6 strains for the class *Bacilli*, 11 strains for the *Actinomycetia*, 7 strains for the *Alphaproteobacteria*, 5 strains for the *Betaproteobacteria*, 4 strains for the *Gammaproteobacteria*, 1 strain for the *Deltaproteobacteria*, 1 strain for the *Verrucomicrobiae*, 1 strain for the *Shpingobacteriia*, 2 strains for the *Cytophagia* and 3 strains 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 *Actinobacteria* (Fig. 2) were found to belong to 1 class 5 separate genera: *Streptomyces* (7 species), *Mycolicibacterium* (1 species), *Smaragdicoccus* (1 species), *Nocardiopsis* (1 species), *Nocardia* (1 species).

Fig. 3 shows phylogenetic assignment of 17 strains of the phylum *Proteobacteria* belong to 4 classes 14 separate genera: *Aquabacterium* (1 species), *Azospirillum* (2 species), *Corallococcus* (1 species), *Devosia* (1 species), *Duganella* (1 species), *Erythrobacter* (1 species), *Methylopila* (1 species), *Novosphingobium* (2 species), *Pectobacterium* (1 species), *Prolinoborus* (1 species), *Pseudomonas* (3 species), *Pseudorhodoferax* (1 species), *Simplicispira* (1 species) and *Sphingobium* (1 species).

Fig. 4 shows phylogenetic assignment of 13 strains of

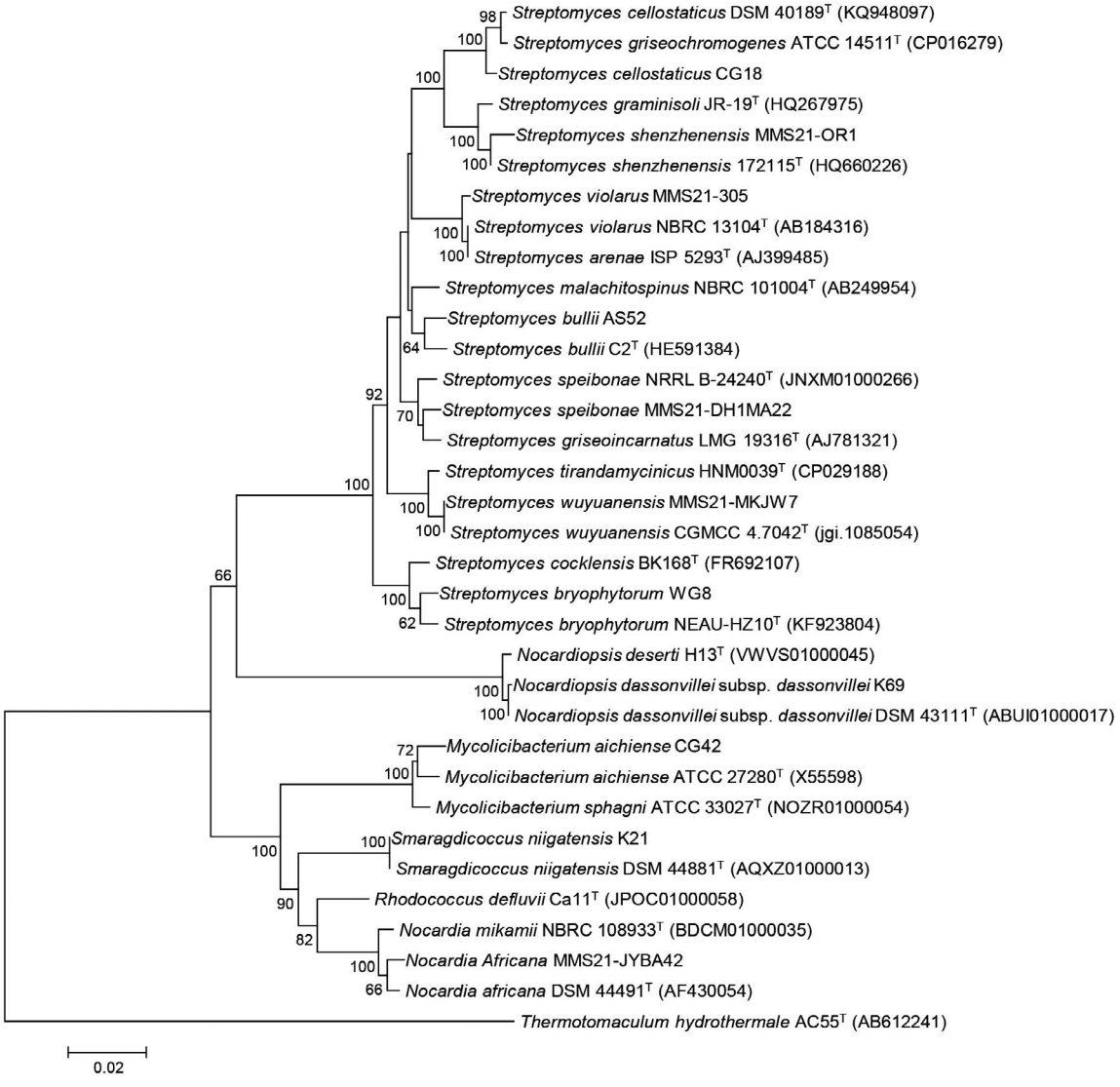


Fig. 2. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Actinobacteria*. Bootstrap values (>60%) are shown in the neighbor-joining method. Bar, 0.02 substitutions per nucleotide position.

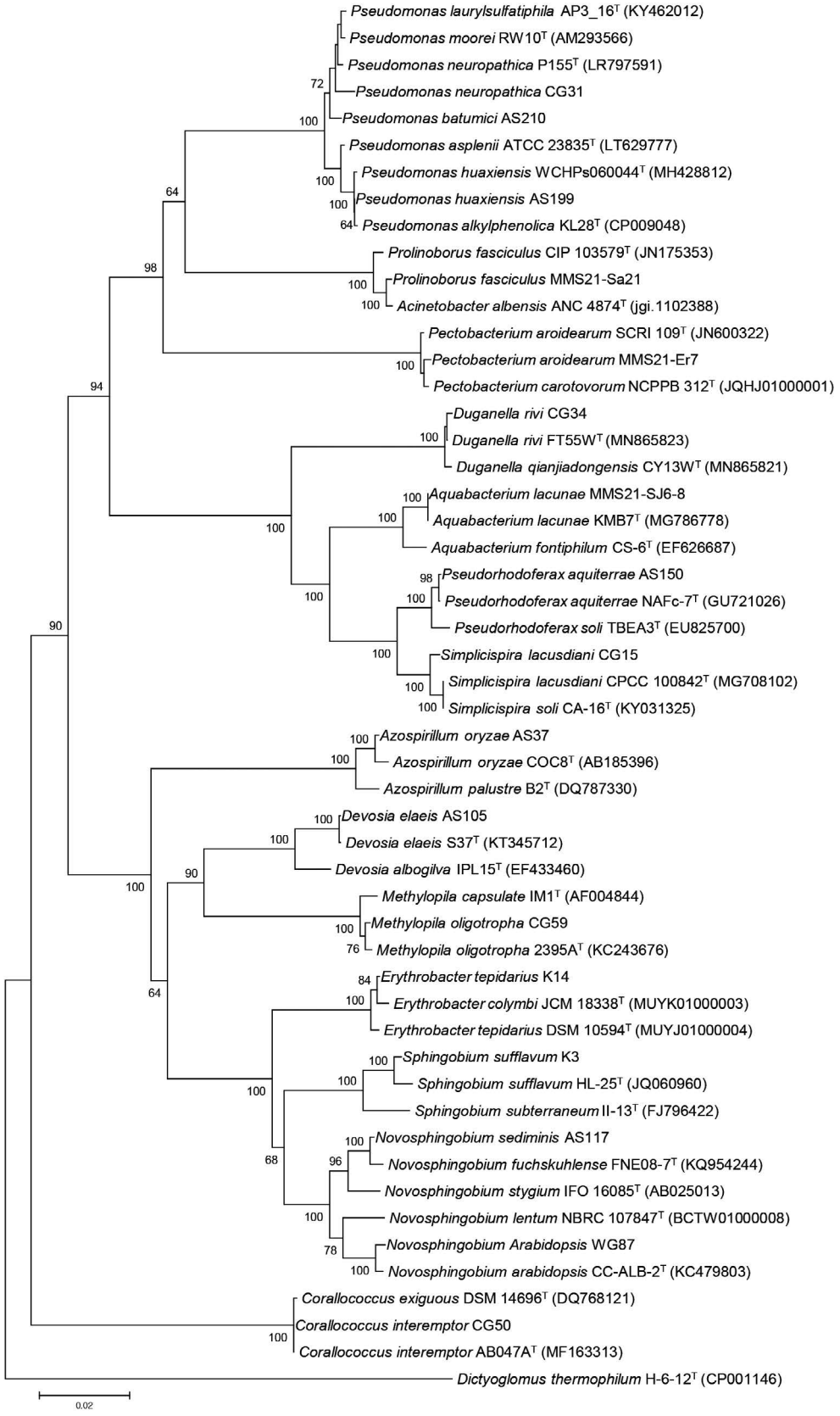


Fig. 3. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Proterobacteria*. Bootstrap values are greater than 60% are shown the neighbor-joining tree method. Bar, 0.02 substitutions per nucleotide position.

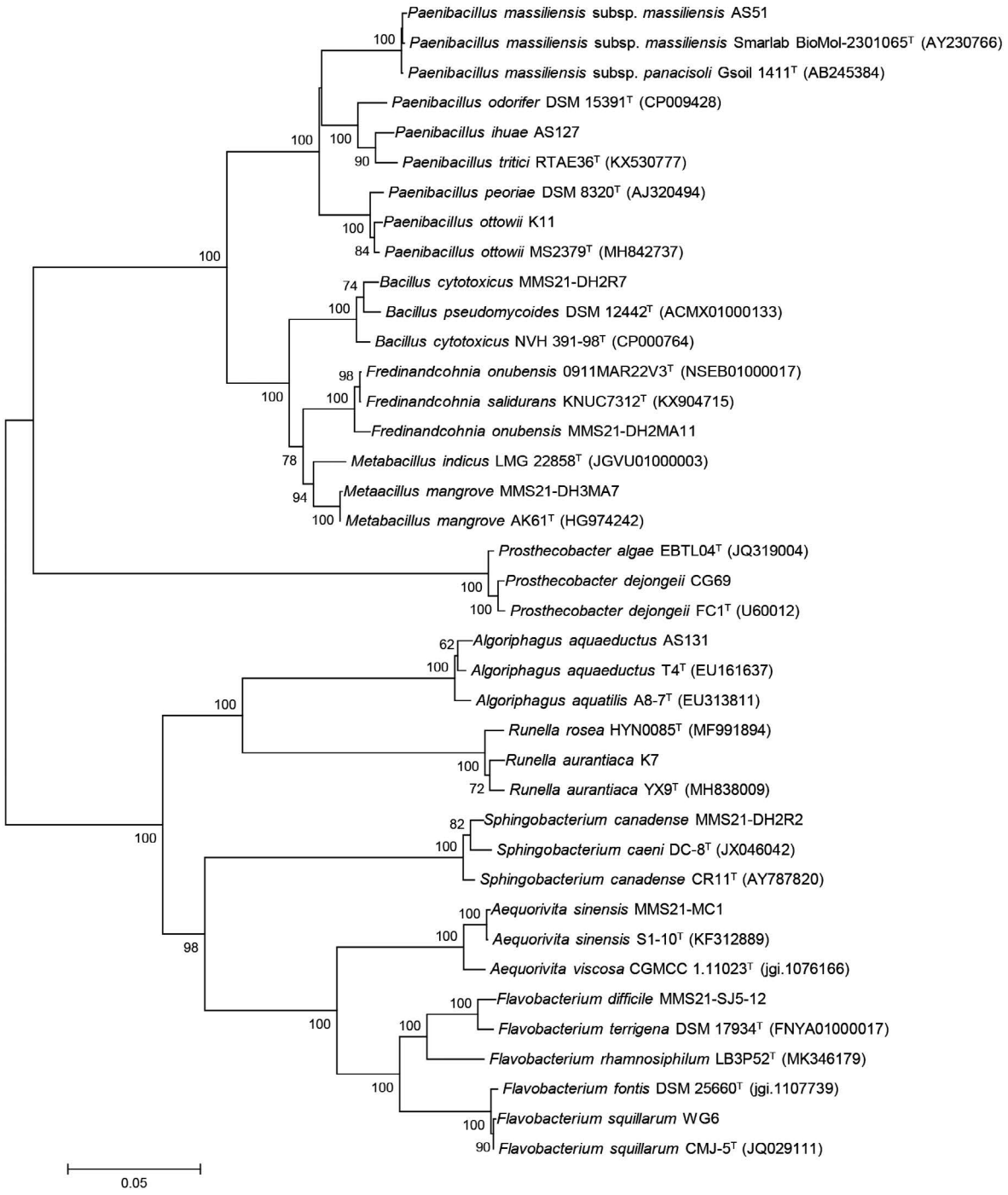


Fig. 4. Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between the strains in the phylum *Firmicutes*, *Bacteroidota* and *Verrucomicrobia*. Bootstrap values (>60%) are shown in the neighbor-joining tree. Bar, 0.05 substitutions per nucleotide position.

the phyla *Firmicutes*, *Bacteroidota* and *Verrucomicrobia* belong to 5 classes 10 separate genera: *Aequorivita* (1 species), *Algoriphagus* (1 species), *Bacillus* (1 species), *Flavobacterium* (2 species), *Fredinandcohnia* (1 species), *Metabacillus* (1 species), *Paenibacillus* (3 species), *Prosthecobacter* (1 species), *Runella* (1 species) and *Sphingobacterium* (1 species).

Here we report 41 unrecorded bacterial species in Korea belonging to 10 classes of 5 phyla.

Description of *Paenibacillus massiliensis* subsp. *massiliensis* AS51

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and white color after 3 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. Utilizes D-glucose, L-arabinose, D-mannose and D-mannitol. Does not utilize *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain AS51 (=NNIBR2021642BA18) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Streptomyces bullii* AS52

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous and white color after 3 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. Utilizes D-glucose, L-arabinose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize D-mannose, D-mannitol, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain AS52 (=NNIBR2021642BA19) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Streptomyces cellostaticus* CG18

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous and white → gray color after 3 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. Utilizes D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid and malic acid. Does not utilize D-mannitol, capric acid, trisodium citrate and phenylacetic acid. Strain CG18 (=NNIBR2021 642BA20) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Sphingobium sufflavum* K3

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes D-glucose and malic acid. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain K3 (=NNIBR2021642BA21) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Sphingobium sufflavum* K7

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and pink color after 3 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. Utilizes D-glucose, D-mannose, *N*-acetyl-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 K7 (=NNIBR2021642BA22) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Paenibacillus ottowii* K11

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and white color after 3 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. Utilizes D-glucose, D-mannose, D-mannitol and D-maltose. Does not utilize L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain K11 (=NNIBR2021642BA23) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Erythrobacter tepidarius* K14

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and orange color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes D-glucose, D-mannose and phenylacetic acid. Does not utilize L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate. Strain K14 (=NNIBR2021642BA24) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Flavobacterium squillarum* WG6

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 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-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain WG6 (=NNIBR2021642BA25) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Streptomyces bryophytorum* WG8

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous and ivory color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate and adipic acid. Does not utilize L-arabinose, D-mannitol, D-maltose, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain WG8 (=NNIBR2021642BA26) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Duganella rivi* CG34

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and white color after 3 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 and β-galactosidase. Utilizes D-glucose and L-arabinose. Does not utilize D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain CG34 (=NNIBR2021642BA27) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Methylopila oligotropha* CG59

Cells are Gram-staining-negative, non-flagellated and oval-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes potassium gluconate and malic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain CG59 (=NNIBR2021642BA28) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Novosphingobium arabidopsis* WG87

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, positive for urease, β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase. Utilizes D-glucose, L-arabinose, D-mannose and potassium gluconate. Does not utilize D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain WG87 (=NNIBR2021642BA29) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Azospirillum oryzae* AS37

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase, urease, esculin hydrolysis, β-galactosidase, but negative for indole production, glucose fermentation, gelatinase. Utilizes D-glucose, L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Does not utilize D-mannose and D-maltose. Strain AS37 (=NNIBR2021642BA30) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Simplicispira lacusdiani* CG15

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and white color after 3 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. Utilizes D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, adipic acid and malic acid. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. Strain CG15 (=NNIBR2021642BA31) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Mycolicibacterium aichiense* CG42

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, adipic acid, malic acid and trisodium citrate. Does not utilize *N*-acetyl-glucosamine, D-maltose, capric acid and phenylacetic acid. Strain CG42 (=NNIBR2021642BA32) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Corallococcus interemptor* CG50

Cells are Gram-staining-negative, non-flagellated and coccoid-shaped. Colonies are swarming and dark orange color after 3 days on R2A at 25°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-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain CG50 (=NNIBR2021642BA33) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Smaragdicoccus niigatensis* K21

Cells are Gram-staining-positive, non-flagellated and coccoid-shaped. Colonies are circular and dark green color after 3 days on R2A at 25°C. In API 20NE, positive for urease, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, β-galactosidase. Utilize *N*-acetyl-glucosamine and malic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain K21 (=NNIBR2021642 BA34) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Nocardiopsis dassonvillei* subsp. *dassonvillei* K69

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous and white color after 3 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. Utilize *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 K69 (=NNIBR2021642BA35) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Pseudomonas neuropathica* CG31

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are irregular raised undulate and cream color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction, arginine dihydrolase and esculin hydrolysis, but negative for indole production, glucose fermentation, urease, gelatinase, β-galactosidase. Utilize capric acid and malic acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, trisodium citrate and phenylacetic acid. Strain CG31 (=NNIBR2021642BA36) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Prosthecobacter dejongeii* CG69

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and cream color after 3 days on R2A at 25°C. In API 20NE, positive for urease, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, β-galactosidase. Utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain CG69 (=NNIBR2021642BA37) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Devosia elaeis* AS105

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and cream color after 3 days on R2A at 25°C. In API 20NE, positive for esculin hydrolysis, weak positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and D-maltose. Does not utilize potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain AS105 (=NNIBR 2021642BA38) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Novosphingobium sediminis* AS117

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and yellow color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate and adipic acid. Does not utilize *N*-acetyl-glucosamine, capric acid, malic acid, trisodium citrate and phenylacetic acid. Strain AS117 (=NNIBR2021642BA 39) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Paenibacillus ihuae* AS127

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and yellow color after

3 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, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate and malic acid. Does not utilize *N*-acetyl-glucosamine, capric acid, adipic acid, trisodium citrate and phenylacetic acid. Strain AS127 (=NNIBR2021642BA40) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Algoriphagus aquaeductus* AS131

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and red color after 3 days on R2A at 25°C. In API 20NE, positive for gelatinase and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis. Utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose and phenylacetic acid. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid and trisodium citrate. Strain AS131 (=NNIBR2021642BA41) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Pseudorhodoferax aquiterrae* AS150

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and white color after 3 days on R2A at 25°C. In API 20NE, positive for nitrate reduction and urease, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, β-galactosidase. Utilize L-arabinose, D-mannitol, D-maltose, potassium gluconate, adipic acid and malic acid. Does not utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, capric acid, trisodium citrate and phenylacetic acid. Strain AS150 (=NNIBR2021642BA 42) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Pseudomonas huaxiensis* AS199

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and red color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilize D-glucose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate and phenylacetic acid. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose and adipic acid. Strain AS199 (=NNIBR2021642BA43) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Pseudomonas batumici* AS210

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular and cream color after 3 days on R2A at 25°C. In API 20NE, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase and β-galactosidase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid and trisodium citrate. Does not utilize *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain AS210 (=NNIBR2021642BA44) has been isolated from a freshwater algae sample, Anseong-si, Korea.

Description of *Flavobacterium difficile* MMS21-SJ5-12

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 and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-SJ5-12 (=NNIBR2021642BA51) has been isolated from a freshwater sample, Okcheon-gun, Korea.

Description of *Streptomyces violarus* MMS21-305

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are filamentous, raised, undulate and yellow color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, urease, glucose fermentation, gelatinase and β-galactosidase, but negative for esculin hydrolysis, indole production and arginine dihydrolase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose and adipic acid. Does not utilize capric acid and phenylacetic acid. Strain MMS21-305 (=NNIBR2021642BA46) has been isolated from a freshwater sample, Gimhae-si, Korea.

Description of *Streptomyces wuyuanensis* MMS21-MKJW7

Cells are Gram-staining-positive and rod-shaped. Colonies are filamentous, raised, undulate and brown color after 3 days on R2A at 30°C. In API 20NE, positive for glucose fermentation, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease and esculin hydrolysis. Utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate and malic acid. Does not utilize L-arabinose,

adipic acid, D-mannitol, capric acid, trisodium citrate and phenylacetic acid. Strain MMS21-MKJW7 (=NNIBR 2021642BA45) has been isolated from a freshwater sample, Gimhae-si, Korea.

Description of *Streptomyces speibonae* MMS21-DH1MA22

Cells are Gram-staining-positive and rod-shaped. Colonies are circular, convex, entire and gray color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction and glucose fermentation, but negative for indole production, esculin hydrolysis, β-galactosidase, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-DH1MA22 (=NNIBR2021642BA 48) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Prolinoborus fasciculus* MMS21-Sa21

Cells are Gram-staining-negative, 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 and urease, but negative for esculin hydrolysis, β-galactosidase, indole production, glucose fermentation, arginine dihydrolase and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-Sa21 (=NNIBR2021 642BA7) has been isolated from a freshwater sample, Gangneung-si, Korea.

Description of *Bacillus cytotoxicus* MMS21-DH2R7

Cells are Gram-staining-positive, flagellate and rod-shaped. Colonies are rhizoid, raised, undulate and white color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for arginine dihydrolase, urease, glucose fermentation, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production and esculin hydrolysis. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol and potassium gluconate. Does not utilize capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-DH2R7 (=NNIBR2021642BA1) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Sphingobacterium canadense* MMS21-DH2R2

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow color after 2 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and β-galactosidase, but negative for indole production and gelatinase. Utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine and D-maltose. Does not utilize D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, adipic acid and phenylacetic acid. Strain MMS21-DH2R2 (=NNIBR2021642BA2) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Metabacillus mangrovi* MMS21-DH3MA7

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and white color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for arginine dihydrolase, esculin hydrolysis, urease, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production and glucose fermentation. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose and potassium gluconate. Does not utilize capric acid, malic acid, trisodium citrate, adipic acid and phenylacetic acid. Strain MMS21-DH3MA7 (=NNIBR2021642BA3) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Fredinandcohnia onubensis* MMS21-DH2MA11

Cells are Gram-staining-positive, flagellate and rod-shaped. Colonies are irregular, flat, entire and white color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for nitrate reduction, esculin hydrolysis, gelatinase and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. Utilize L-arabinose, potassium gluconate, *N*-acetyl-glucosamine and D-maltose. Does not utilize D-glucose, D-mannose, D-mannitol, capric acid, malic acid, trisodium citrate, adipic acid and phenylacetic acid. Strain MMS21-DH2MA11 (=NNIBR2021642BA49) has been isolated from a freshwater sample, Gongju-si, Korea.

Description of *Aequorivita sinensis* MMS21-MC1

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are beige color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for urease and esculin hydrolysis, but negative for nitrate reduction, indole production, β-galactosidase, glucose fermentation, arginine dihydrolase and gelatinase. Utilize D-mannose, D-mannitol and trisodium citrate. Does not utilize D-glucose, L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, D-maltose, adipic acid and

phenylacetic acid. Strain MMS21-MC1 (=NNIBR2021 642BA11) has been isolated from a freshwater sample, Asan-si, Korea.

Description of *Pectobacterium aroidearum* MMS21-Er7

Cells are Gram-staining-negative, flagellate and rod-shaped. Colonies are circular, flat, undulate and white color after 3 days on R2A at 30°C. In API 20NE, positive for glucose fermentation, but negative for esculin hydrolysis, β-galactosidase, nitrate reduction, indole production, arginine dihydrolase, urease and gelatinase. Utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine and D-maltose. Does not utilize, adipic acid, phenylacetic acid, potassium gluconate, capric acid, malic acid and trisodium citrate. Strain MMS21-Er7 (=NNIBR 2021642BA10) has been isolated from a freshwater sample, Daejeon, Korea.

Description of *Aquabacterium lacunae* MMS21-SJ6-8

Cells are Gram-staining-negative, flagellate and rod-shaped. Colonies are circular, convex, entire and white color after 3 days on marine agar 2216 at 30°C. In API 20NE, positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase and β-galactosidase. Utilize D-glucose. Does not utilize L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-SJ6-8 (=NNIBR2021642BA47) has been isolated from a freshwater sample, Okcheon-gun, Korea.

Description of *Streptomyces shenzhenensis* MMS21-OR1

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and brown color after 3 days on R2A at 30°C. In API 20NE, positive for urease and esculin hydrolysis, but negative for nitrate reduction, β-galactosidase, indole production, glucose fermentation, arginine dihydrolase, and gelatinase. Utilize D-mannose, D-mannitol and trisodium citrate. Does not utilize *N*-acetyl-glucosamine, potassium gluconate, capric acid, malic acid, D-glucose, L-arabinose, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-OR1 (=NNIBR2021642BA9) has been isolated from a freshwater sample, Cheongju-si, Korea.

Description of *Nocardia africana* MMS21-JYBA42

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are punctiform, convex, entire and peach color after 3 days on R2A at 30°C. In API 20NE, positive for nitrate reduction, but negative for esculin hydrolysis, β-galactosidase, indole production, glucose fermentation, arginine dihydrolase, urease and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, malic acid, trisodium citrate, *N*-acetyl-glucosamine, D-maltose, adipic acid and phenylacetic acid. Strain MMS21-JYBA42 (=NNIBR2021642BA8) has been isolated from a freshwater sample, Gangneung-si, Korea.

Conflicts of Interest

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

Acknowledgements

This research was supported by the "Survey of freshwater organisms (Prokaryotes)" (NNIBR202101204) research program of the Nakdonggang National Institute of Biological Resources.

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Submitted: December 19, 2022
Revised: February 19, 2024
Accepted: February 22, 2024

Journal of Species Research 13(4):367-372, 2024

Open Access

Three species of the subgenus *Bessobates* Townes, Townes & Gupta of *Netelia* Gray (Hymenoptera: Ichneumonidae: Tryphoninae) new to South Korea

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Three newly recorded species of the subgenus *Bessobates* of *Netelia* Gray, 1860 are reported. Three species, *Netelia* (*Bessobates*) *longipad*, *N.* (*B.*) *pallescens* and *N.* (*B.*) *yakushimensis*, are recorded for the first time from South Korea. Photographs and diagnoses of three unrecorded species of this subgenus are provided.

Keywords: *Bessobates*, Korea, *Netelia*, new record, taxonomy

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DOI:10.12651/JSR.2024.13.4.367

Introduction

Netelia Gray, 1860 is one of largest nocturnal genus within the family Ichneumonidae and its distribution is worldwide. Most *Netelia* are known to be ectoparastic koinobionts of lepidopteran larvae. This genus *Netelia* is uniformly brownish yellow Darwin Wasps (Konish, 2014) and this genus can be best identified by specialized structures of the male genitalia (Townes, 1938). More than 330 species have so far been recorded and divided into 12 subgenera worldwide. Until now, 31 species of eight subgenera recorded from South Korea (Lee, 2021). Among them, the subgenus *Bessobates* was reported as a genus by Townes *et al.* (1961) based on *Parabatus deceptor*. 22 species have been recognized from the Holarctic, Oriental and Neotropical regions.

In this study, three South Korean species of *Bessobates* are revised. Diagnoses and photographs of the South Korean species are provided.

Materials and Methods

Specimens are preserved in the Insect Inquiry · Education Institute in Daegu National University of Education (**DNUE-IIEI**, Daegu, Korea), Nakdonggang National Institute of Biological Resources (**NNIBR**, Sangju, Korea). Images of specimens of the new species were taken using an AxioCam MRc5 camera attached to a stereo microscope (Zeiss SteREO Discovery. V20; Carl Zeiss, Göttingen, Germany), processed using AxioVision SE64 software (Carl Zeiss), and optimized with a Delta imaging system (i-solution, IMT i-Solution Inc. Vancouver, Canada) and a Leica MC190 HD Camera attached to a Leica M125 Microscope (Leica Microsystems, Germany) with images, processed using LEICA LAS X software (Leica). Morphological terminology follows mostly that of Gauld (1991) and terminology for the male genitalia follows that of Snodgrass (1941). Dissections of the genitalia were prepared following Konishi (2005) and head and mesosoma were measured by the methods of Konishi (1985). Konishi's key was used to distinguish between these three species (Konish, 2014). The following indices (Gauld and Michell, 1981) are used:

Geno-orbital index = maximum breadth of eye in profile / maximum breadth of gena in same line

Nervellar index of hind wing = length of Cu1 between cu-a and M / length of cu-a

Abbreviations are as follows: **NIAES**, Laboratory of Insect Systematics, National Institute for Agro-Environmental Sciences, Tsukuba, Japan; **GN**, Gyeongsangnam-do; **GW**, Gangwon-do; **JJ**, Jeju-do.

Systematic Accounts

Family Ichneumonidae Latreille, 1802 맵시벌과
Subfamily Tryphoninae Shuckard, 1840
뭉툭맵시벌아과

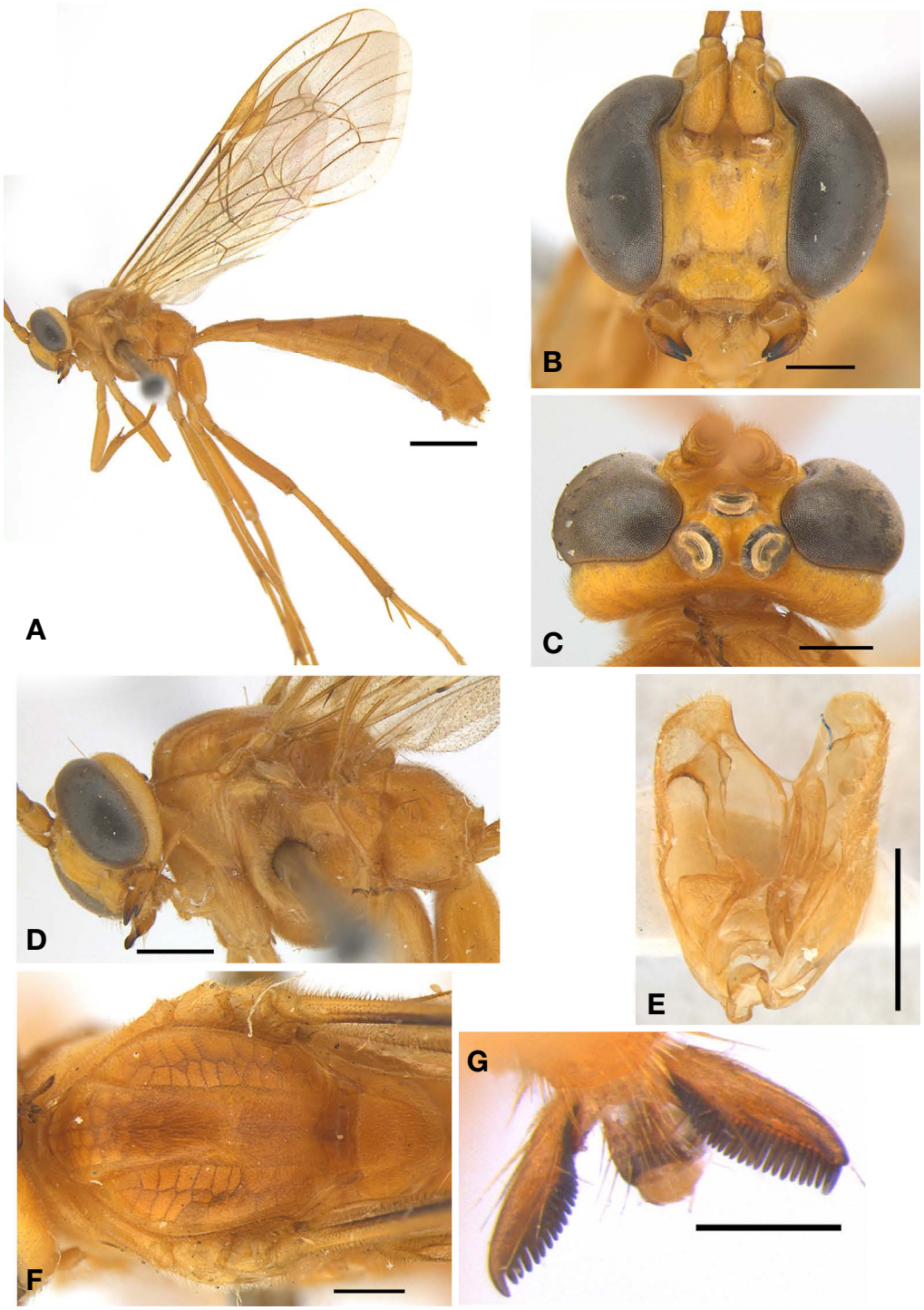


Fig. 1. *Netelia* (*Bessobates*) *longipad* A, Habitus, lateral view; B, Head, frontal view; C, Head, dorsal view; D, Head and mesosoma, lateral view; E, Male genitalia; F, Mesoscutum, dorsal view; G, Tarsal claws. Scale bars: 2 mm: A; 1 mm: D, E; 0.5 mm: B, C, F; 0.2 mm: G.

Genus *Netelia* Gray, 1860 자루맵시벌속

Netelia Gray, 1860: 341. Type species: *Paniscus inquinatus* Gravenhorst, 1829

Subgenus *Bessobates* Townes, Townes & Gupta, 1961 등검정자루맵시벌아속

Bessobates Townes, Townes & Gupta, 1961: 1−522. Type species: *Parabatus deceptor*

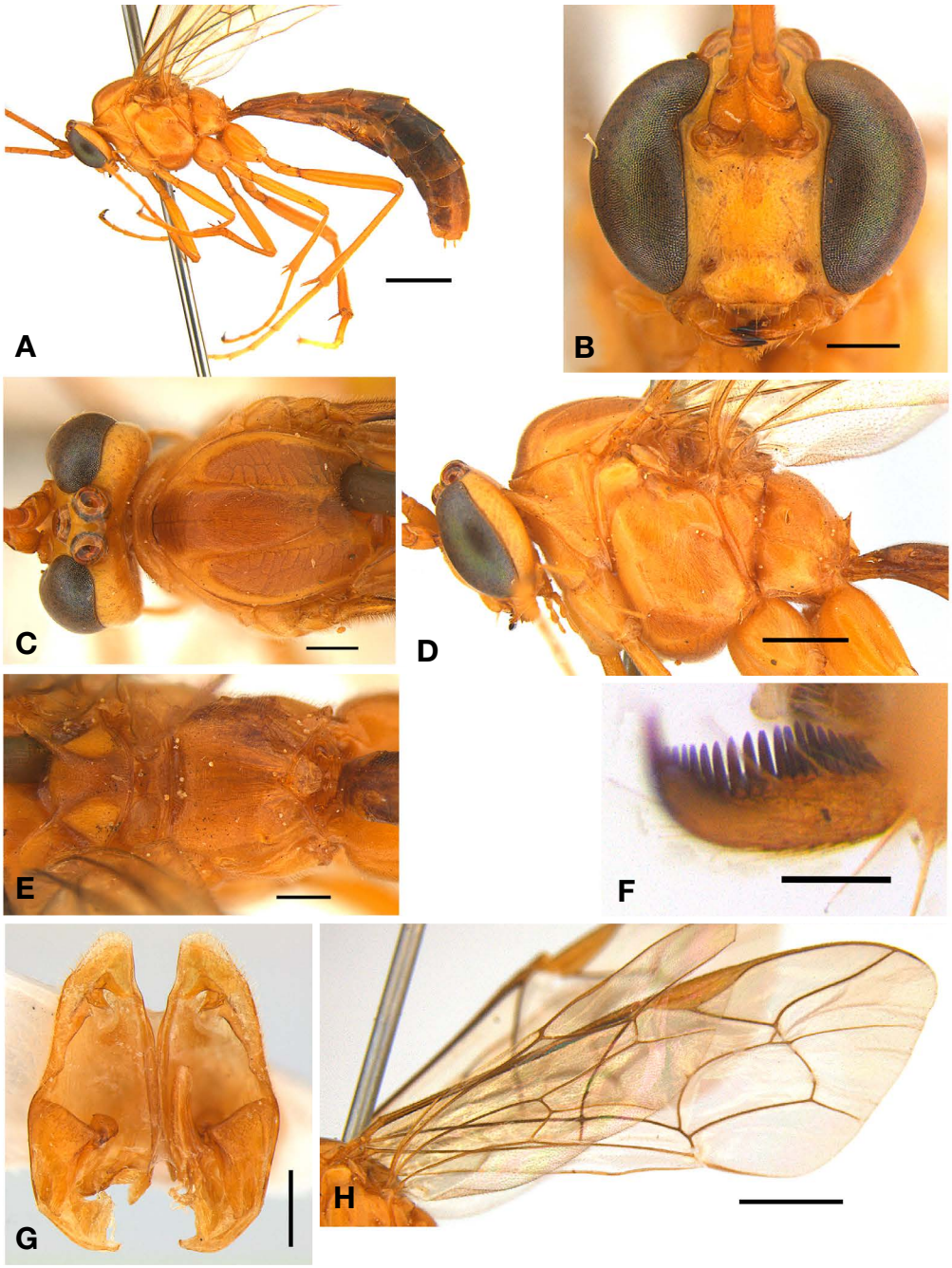


Fig. 2. *Netelia* (*Bessobates*) *pallescens* A, Habitus, lateral view; B, Head, frontal view; C, Head and mesoscutum, dorsal view; D, Head and mesosoma, lateral view; E, Propodeum, dorsal view; F, Hind tarsal claw; G, Male genitalia; H, Fore wing. Scale bars: 2 mm: A, H; 0.5 mm: B–E, G; 0.1 mm: F.

Diagnosis. Occipital carina absent. Lateral carina of scutellum not extending to apex of scutellum. Cu-a almost opposite Rs&M; 3r-m present. In female, the short ovipositor is the unique character, as long as apical depth of metasomal tergite. In male, the overhang of the subapical sclerotized plate of the paramere and the strongly curved basal apodeme of the aedeagus.

***Netelia* (*Bessobates*) *longipad* Konishi, 2014**
끝긴등검정자루맵시벌 (신칭) (Fig. 1)
Netelia (*Bessobates*) *longipad* Konishi, 2014: 322. Type: male; TD: NIAES.

Diagnosis. Vertex evenly rounded in dorsal view; geno-orbital index 2.0–2.2; antenna with 50–57 flagel-

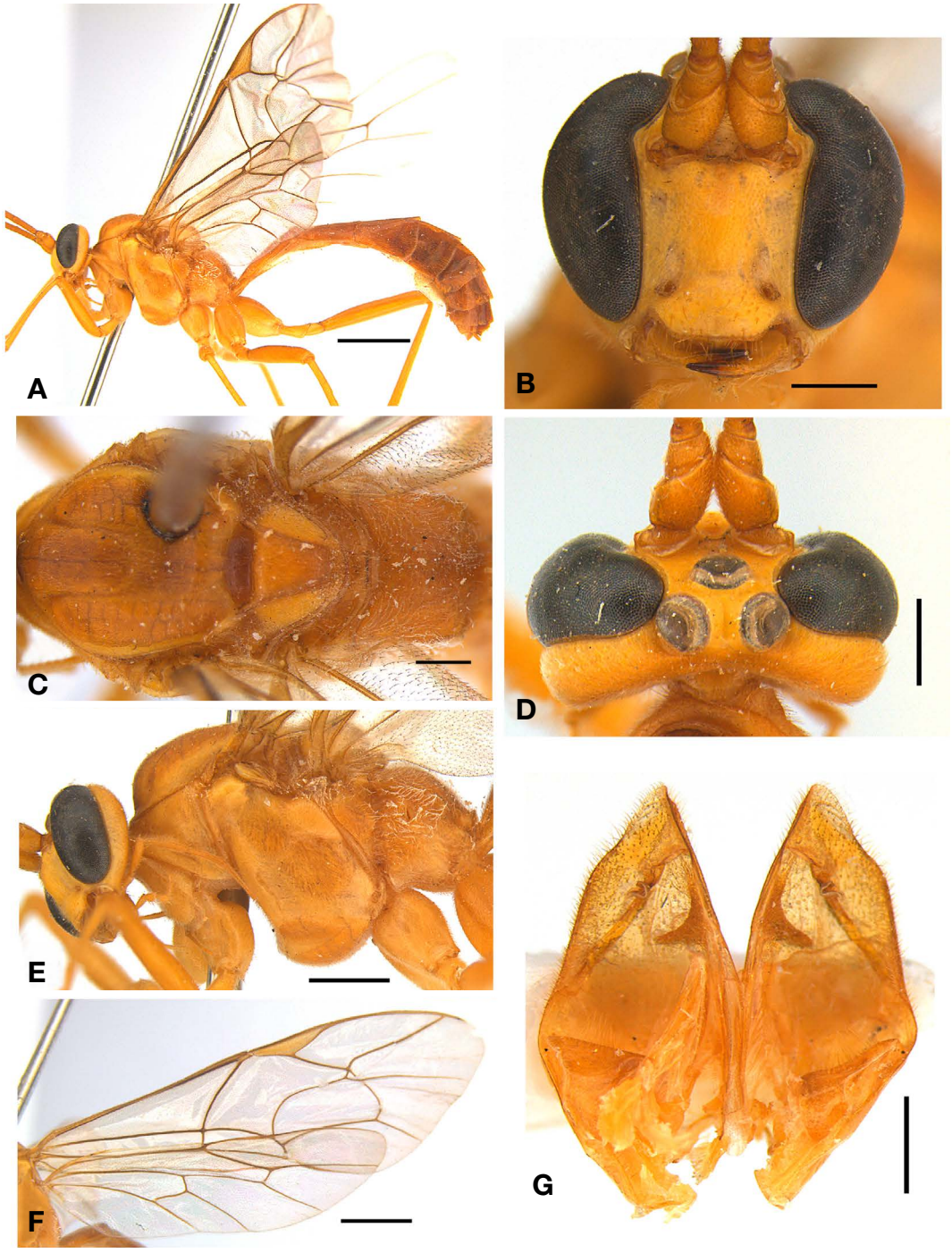


Fig. 3. *Netelia* (*Bessobates*) *yakushimensis* A, Habitus, lateral view; B, Head, frontal view; C, Mesosoma, dorsal view; D, Head, dorsal view; E, Head and mesosoma, lateral view; F, Wings; G, Male genitalia. Scale bars: 2 mm: A, F; 0.5 mm: B–E, G.

lomeres. Upper tooth of mandible much longer than lower tooth. Scutellar lateral carina fading out basal 1/3 of scutellum; propodeum weakly trans-striate on about basal 0.7, with weak crest; nervellar index 0.7–0.9. Dorsal and ventral margins of paramere slightly emarginated subapically; digitus truncate and ventro-apical corner without any teeth; pad long, extending anterior area and meeting apical 2/3 of paramere. Occiput and interocellar area light brownish yellow. Mesosternum and mesopleuron with light brown markings irregularly.

Material examined. [South Korea] 1♂, [GN] Hadong-gun, Bukcheon-myeon, Jikjeon-ri, Mt. Emyeonsan, 14–

15.v.1999, J.S. Park (DNUE_IIEI); 1♂, Hadong-gun, Cheongam-myeon, Gunghang-ri, Jusan, 1−2.vi.2002, E.S. Lee (DNUE_IIEI); 1♂, Ulju-gun, Ungchon-myeon, Eunhyeon-ri, Mujaechi 1 Wetland, 1−2.vi.2001, J.S. Park (DNUE_IIEI); 1♂, ditto, J.K. Choi (NNIBR: NNIBRIN213162).
Distribution. South Korea (new record), Japan.

***Netelia* (*Bessobates*) *pallescens* (Schmiedeknecht, 1910)** 연노랑등검정자루맵시벌 (신칭) (Fig. 2)
Parabatus pallescens Schmiedeknecht, 1910: 1851. Type: male; TD: SCHMIEDEKNE.

Diagnosis. Vertex evenly rounded in dorsal view; geno-orbital index 2.2−2.7; antenna with 43−53 flagellomeres. Scutellar lateral carina fading out basal 1/4 of scutellum; propodeum weakly or distinctly trans-striate in middle, with weak crest; nervellar index 0.4−0.5. Paramere subelliptic, dorsal and ventral margins of paramere slightly emarginated subapically; dorso-apical corner of digitus rounded and ventro-apical corner with 1−2 teeth; membrane of median surface with strip of minutely papillate cuticle, long and weakly curved; pad large situated at apical 1/4 of paramere in middle. Mesoscutum with light brown stripes on each of median and lateral lobes; mesosternum and mesopleuron with brownish yellow markings.
Material examined. [South Korea] 1♂, [GN] Hadong-gun, Akyang-myeon, Deungchon-ri, Hyeongjaebong, 10−11.v.2002, T.H. Ahn (DNUE_IIEI); 1♂, [GW] Ganseong, Geonbongsa, 22.v.1992, J.W. Lee (NNIBR: NNIBRIN166271); 1♂, [JJ] Jeju-si, Yongsil, Giam, 2.vi.1968 (DNUE_IIEI).
Distribution. South Korea (new record), Japan, Germany, Ukraine and United Kingdom.

***Netelia* (*Bessobates*) *yakushimensis* Konishi, 2014** 작은등검정자루맵시벌 (신칭) (Fig. 3)
Netelia (*Bessobates*) *yakushimensis* Konishi, 2014: 339. Type: male; TD: NIAES.

Diagnosis. Vertex evenly rounded in dorsal view; geno-orbital index 2.6−2.9; antenna with 51−53 flagellomeres. Scutellar lateral carina fading out beyond middle of scutellum; propodeum trans-striate on basal 0.65, with crest or without and represented by rounded swelling; nervellar index 0.5−0.6. Paramere long, apical margin of paramere acutely rounded; dorso-apical corner of digitus rounded off and ventral-apical corner with teeth; membrane of median surface with subtriangular area of minutely papillate cuticle; pad subtriangular, situated at apical 1/4 of paramere in middle. Mesoscutum with brown stripes on each of median and lateral lobes and a brown spot situated behind median stripe or fused anterioly; mesopleuron with yellow markings.
Material examined. [South Korea] 1♂, [GN] Hadong-gun, Akyang-myeon, Deungchon-ri, Hyeongjaebong, 10−11.v.2002, J.S. Shin (NNIBR: NNIBRIN166272); 2♂♂, [GW] Ganseong, Geonbongsa, 22.v.1992, J.W. Lee (DNUE_IIEI); 1♂, Samcheok-si, Hajang-myeon, Galjeon-ri, 16.v-5.vi.2007, P. Tripotin (DNUE_IIEI); 1♂, [JJ] Jeju-si, Yongsil, Giam, 2.vi.1968 (DNUE_IIEI); 1♂, Jeju-si, Hallasan, 22.v.1968, S.M. Lee (DNUE_IIEI).
Distribution. South Korea (new record), Japan.

Conflicts of Interest

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

Acknowledgements

I am deeply grateful to anonymous reviewers for reviewing this manuscript. Reviewers provided constructive criticism that improved this paper. This work was supported by a grant from the Nakdonggang National Institute of Biological Resources (NNIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NNIBR202201201, NNIBR20241204).

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Submitted: July 2, 2024
Revised: October 14, 2024
Accepted: October 15, 2024

Journal of Species Research 13(4):373-377, 2024

Open Access

New record of two spirotrichous ciliate species (Protozoa, Ciliophora) collected from coasts of South Korea

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Two newly recorded ciliates, *Aspidisca major* (Madsen, 1931) Kahl, 1932 and *Metaurostylopsis rubra* Song and Wilbert, 2002, were collected from eastern and southern coasts of South Korea, respectively. The morphology of these two species were studied based on observations of protargol-stained specimens. The morphological characteristics of the two species are as follows: (1) *Aspidisca major* is characterized by a size of 76−78 × 42−49 μm after protargol impregnation, two distinct macronuclei, seven frontoventral cirri in "*polystyla*-arrangement", and dorsal kinety 1 extends to posterior end of dorsal kinety 2; and (2) *Metaurostylopsis rubra* is characterized by a size of 68−77 × 49−58 μm size after protargol impregnation, brick-reddish body color, and large number of marginal rows. In the present study, we provide a brief diagnosis, remarks, and photomicrographs.

Keywords: *Aspidisca major*, marine ciliates, *Metaurostylopsis rubra*, taxonomy

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DOI:10.12651/JSR.2024.13.4.373

Introduction

The genera *Aspidisca* Ehrenberg, 1830 and *Metaurostylopsis* Song *et al*., 2001 are widely distributed globally in marine ecosystems. Up to date, about 60 nominal *Aspidisca* species and eight *Metaurostylopsis* species have been recorded (Wu and Curds, 1979; Fernandez-Leborans de Zaldumbide, 1987; Valbonesi, 1996; Berger, 2001; Song and Wilbert, 2002; Lei *et al*., 2005; Shao *et al*., 2008a; 2008b; Jankowski, 2009; Shen *et al*., 2010; Chen *et al*., 2011; Jung *et al*., 2011; Jiang *et al*., 2013; Lu *et al*., 2016; Song *et al*., 2020; Zheng *et al*., 2022; Choi *et al*., 2024). According to the National Institution of Biological Resources (2023), only 11 *Aspidisca* species (*A. aculeata* (Ehrenberg, 1838) Kahl, 1932; *A. cicada* (Müller, 1786) Claparède and Lachmann, 1858; *A. dentata* Kahl, 1928; *A. hexeris* Quennerstedt, 1869; *A. leptaspis* Fresenius, 1865; *A. lynceus* (Müller, 1773) Ehrenberg, 1830; *A. orthopogon* Deroux and Tuffrau, 1965; *A. polypoda* (Dujardin, 1841) Kahl, 1932; *A. polystyla* Stein, 1859; *A. steini* (Buddenbrock, 1920) Kahl, 1932; and *A. turrita* (Ehrenberg, 1838) Claparède and Lachmann, 1858) and only 4 *Metaurostylopsis* species (*M. cheni* Chen *et al*., 2011; *M. marina* (Kahl, 1932) Song *et al*., 2001; *M. salina* Lei *et al*., 2005; and *M. struederkypkeae* Shao *et al*., 2008) have been recorded in South Korea, indicating undersampling of the Korean ciliate fauna (Shin and Kim, 1988; Lei *et al*., 2005; Li *et al*., 2010; Park *et al*., 2012; Kim *et al*., 2016; Kim and Jung, 2018; Kim *et al*., 2018; 2020; Choi *et al*., 2020; Choi *et al*., 2023). Therefore, this study aims to further explore the diversity and distribution of these genera in Korean marine environments.

During a field survey, we identified two species, 1) *Aspidisca major* (Madsen, 1931) Kahl, 1932; and 2) *Metaurostylopsis rubra* Song and Wilbert, 2002, previously unrecorded in Korea. In this paper, we provide brief diagnosis, remarks, photomicrographs, and information about collection sites and conditions.

Materials and Methods

The detailed information about collection sites and conditions are provided in 'Material examined' section for each species. After gently stirring up surface marine water, samples were collected with bottom sediments. All samples were transferred to the laboratory and kept in Plant culture dishes at room temperature (ca. 20°C) with 1−3 autoclaved rice grains. The morphology of each species was studied using a stereomicroscope (SZ11; Olympus, Tokyo, Japan), and photomicrographs were captured

using a digital camera (DP74; Olympus). The protargol powder was synthesized using the method of Pan *et al.* (2013) and Kim and Jung (2017). The protargol-impregnated specimens were prepared using the 'procedure A' method of Foissner (2014). The differential through-focal images of the protargol-impregnated specimens were merged using Helicon Focus ver. 8.1.0 software (Helicon-Soft Ltd, Kharkiv, Ukraine). The basic terminology and taxonomic classification mainly followed Lynn (2008), Song *et al.* (2001), and Wu and Curds (1979).

Results and Discussion

Phylum Ciliophora Doflein, 1901
Class Spirotrichea Bütschli, 1889
Order Euplotida Small and Lynn, 1985
Suborder Euplotina Jankowski, 1979
Family Aspidiscidae Ehrenberg, 1830
Genus *Aspidisca* Ehrenberg, 1830

1. *Aspidisca major* (Madsen, 1931) Kahl, 1932 (Fig. 1)

Material examined. Marine water (salinity 37.8‰, temperature 18.3°C) collected from Sodol beach, Jumun-ri, Jumunjin-eup, Gangneung-si, Gangwon-do, Korea (37° 54′24.6″N, 128°49′41.7″E) on May 15, 2023.

Diagnosis. Size 76−78 × 42−49 μm after protargol impregnation (n = 3); body bean-shaped; cortex rigid; peristomial spur and projections along left margin on ventral side lacking (Fig. 1A); anterior adoral zone of membranelles (AZM1) about 4 μm long with 4 membranelles, posterior adoral zone of membranelles (AZM2) about 24 μm long after protargol impregnation and with 15−18 membranelles; invariably seven frontoventral cirri in "*polystyla*-arrangement"; five transverse cirri, cilia of each transverse cirrus not separated (Fig. 1A); four dorsal kineties with 25−28, 18−22, 19−23, and 22−24 dikinetids in dorsal kineties 1−4, respectively (Fig. 1B); dorsal kinety 1 distinctly curved to right posteriorly and ends near posterior end of dorsal kinety 2 (Fig. 1C); two ellipsoid macronuclei, micronucleus not observed (Fig. 1D).

Distribution. Germany, South Korea

Remarks. The Korean population of *Aspidisca major* corresponds with the German population redescribed by Kahl (1932) in all available aspects. *Aspidisca major* is a special species among the congeners in having two macronuclei (Fig. 1D). Because of this feature, *A. major*, *A. binucleata* Kahl, 1932, *A. fusca* Kahl, 1928, and *A. mutans* Kahl, 1932 can be easily distinguished from other members of the genus *Aspidisca*. However, *A. major* differs from *A. binucleata* in the number of frontoventral cirri (7 vs. 9). *Aspidisca mutans* differs from *A. major* by the number of frontoventral cirri (12 vs. 7) and the number of transverse cirri (7 vs. 5) (Kahl, 1932). *Aspidisca fusca* differs from *A. major* by the presence of a peristomial spur

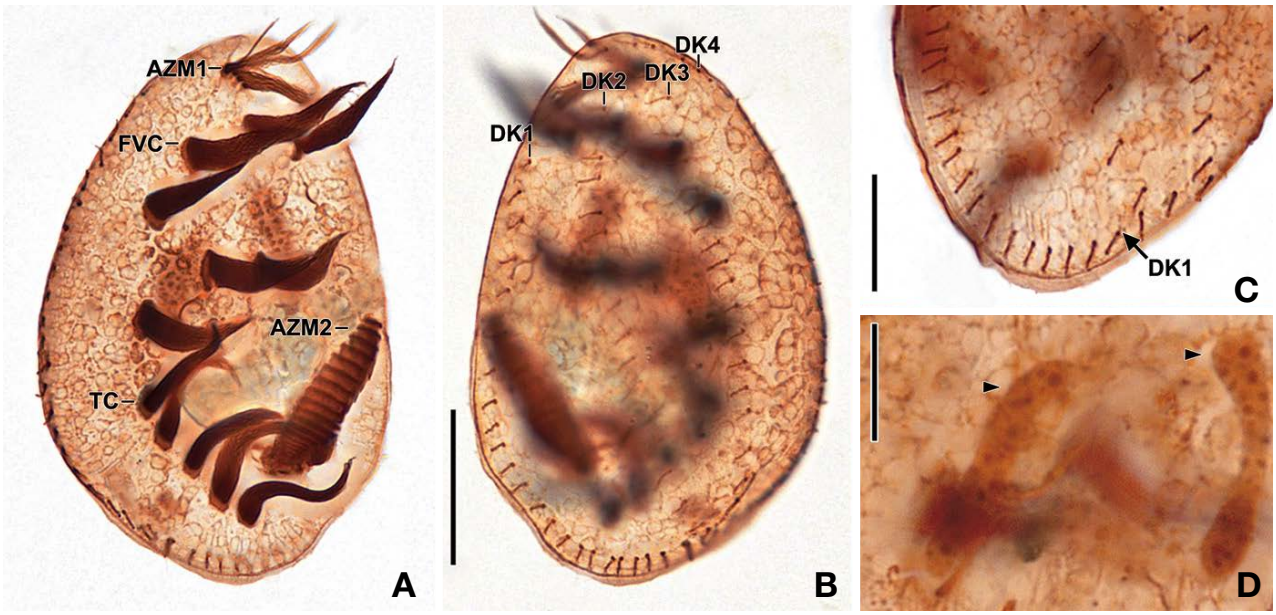


Fig. 1. *Aspidisca major* after protargol impregnation (A−D). A, B. Ventral (A) and dorsal (B) view, showing the body shape, anterior and posterior portion of adoral zone of membranelles, the transverse cirri, the frontoventral cirri in "*polystyla*-arrangement", and dorsal kineties 1−4. C. Dorsal view showing the arrangement of dorsal kineties. Dorsal kinety 1 curved posteriorly and extends to posterior end of dorsal kinety 2. D. Two ellipsoidal macronuclei (arrowheads). AZM1 and 2, anterior and posterior adoral zone of membranelles; DK1−4, dorsal kineties 1−4; FVC, frontoventral cirri; TC, transverse cirri. Scale bars = 20 μm (A, B); 10 μm (C, D).

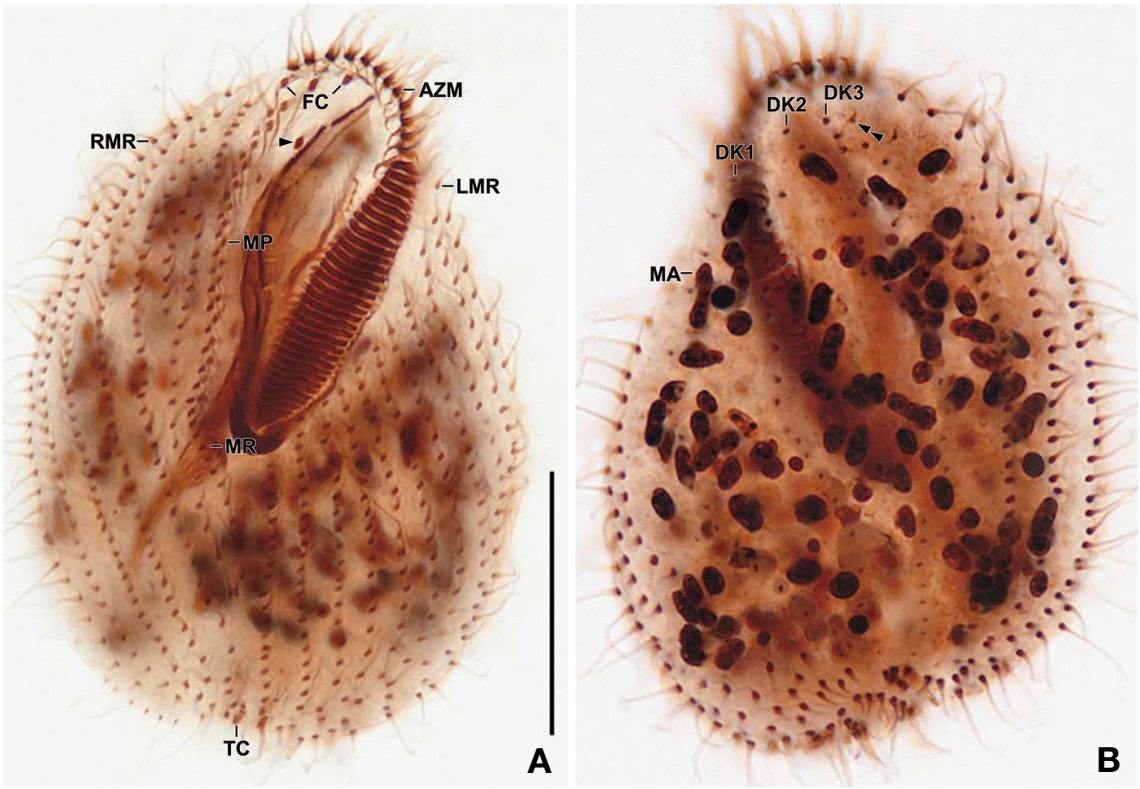


Fig. 2. *Metaurostylopsis rubra* after protargol impregnation (A, B). A. Ventral view showing the body shape, adoral zone of membranelles, frontal and transverse cirri, midventral pairs and row, and left and right marginal rows. Arrowhead denotes one buccal cirrus. B. Dorsal view showing the dorsal kineties 1–3 and macronuclear nodules. Double arrowhead denotes fragment-like kineties anterior to the leftmost right marginal row. AZM, adoral zone of membranelles; DK1–3, dorsal kineties 1–3; FC, frontal cirri; LMR, left marginal rows; MA, macronuclear nodules, MP, midventral pairs; MR, midventral row; RMR, right marginal rows; TC, transverse cirri. Scale bar = 30 μm.

(present vs. absent) (Wu and Curds, 1979). Considering the arrangement of frontoventral cirri, number of membranelles in AZM1 and the presence of peristomial spur, the Korean population of *A. major* is similar to *A. steini* however, they differ mainly in the number of macronuclei (two vs. one) and larger body size (76–78 × 42–49 μm vs. 20–35 × 15–27 μm) (Wu and Curds, 1979; Song and Wilbert, 1997).

Voucher slides. Two slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korea (MABIK PR00045067, MABIK PR00045068).

Order Urostylida Jankowski, 1979
Family Urostylidae Bütschli, 1889
Genus *Metaurostylopsis* Song, Petz and Warren, 2001

2. *Metaurostylopsis rubra* Song and Wilbert, 2002 (Fig. 2)

Material examined. Marine water (salinity 36.1‰, temperature 16.8°C) collected from Sanyang-eup, Tongyeong-si, Gyeongsangnam-do, South Korea (34°49′16″ N, 128°26′27″E) on April 17, 2023.

Diagnosis. Size 68–77 × 49–58 μm after protargol impregnation (n = 3); body oval to ellipsoidal with brick-reddish color; body flexible and slightly contractile; adoral zone of membranelles occupies about 50% of body length; 36–44 adoral membranelles; 1 buccal cirrus; 3 frontal, 8–9 frontoterminal, and 5–6 transverse cirri; 13–16 midventral pairs and a midventral row with about 8–12 cirri, extending to the midline of body; 10–12 left and 6–7 right marginal rows (Fig. 2A); three dorsal kineties (Fig. 2B); and about 70 macronuclear nodules.

Distribution. Antarctic, South Korea

Remarks. The Korean population of *Metaurostylopsis rubra* is similar to the type population of *M. rubra* described by Song and Wilbert (2002) in most features except for the slightly non-overlapping number of midventral pairs (13–16 vs. 8–11) and left marginal rows (10–12 vs. 6–9) (Song and Wilbert, 2002). *Metaurostylopsis rubra* can be easily distinguished from other congeners by the cell color and the number of midventral pairs and left marginal rows (Fig. 2A). Since the number of living

cells were not enough to examine in detail, we cannot determine whether the reddish color of the cells is due to the reddish cytoplasm or the cortical granules. Among the congeners, there is no species with more than six right marginal rows (Song *et al*., 2020). Also, only two species, *M. struederkypkeae* and *M. parastruederkypkeae* Lu *et al*., 2016, have reddish body color like *M. rubra*, but they differ from the present Korean population by the number of right marginal rows (3 vs. 3–5 vs. 6–7) (Shao *et al*., 2008b; Lu *et al*., 2016; Song *et al*., 2020).

Voucher slides. Two slides with protargol-impregnated specimens were deposited at the National Marine Biodiversity Institute of Korea (MABIK PR00045065, MABIK PR00045066).

Conflicts of Interest

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

Acknowledgements

This work was supported by the management of Marine Fishery Bio-resources Center (2024) funded by the National Marine Biodiversity Institute of Korea (MABIK), the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBRE202406), and 2024 Academic Research Support Program in Gangneung-Wonju National University.

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Submitted: July 9, 2024
Revised: August 6, 2024
Accepted: August 6, 2024

Journal of Species Research 13(4):378-384, 2024

Open Access

Report of five unrecorded bacterial species in Korea belonging to the genus *Bradyrhizobium*

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Symbiotic bacteria belonging to the genus *Bradyrhizobium* were generally called 'rhizobia' which can fix nitrogen by nodulating legumes. Between 2000 and 2001, numerous *Bradyrhizobium* strains were isolated from root nodules. However, due to challenges in identification, many of these isolates remained unreported. Recent advances in phylogenetic analyses based on whole genome sequencing have resolved these difficulties in species identification. Consequently, five of these strains have now been re-identified and are described as previously unrecorded species in the Republic of Korea. As a result, we report *Bradyrhizobium elkanii* Glm-3 (=KACC 10989), *Bradyrhizobium australafricanum* Glm-4 (=KACC 10990), *Bradyrhizobium huanghuaihaiense* Glm-7 (=KACC 10993), these were isolated from root nodules of *Glycine max*, *Bradyrhizobium frederickii* Kus-5 (=KACC 11016) isolated from root nodules of *Kummerowia striata* and *Bradyrhizobium diazoefficiens* Leb-14 (=KACC 11026) isolated from root nodules of *Lespedeza bicolor*.

Keywords: *Bradyrhizobium*, Rhizobia, unrecorded species

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DOI:10.12651/JSR.2024.13.4.378

Introduction

The genus *Bradyrhizobium* was established by Jordan DC (1982), belongs to the family *Nitrobacteracea*. This genus comprises 72 validly published species, with *Bradyrhizobium japonicum* as the type species (https://lpsn.dsmz.de/genus/microbacterium; accessed 24 June 2024). They are a Gram-negative, non-spore-forming, rod-shaped and either motile or non-motile. The genus *Bradyrhizobium* is known as a predominant symbiont of legumes by nodulating (Parker, 2015) and most of them have ability to biological nitrogen fixation (BNF) (Vanlnsberghe *et al*., 2015). They are found mainly in soil, rhizosphere and plant root nodules, and are classified by their or and characteristics (Parker, 2015; Tao *et al*., 2021). The significance of *Bradyrhizobium* has led to studies on its diversity in Korea, many of strains were isolated from legumes or agricultural soils (Kwon *et al*., 2005; Kim *et al*., 2022). However, only six species are currently reported in Korea according to the National List of Species of Korea (https://species.nibr.go.kr; accessed 24 June 2024). Because *Bradyrhizobium* cannot be identified based on 16S rRNA region sequences alone, most of them are not identified with the correct scientific name (Vinuesa *et al*., 2005; Delamuta *et al*., 2013). In this study, we focused on re-identification of *Bradyrhizobium* strains isolated from plant in Korea during 2000−2001. These strains were preserved in Korea Agricultural Culture Collection (KACC) and describe the taxonomic properties of five unrecorded species of the genus *Bradyrhizobium*.

Materials and Methods

All strains have been deposited at the Korean Agricultural Culture Collection (KACC) which were isolated from nodules of *Glycine max*, *Lespedeza bicolor* or *Kummerowia striata* in 2001−2002 reported by Qian *et al*. (2003). The designated strain IDs, isolation sources and identification results are summarized in Table 1. All cultured strains were maintained in glycerol suspension (15%, v/v) at −80°C.

The 16S rRNA and *recA* gene sequencing of the strains pure-cultured by the above procedure was conducted by Macrogen. For getting the sequence of *recA* gene region, all strains were amplified with the TSrecAf and TSrecAr primer sets described by Stępkowski *et al*. (2005).

These gene sequences of the type strains of the *Bradyrhizobium* species were obtained from NCBI database (https://www.ncbi.nlm.nih.gov/genbank/). Phylogenetic

Table 1. The taxonomic affiliations of isolated strains belonging to the genus *Bradyrhizobium*.

Genus	Strain code	KACC[a] ID	Identification	16S rRNA similarity (%)	*recA* similarity (%)	Host	Location	Culture conditions
Bradyrhizobium	Glm-3	10989	*Bradyrhizobium elkanii*	100	100	*Glycine max*	Suwon-si, Gyeonggi-do	R2A[b] medium, 28°C, 5 days
	Glm-4	10990	*Bradyrhizobium ferriligni*	99.6	99.6	*Glycine max*	Suwon-si, Gyeonggi-do	
	Glm-7	10993	*Bradyrhizobium huanghuaihaiense*	100	100	*Glycine max*	Suwon-si, Gyeonggi-do	
	Kus-5	11016	*Bradyrhizobium frederickii*	100	97.7	*Kummerowia striata*	Suwon-si, Gyeonggi-do	
	Leb-14	11026	*Bradyrhizobium diazoefficiens*	100	98.2	*Lespedeza bicolor*	Suwon-si, Gyeonggi-do	

[a]Korean Agricultural Culture Collection (KACC).
[b]R2A agar medium containing yeast extract 0.05% (w/v), peptone 0.05% (w/v), casamino acid 0.05% (w/v), dextrose 0.05% (w/v), soluble starch 0.05% (w/v), dipotassium phosphate 0.03% (w/v), magnesium sulfate 0.005% (w/v), sodium pyruvate 0.03% (w/v) and agar 1.5% (w/v).

trees were reconstructed with three different algorithms, neighbor-joining (NJ) (Saitou and Nei, 1987), maximum-likelihood (ML) (Felsenstein, 1981) and maximum-parsimony (MP) (Fitch, 1971) algorithm, in MEGA X (Tamura *et al*., 2021). Evolutionary distance matrices for the neighbor-joining and maximum-likelihood analyses were evaluated using Kimura 2 parameter model. Bootstrap analyses with 1,000 times were carried out for stability evaluation of tree topology (Felsenstein, 1985). Cell morphology was observed by a phase-contrast microscope (AX10; Carl Zeiss) and a transmission electron microscope (TEM; LEO 912AB; LEO Electron) after being grown on Reasoner's 2A agar medium at 28°C for 5 days. Gram staining was tested using a Gram staining kit (Sigma Aldrich, USA) according to the manufacturer's instructions. Catalase activity was tested by adding 3% (v/v) hydrogen peroxide solution (bioMérieux, France) and observing for bubbling, while oxidase activity was assessed by applying 1% (w/v) tetramethyl-p-phenylenediamine and noting any color change. Additional biochemical properties was determined using API 20NE kits (bioMérieux, France) according to the manufacturer's recommendations. In addition, media growth studies were performed. The media used were yeast mannitol agar (YMA), Reasoner's 2A (R2A) agar, nutrient agar (NA), potato dextrose agar (PDA), trypticase soy agar (TSA), yeast-peptone-dextrose (YPD) agar, Luria-Bertani (LB) agar, and marine agar solid media. The YMA medium consisted of 1 g/L yeast extract (BD Difco, USA), 2 g/L casamino acids (BD Difco, USA), 20 g/L Bacto Agar (BD Difco, USA), 1 g/L beef extract (Sigma Aldrich, USA), and 10 g/L maltose (Sigma Aldrich, USA). Other media ingredients were sourced from BD Difco in the USA and were prepared according to their respective instructions. All strains were inoculated on each solid media, and monitored at 3, 5, 7, and 10 days at 28°C.

Results and Discussion

The results of 16S rRNA and *recA* gene sequences analysis show that five strains were confirmed as unrecorded *Bradyrhizobium* species in Korea (Table 1). The phylogenetic trees between the isolated strains and closely related *Bradyrhizobium* type strains were presented in Figs. 1 and 2. The isolation of *Bradyrhizobium* strains obtained from *Lespedeza bicolor* and *Kummerowia striata* is taxonomically important report. On the other hand, the isolates isolated from *Glycine max* could be useful for agriculture.

Cellular morphology and the presence of flagella, which were examined by transmission electron microscope, were presented in Fig. 3. Flagella of *Bradyrhizobium elkanii* Glm-3 were only observed on this bacterium in dense clusters presented in Fig. 3. All of strains confirmed flagel-

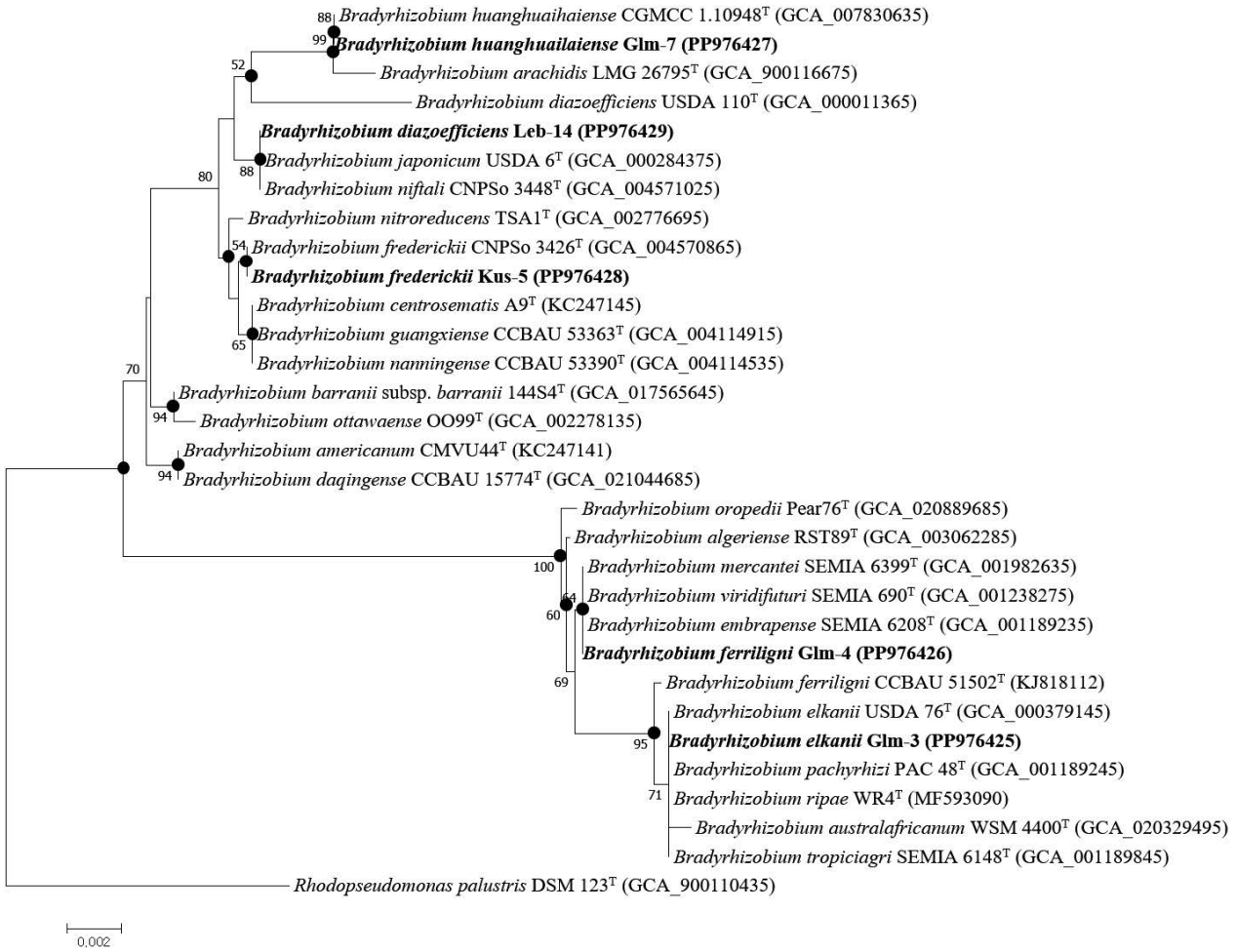


Fig. 1. Neighbor-joining phylogenetic tree showing the phylogenetic relationships of strains reported in this study and related species of *Bradyrhizobium*, based on 16S rRNA gene sequences. Numbers on nodes correspond to bootstrap values for branches (1,000 replicates); only values over 50% are shown. Filled circles indicate the corresponding nodes that were also recovered in trees constructed using the maximum-likelihood and maximum-parsimony algorithms. Scale bar, 0.002 substitutions per nucleotide.

lum depending on species. The phenotypic characteristics among five strains are listed in Table 2. The detailed description of each of *Bradyrhizobium* strains were described below.

Description of *Bradyrhizobium elkanii* Glm-3 (=KACC 10989)

Cells are Gram-negative, flagellated or non-flagellated, non-spore-forming rods (0.8 μm × 1.5 μm). Colonies are circular and white colored with less than 1 mm in diameter within 5 days at 28°C on R2A medium. Catalase-negative and oxidase-positive. Positive for nitrate reduction and urease activity; but negative for indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase activity. According to API 20 NE test results, all assimilations were negative; 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. It grow on YMA, R2A, NA, PDA media within 3 days at 28°C; but weakly grow on YPD, TSA, LB, Marine agar media within 5 days or more at 28°C in the aerobic condition. Strain Glm-3 (=KACC 10989) was isolated from *Glycine max* sampled from Suwon-si, Gyeonggi-do. The GenBank accession number for the 16S rRNA gene and *recA* gene sequence of strain Glm-3 is PP986425 and PP990516, respectively.

Description of *Bradyrhizobium ferriligni* Glm-4 (=KACC 10990)

Cells are Gram-negative, flagellate, non-spore-forming rods (0.7 μm × 1.7 μm). Colonies are circular and white colored with less than 1 mm in diameter within 5 days at 28°C on R2A medium. Catalase-negative and oxidase-

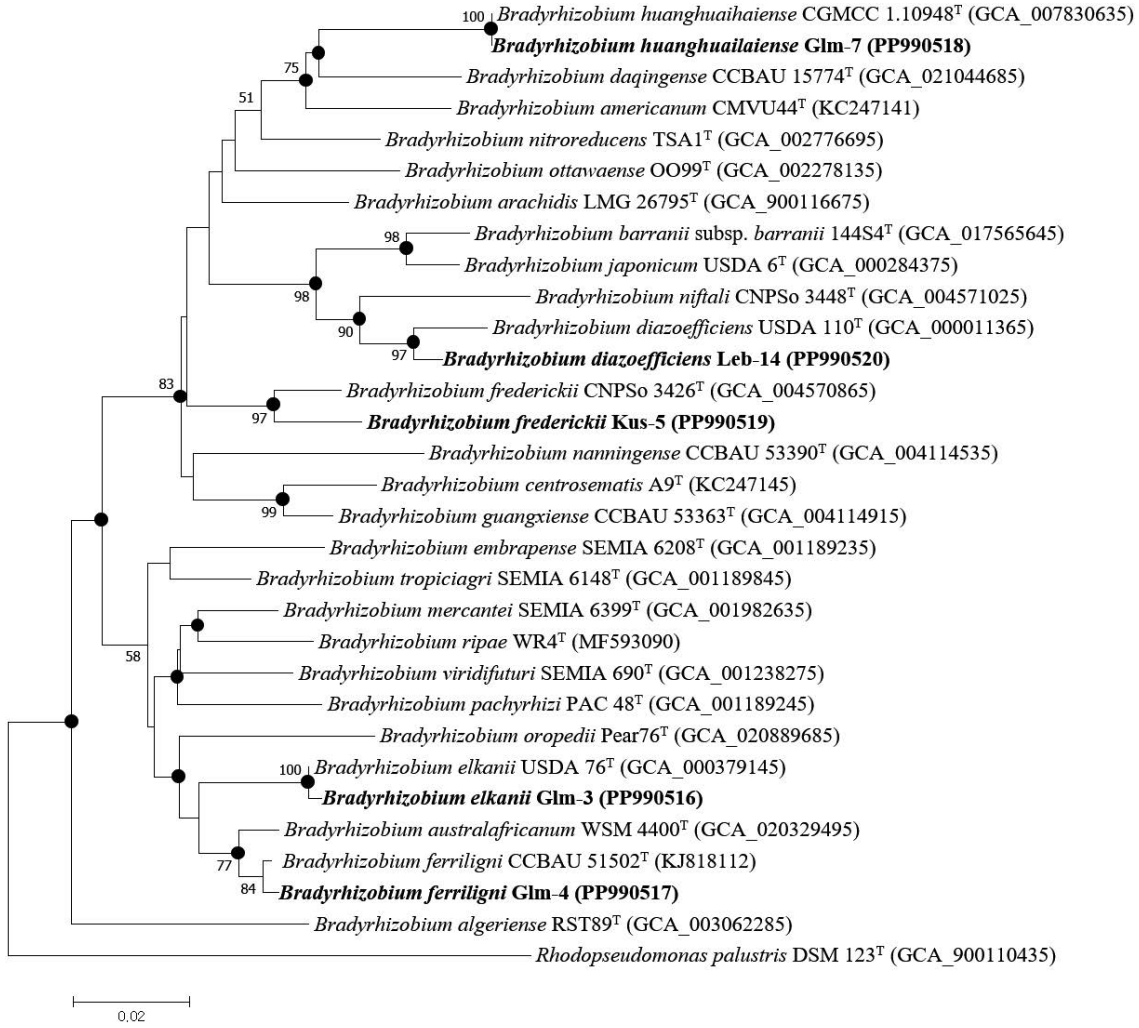


Fig. 2. Neighbor-joining phylogenetic tree showing the phylogenetic relationships of strains reported in this study and related species of *Bradyrhizobium*, based on *recA* gene sequences. Numbers on nodes correspond to bootstrap values for branches (1,000 replicates); only values over 50% are shown. Filled circles indicate the corresponding nodes that were also recovered in trees constructed using the maximum-likelihood and maximum-parsimony algorithms. Scale bar, 0.02 substitutions per nucleotide.

positive. Positive for urease activity; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase activity. According to API 20 NE test results, it assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine and D-maltose; but does not assimilate potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. It grow on YMA, R2A, NA and PDA media within 3 days at 28°C; but weakly grow on YPD, TSA, LB and Marine agar media within 5 days or more at 28°C in the aerobic condition. Strain Glm-4 (=KACC 10990) was isolated from *Glycine max* sampled from Suwon-si, Gyeonggi-do. The GenBank accession number for the 16S rRNA gene and *recA* gene sequence of strain Glm-4 is PP986426 and PP990517, respectively.

Description of *Bradyrhizobium huanghuaihaiense* Glm-7 (=KACC 10993)

Cells are Gram-negative, flagellated, non-spore-forming rods (0.8 μm × 1.8 μm). Colonies are circular and white colored with 1 mm in diameter within 5 days at 28°C on R2A medium. Catalase-positive and oxidase-positive. Positive for urease activity; but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase activity. According to API 20 NE test results, all assimilations were negative; D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. It grow on YMA, R2A, NA and PDA media within 3 days at 28°C; but weakly grow on YPD, LB and Marine agar media within

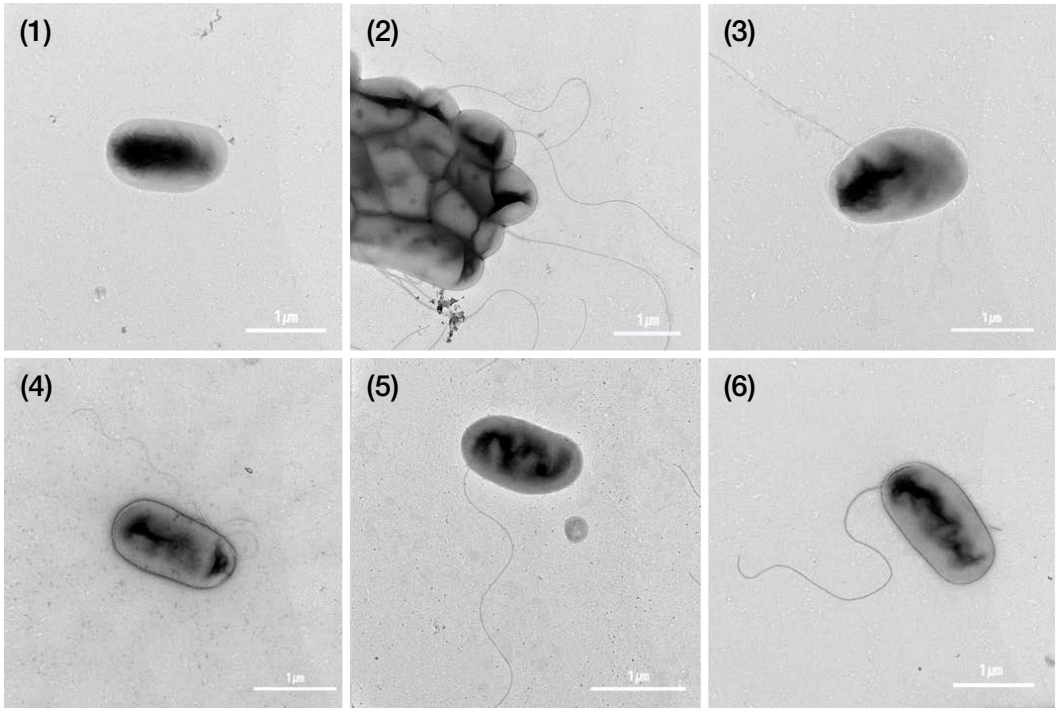


Fig. 3. Transmission electron micrographs of the *Bradyrhizobium* strains. Strain: 1 and 2, *Bradyrhizobium elkanii* Glm-3 (=KACC 10989); 3, *Bradyrhizobium australafricanum* Glm-4 (=KACC 10990); 4, *Bradyrhizobium huanghuaihaiense* Glm-7 (=KACC 10993); 5, *Bradyrhizobium frederickii* Kus-5 (=KACC 11016); 6, *Bradyrhizobium diazoefficiens* Leb-14 (=KACC 11026).

Table 2. Differential phenotypic characteristics among the isolated strains classified into the genus.

Characteristics	1	2	3	4	5
Cell shape	Rod	Rod	Rod	Rod	Rod
Cell size (μm)	0.8−1.0×1.5	0.7−1.1×1.2−1.7	0.8−1×1.5−1.8	0.9×1.5	0.9×1.5
Flagellum	Present	Present	Present	Present	Present
Catalase/oxidase	−/+	−/+	+/+	+/+	−/+
Activity:					
arginine dihydrolase	−	−	−	−	+
nitrate reduction	+	−	−	+	−
urease activity	+	+	+	+	+
Assimilation:					
D-glucose	−	+	−	−	−
L-arabinose	−	+	−	−	−
D-mannose	−	+	−	−	−
D-mannitol	−	+	−	−	−
N-acetylglucosamine	−	+	−	−	−
D-maltose	−	+	−	−	−

Strain: 1, *Bradyrhizobium elkanii* Glm-3 (=KACC 10989); 2, *Bradyrhizobium ferriligni* Glm-4 (=KACC 10990); 3, *Bradyrhizobium huanghuaihaiense* Glm-7 (=KACC 10993); 4, *Bradyrhizobium frederickii* Kus-5 (=KACC 11016); 5, *Bradyrhizobium diazoefficiens* Leb-14 (=KACC 11026).
All strains are positive for oxidase and urease activity, but negative for Gram-staining, aesculin hydrolysis, gelatin hydrolysis glucose fermentation, indole production and β-galactosidase activity; +, positive; −, negative.

5 days or more at 28°C in the aerobic condition. The TSA media did not grow. Strain Glm-7 (=KACC 10993) was isolated from *Glycine max* sampled from Suwon-si, Gyeonggi-do. The GenBank accession number for the 16S rRNA gene and *recA* gene sequence of strain Glm-3 is PP986427 and PP990518, respectively.

Description of *Bradyrhizobium frederickii* Kus-5 (=KACC 11016)

Cells are Gram-negative, flagellated, non-spore-forming rods (0.9 μm × 1.5 μm). Colonies are circular and white colored with 1 mm in diameter within 5 days at 28°C on R2A medium. Catalase-positive and oxidase-positive. Positive for nitrate reduction and urease activity; but negative for indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase activity. According to API 20 NE test results, all assimilations were negative; D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. It grow on YMA, R2A, NA and PDA media within 3 days at 28°C; but weakly grow on LB and Marine agar media within 5 days or more at 28°C in the aerobic condition. The YPD and TSA media did not grow. Strain Kus-5 (=KACC 11016) was isolated from *Kummerowia striata* sampled from Suwon-si, Gyeonggi-do. The GenBank accession number for the 16S rRNA gene and *recA* gene sequence of strain Kus-5 is PP986428 and PP990519, respectively.

Description of *Bradyrhizobium diazoefficiens* Leb-14 (=KACC 11026)

Cells are Gram-negative, flagellated, non-spore-forming rods (0.9 μm × 1.5 μm). Colonies are circular and white colored with 1 mm in diameter within 5 days at 28°C on R2A medium. Catalase-negative and oxidase-positive. Positive for arginine dihydrolase and urease activity; but negative for nitrate reduction, indole production, glucose fermentation, aesculin hydrolysis, gelatin hydrolysis and β-galactosidase activity. According to API 20 NE test results, all assimilations were negative; D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. It grow on YMA, R2A, NA and PDA media within 3 days at 28°C; but weakly grow on LB and Marine agar media within 5 days or more at 28°C in the aerobic condition. The YPD and TSA media did not grow. Strain Leb-14 (=KACC 11026) was isolated from *Lespedeza bicolor* sampled from Suwon-si, Gyeonggi-do. The GenBank accession number for the 16S rRNA gene and *recA* gene sequence of strain Glm-3 is PP986429 and PP990520, respectively.

Conflicts of Interest

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

Acknowledgements

This study was carried out with the support (PJ017286) of National Institute of Agricultural Sciences, Rural Development Administration, Republic of Korea.

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Submitted: July 9, 2024
Revised: October 22, 2024
Accepted: October 24, 2024

Journal of Species Research 13(4):385-390, 2024

Open Access

Characterization of two unrecorded yeast species, *Starmerella apicola* and *Symmetrospora symmetrica*, isolated from *Apis mellifera* and *Citrus sunki* in Republic of Korea

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The purpose of this study is to isolate and identify wild yeasts from *Apis mellifera* and *Citrus sunki* samples collected in Okcheon-gun and Jeju-si, Republic of Korea. Among ten strains, one strain had been previously reported, but nine strains were unreported in the Republic of Korea. To identify the wild yeast strains, pairwise sequence comparisons of the D1/D2 region of the 26S rRNA gene sequence were conducted using the Basic Local Alignment Search Tool (BLAST) (Fell *et al*., 2000). Cell morphologies were observed using a phase contrast microscope, and assimilation tests were performed using the API 20C AUX kit. The two unrecorded yeast strains, MBEE-1 and MMD-1, belong to the genus *Starmerella* (family *Saccharomycetales* incertae sedis, Order *Saccharomycetales*, Class *Saccharomycetes*) and the genus *Symmetrospora* (family *Symmetrosporaceae*, Order *Cystobasidiomycetes* incertae sedis, Class *Cystobasidiomycetes*), respectively. Both strains had oval-shaped and polar budding cells. This research elucidated the morphological and biochemical properties of these two previously unreported yeast species in Korea.

Keywords: 26s rRNA, *Starmerella*, *Symmetrospora*, unrecorded yeasts

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DOI:10.12651/JSR.2024.13.4.385

Introduction

In this study, wild yeasts were isolated from *Apis mellifera* and *Citrus sunki* samples collected in Okcheon-gun and Jeju-si, Republic of Korea. As a result of isolation and identification, many yeast strains were found to be previously reported species, while a few were identified as unrecorded species. The unreported species were classified within the genera *Starmerella* and *Symmetrospora*. The genus *Starmerella* is a member of the class *Saccharomycetes* within the phylum *Ascomycota*. This genus comprises 42 distinct species, with *Starmerella bombicola* designated as the type species (https://www.mycobank.org). The genus *Symmetrospora* belongs to the class *Cystobasidiomycetes* incertae sedis within the phylum *Basidiomycota*. This genus includes 16 distinct species, with *Symmetrospora gracilis* as the type species (https://www.mycobank.org).

The genus *Starmerella* was described to accommodate the sexual state of *Candida bombicola* (Rosa and Lachance, 1998). The cells range from spherical to ellipsoidal, and neither hyphae nor pseudohyphae are produced. During sexual reproduction, it is found that the conjugated asci usually form a single, roughened, asymmetrical ascospore (Teixeira *et al*., 2003; Santos *et al*., 2018; Shibayama *et al*., 2024). Coenzyme Q-9 is present, and the diazonium blue B reaction is negative. The type species is *Starmerella bombicola* (Kurtzman *et al*., 2011).

The genus *Symmetrospora*, which belongs to the family *Symmetrosporaceae*, is primarily defined by phylogenetic analysis of seven genes and an expanded LSU rRNA gene dataset. This analysis positions *Symmetrospora* as a sister clade to *Erythrobasidiales* within *Cystobasidiomycetes*. Characteristically, pseudohyphae and true hyphae are not observed in this genus, and ballistoconidia may be present or absent. When present, the ballistoconidia are typically symmetrical or nearly symmetrical, and ellipsoidal or ovoid in shape. The major coenzyme Q system is CoQ-10 (Wang *et al*., 2015).

As a result of this study, two previously unrecorded yeast strains were discovered in domestic ecosystems, and their phenotypic characteristics were thoroughly investigated.

Materials and Methods

Samples were collected from Okcheon-gun and Jeju-si, Republic of Korea. Each sample was thoroughly crushed, serially diluted in distilled water, and the resulting suspension was spread on Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA). The plates were then incubated at 25°C and 10°C for 3 days. The strains are preserved in a metabolically inactive state at the Korean Agricultural Culture Collection, Republic of Korea.

The cell morphologies of the strains were observed using a LEICA (DM500) microscope, with yeast strains incubated on YPD agar for 3 days. Phase contrast microscope images and colonies of strains MBEE-1 and MMD-1 are shown in Fig. 1. To characterize the biochemical features, the API 20C AUX (bioMérieux) test was performed according to the manufacturer's instructions.

The genomic DNA was extracted after incubation on YPD agar for 3 days. The D1/D2 region of the 26S rRNA gene sequence was amplified by PCR using NL1 (5′-GCATATCAATAAGCGGAGGAAA AG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) primers (Kurtzman and Robnett, 1998). Pairwise sequence comparisons were performed using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) to align with sequences of related species retrieved from GenBank. The MYCOBANK (https://www.mycobank.org/) database

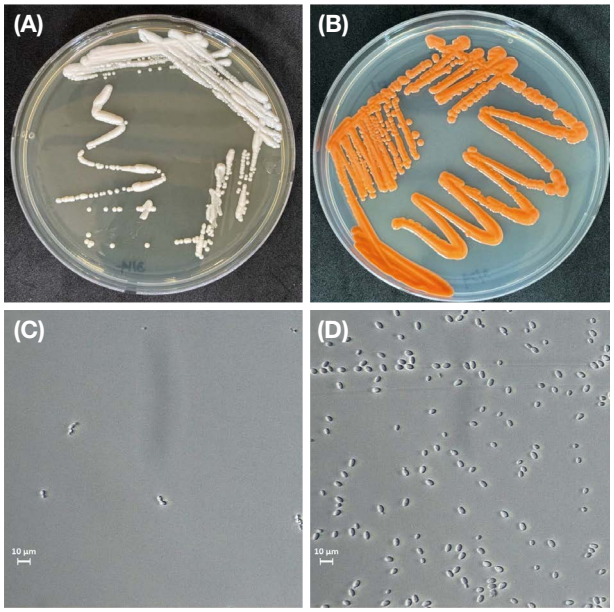


Fig. 1. Morphology of cells from the unrecorded strains MBEE-1 and MMD-1 incubated at 25°C. All strains were grown after 3 days on YPD agar. The colonies of *Starmerella apicola* MBEE-1 (A) and *Symmetrospora symmetrica* MMD-1 (B). The budding cells of *Starmerella apicola* MBEE-1 (C) and *Symmetrospora symmetrica* MMD-1 (D). Bars, 20 μm and 10 μm, respectively.

Table 1. Yeasts isolated strains from soil in Republic of Korea. All strains were cultured under 25°C and 10°C for 3 days.

Phylum	Class	Order	Family	Sample	Strain ID	Most closely related species	26S rRNA similarity	Record in Korea
Ascomycota	*Saccharomycetes*	*Saccharomycetales*	*Saccharomycetales* incertae sedis	*Apis mellifera*	MBEE-1	*Starmerella apicola*	479/480 (99%)	Unreported
					MBEE-4	*Starmerella apicola*	479/480 (99%)	Unreported
					MBEE-5	*Starmerella apicola*	479/480 (99%)	Unreported
					PBEE1-3	*Starmerella apicola*	479/480 (99%)	Unreported
					PBEE3-1	*Starmerella apicola*	479/480 (99%)	Unreported
					PBEE1-5	*Zygosaccharomyces rouxii*	581/581 (100%)	Reported
Basidiomycota	*Cystobasidiomycetes*	*Cystobasidiomycetes* incertae sedis	*Symmetrosporaceae*	*Citrus sunki*	MMD-1	*Symmetrospora symmetrica*	618/620 (99%)	Unreported
					PMD-2	*Symmetrospora symmetrica*	618/620 (99%)	Unreported
					PMD-3	*Symmetrospora symmetrica*	618/620 (99%)	Unreported
					PMD-4	*Symmetrospora symmetrica*	618/620 (99%)	Unreported

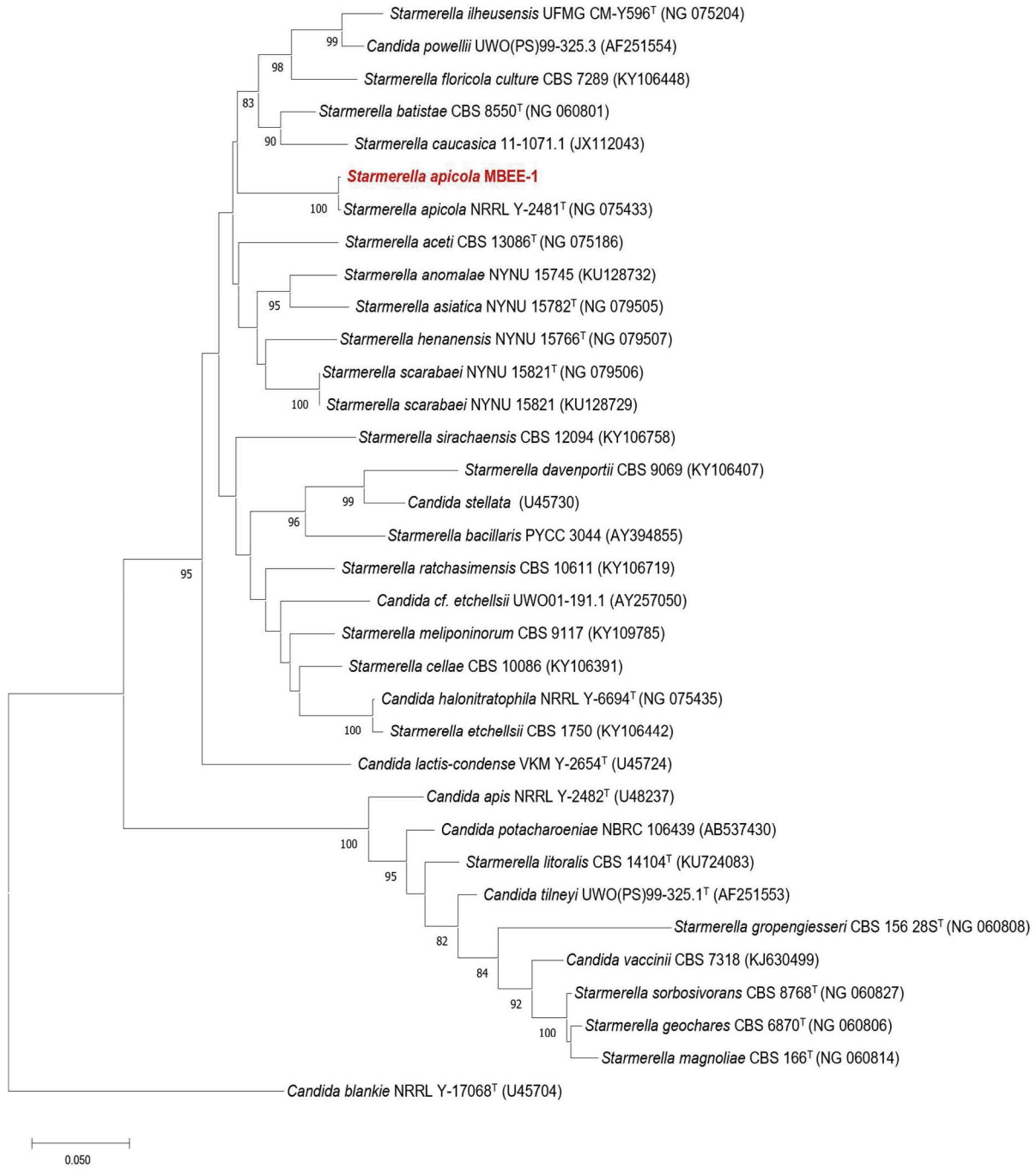


Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains MBEE-1 with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.05 substitutions per nucleotide position.

was used to identify strain types for each species, and closely related strains' gene sequences were obtained from the NCBI (https://www.ncbi.nlm.nih.gov/) for the 26S rDNA in a row.

The phylogenetic trees based on the D1/D2 domain of the LSU rRNA gene sequence were reconstructed using the neighbor-joining algorithm (Saitou and Nei, 1987) of the MEGA 11 program (Tamura *et al*., 2021). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983) for neighborhood bond analysis, and the confidence levels of the clades were estimated through bootstrap analysis with 1,000 replicates (Felsenstein, 1985).

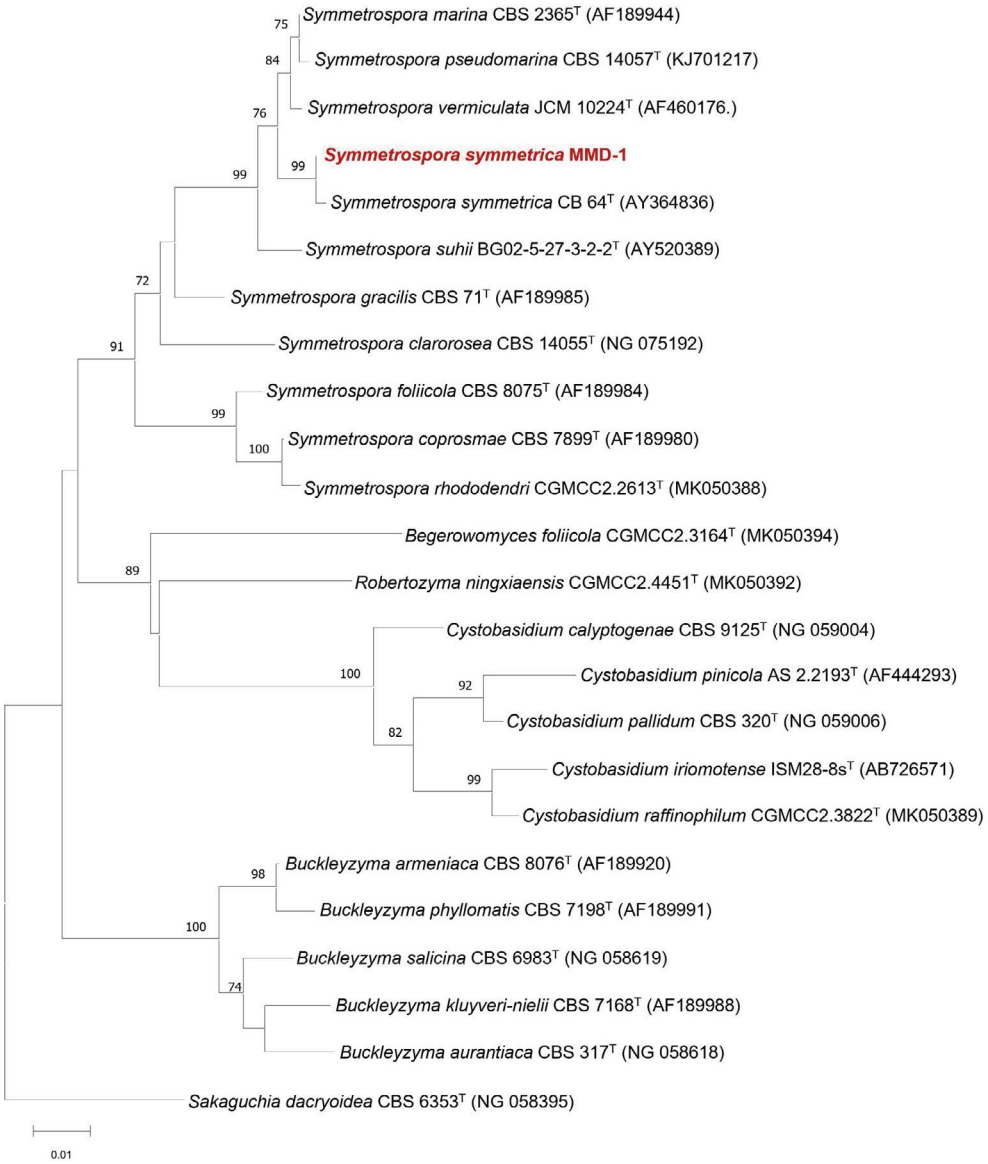


Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain MMD-1 with closely related species. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.01 substitutions per nucleotide position.

Results and Discussion

Among the ten wild yeast strains analyzed, six were isolated from *Apis mellifera* samples collected in Okcheon-gun, while the remaining four were derived from *Citrus sunki* samples obtained in Jeju-si. The yeast strains were identified by analyzing D1/D2 domain of the 26S rRNA gene sequence similarities, which were calculated using NCBI BLAST. Based on the D1/D2 domain of the 26S rRNA gene sequence, four yeast strains were identified as previously unrecorded species in Korea. The taxonomic composition and identification results are listed in Table 1.

Of the ten strains, the six strains isolated from *Apis mellifera* all belonged to the *Saccharomycetales incertae* sedis family of the phylum *Ascomycota*, while the four strains isolated from *Citrus sunki* all belonged to the *Symmetrosporaceae* family of the phylum *Basidiomycota*. The unrecorded yeast strain MBEE-1 belongs to the phylum *Ascomycota* and genus *Starmerella*, while MMD-1 belongs to the phylum *Basidiomycota* and genus *Symmetrospora*. The phylogenetic tree shows that the isolated strains are closely related to the *Starmerella* species and *Symmetrospora* species with the highest 26S rRNA gene

Table 2. Phenotypic characteristics of strain MBEE-1 and related species.

	1	2	3	4
Growth on				
Temp (°C)	10–25	19–37	25	4–40
Assimilation of:				
Glucose	+	+	+	+
Glycerol	+	+	−	+
L-Arabinose	W	−	−	−
D-Xylose	+	−	−	−
Xylitol	−	−	−	−
D-Galactose	−	−	−	−
Inositol	−	−	−	−
D-Sorbitol	+	W	+	W
Methyl-α-D-Glucopyranoside	−	−	ND	−
N-Acetyl-Glucosamine	−	−	−	−
D-Cellobiose	−	−	−	−
D-Lactose	−	−	−	−
D-Maltose	−	−	−	−
D-Saccharose	+	−	ND	−
D-Trehalose	−	−	−	W
D-Melezitose	−	−	−	−
D-Raffinose	+	+	+	+

Taxa: 1, *Starmerella apicola* MBEE-1; 2, *Starmerella apicola* CBS 2868^T; 3, *Starmerella vitae* CBS 15147^T; 4, Starmerella bombi CBS 5836^T.
+, positive; w, weakly positive; d, delay; −, negative; ND, no data.

Table 3. Phenotypic characteristics of strain MMD-1 and related species.

	1	2	3	4
Growth on				
Temp (°C)	10–25	19–30	19–30	12–40
Assimilation of:				
Glucose	+	+	+	+
Glycerol	+	+	+	−
Calcium 2-Keto-Gluconate	−	ND	−	ND
L-Arabinose	−	−	d	−
D-Xylose	+	−	d	−
Xylitol	W	ND	+	−
D-Galactose	+	−	−	+
Inositol	−	−	−	−
D-Sorbitol	−	−	−	−
Methyl-α-D-Glucopyranoside	−	−	d	−
N-Acetyl-Glucosamine	+	ND	ND	−
D-Cellobiose	−	+	+	−
D-Lactose	−	−	+	−
D-Maltose	−	−	+	−
D-Saccharose	−	ND	−	−
D-Trehalose	W	+	−	W
D-Melezitose	W	+	+	W
D-Raffinose	−	−	d	+

Taxa: 1, *Symmetrospora symmetrica* MMD-1; 2, *Symmetrospora symmetrica* CB 64^T; 3, *Symmetrospora marina* CBS 2365^T; 4, *Symmetrospora vermiculata* JCM 10224^T.
+, positive; w, weakly positive; d, delay; -, negative; ND, no data.

sequence similarity (Figs. 2 and 3), thereby supporting close relationships.

Description of *Starmerella apicola* MBEE-1

Cells are oval-shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, and white-cream colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain MBEE-1 is positive for glucose, glycerol, D-xylose, D-sorbitol, D-saccharose, and D-raffinose; weakly positive for calcium 2-keto-gluconate, L-arabinose, and adonitol; and negative for xylitol, D-galactose, inositol, methyl-α-D-glucopyranoside, *N*-acetyl-glucosamine, D-cellobiose, D-lactose, D-maltose, D-trehalose, and D-melezitose (Table 2).

Strain MBEE-1 (KCTC 37300) was isolated from *Apis mellifera* collected in Okcheon-gun, North Chungcheong Province, Republic of Korea.

Description of *Symmetrospora symmetrica* MMD-1

Cells are circular-shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, and orange cream-colored after 3 days of incubation on YPD agar at 25°C. In the API 20C AUX test, strain MMD-1 is positive for glucose, glycerol, D-xylose, adonitol, D-galactose, and *N*-acetyl-glucosamine; weakly positive for xylitol, D-trehalose, and D-melezitose; but negative for calcium 2-keto-gluconate, L-arabinose, inositol, D-sorbitol, methyl-α-D-glucopyranoside, D-cellobiose, D-lactose, D-maltose, D-saccharose, and D-raffinose (Table 3).

Strain MMD-1 (KCTC 37301) was isolated from *Citrus sunki* collected in Jeju-si, Republic of Korea.

Conflicts of Interest

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

Acknowledgements

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBRE202406 and NIBR202402104) and was also supported by Seoul Women's University (2024).

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Submitted: July 15, 2024
Revised: September 10, 2024
Accepted: September 10, 2024

Journal of Species Research 13(4):391-397, 2024

Open Access

A report of 7 unrecorded haloarchaea species from seawater samples near Dokdo island, Korea

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In July 2023, the seawater samples were collected near Dokdo island to obtain unrecorded haloarchaea in Korea. These samples were suspended in a 20% NaCl (w/v) solution and enriched for one month at 37°C in fresh DB Characterization media No. 2 broth with simply modification. Further, the enriched culture was spread on agar medium to obtain single colonies of halophilic archaea. Phylogenetic analysis based on the 16S rRNA gene showed that the isolates from this study exhibited minimum 98.7% sequence similarity with previously reported species. Finally, 7 haloarchaeal species, which had not been reported in Korea but were validly published under the International Code of Nomenclature of Prokaryotes (ICNP), were obtained. These isolates were classified into the orders *Halobacteriales*. The 7 *Halobacteriales* species were further categorized into the family *Haloferacaceae*, comprising 2 genus, *Haloferax* and *Haloplanus*. Collectively, these unrecorded haloarchaeal species spanned 1 order, 1 family, and 2 genera. This research highlights the potential for discovering previously unknown species in domestic seawater environment. Comprehensive analyses, including Gram staining, cell morphology, physiological and basic biochemical parameters, and phylogenetic analysis, were conducted and are detailed for each species.

Keywords: Dokdo island, Haloarchaea, seawater, unrecorded species

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DOI:10.12651/JSR.2024.13.4.391

Introduction

Extremophiles are microorganisms capable of growing, adapting, and thriving in extreme environments, such as those with high salinity, extreme temperatures, or acidic and alkaline conditions (Rampelotto, 2013). Among these, haloarchaea are the members of the class *Halobacteria*, which belongs to the *Methanobacteriota* phylum of the archaea (Goker and Orea, 2023). They can thrive in hypersaline habitats, including natural brine, the Dead Sea, alkaline salt lakes, marine solar salterns, and rock salt deposits (Kato *et al*., 1995; Stan-Lotter and Fendrihan, 2015). Beyond high salinity, haloarchaea are exposed to additional extreme conditions such as elevated temperatures, UV radiation, severe ionic stresses, and alkaline pH. To survive and grow under such harsh conditions, these archaea produce compounds such as carotenoids, halorhodopsin, and polyhydroxyalkanoates (PHA). Carotenoids are abundant in haloarchaea, which have a role of antioxidants, protect cell to external stress (Oren, 2014; Squillaci *et al*., 2017). Bacterioruberin, a representative C_{50} carotenoid produced by haloarchaea, is capable to membrane stabilizers, contributing to their adaptability, and survival (Grivard *et al*., 2022). PHA is a biopolymer that accumulates as carbon and energy storage inclusions, serving as a survival strategy to overcome physical, geochemical, and environmental stresses in archaea (Kumar and Kim, 2018; Kumar *et al*., 2020). Halorhodopsin is the known anion pump detected in haloarchaea that is energized by light (Oesterhelt, 1998). Haloarchaea thrive in saturated brines with isoosmolar salt concentrations in their cytoplasm. During cell growth, halorhodopsin plays a crucial role by

using light energy to help maintain osmotic balance (Kolbe *et al*., 2000). The unique metabolites and proteins of these halophilic archaea make them valuable for various industrial and research applications (Singh and Singh, 2017). Therefore, discovering haloarchaea from halophilic environment is considered highly valuable.

Research on the microbial biodiversity of Dokdo is limited, particularly regarding the isolation of halophilic archaea from seawater around Dokdo. To contribute to the understanding of domestic biodiversity, this study focuses on unrecorded haloarchaeal species that have not yet been reported in Korea. In this study, we identified 7 species of haloarchaea from the orders *Halobacteriales*, belonging to 2 genera (*Haloferax* and *Haloplanus*) and one family *Haloferacaceae*, based on 16S rRNA gene sequence. The phenotypic characteristics of these species were confirmed their status as previously unrecorded haloarchaea in Korea.

Materials and Methods

Samples were collected from seawater near Dokdo island in July 2023. The obtained seawater samples (5 mL) were mixed in 10 mL of 20% NaCl (v/v) and vortexed enough. The sample solutions (15 mL) were inoculated directly to 100 mL of modified DB characterization medium No. 2 (DBCM2) broth in 500 mL shaking flask as following components (l^{-1}): 833 mL MDS salt water (7 g of KCl, 35 g of $MgSO_4 \cdot 7H_2O$, 30 g of $MgCl_2 \cdot 6H_2O$, 240 g of NaCl, and 5 mL of 1 M $CaCl_2$ solution), 1 mL of $FeCl_2$ solution, 0.05 g of peptone (Oxoid, Hampshire, UK), 0.25 g of yeast extract (BD, Franklin Lakes, NJ, USA), 5 mL of 1 M NH_4Cl, 2 mL of potassium phosphate buffer, 1 mL of trace element solution (2.0 mg of $FeSO_4 \cdot 7H_2O$, 141.5 mg of $ZnSO_4 \cdot 7H_2O$, 57.6 mg of $MnSO_4 \cdot H_2O$, 6.0 mg of H_3BO_3, 190.0 mg of $CoCl_2 \cdot 6H_2O$, 2.0 mg of $CuCl_2 \cdot 2H_2O$, 24.0 mg of $NiCl_2 \cdot 6H_2O$, and 36.0 mg of $Na_2MoO_4 \cdot 2H_2O$ per liter), 3 mL of vitamin solution (3.0 mg of biotin, 4.0 mg of folic acid, 50.0 mg of pyridoxine · HCl, 33.0 mg of thiamine · HCl, 10.0 mg of riboflavin, 33.0 mg of nicotinic acid, 17.0 mg of DL-calcium pantothenate, 17.0 mg of vitamin B_{12}, 13.0 mg of *p*-aminobenzoic acid, and 10 mg of lipoic acid per liter), 10 mL of 1 M sodium pyruvate solution, supplemented 0.25 g of fish peptone (HiMedia, Maharashtra, India), 1 g of D-glucose (Samchun chemical, Pyeongtaek, Korea), and 1 g of sucrose (SAMCHUN CHEMICALS, Pyeongtaek, Korea), and finally adjusted to pH 7.0–7.3 using 1 M Tris-base buffer solution. The DBCM2 agar plate was added with 2% (w/v) agarose (KisanBio, Seoul, Korea). Each enrichment culture was incubated at 37°C for one month. Subsequently, the enriched culture broths were serially diluted with DBCM2 broth. Different colonies were selected and repeatedly subcultured into fresh culture media at intervals of 3–7 days. After more than three subculturing steps, pure colonies were obtained and stored at −80°C in a 20% (w/v) glycerol with 15% (w/v) NaCl stock solution for preservation.

Phenotypic experiments were conducted on cultures grown at 37°C on DBCM2 agar plates. The cell morphology of isolated species was examined using light microscopy (model CX 23; Olympus, Tokyo, Japan) and transmission electron microscopy (LIBRA 120; Carl Zeiss, Oberkochen, Germany). Gram staining of the isolated species was performed using a Gram stain kit (BioWORLD, Dublin, OH) according to the manufacturer's instructions. Each species was cultured in DBCM2 broth with NaCl concentrations ranging from 5% to 30% (w/v), in increments of 5%, for 3–7 days at 37°C and 200 rpm to evaluate cell growth at varying salinities. The pH range for cell growth was assessed by growing the cells for 3–7 days at 30°C and 200 rpm, using buffer solutions with pH increments of 1.0 unit from pH 4.0 to 12.0: 100 mM of CH_3COOH/CH_3COONa buffer (pH 4.0–6.0), 100 mM of NaH_2PO_4/Na_2HPO_4 buffer (pH 7.0–8.0), 100 mM $NaHCO_3/Na_2CO_3$ buffer (pH 9.0–10.0), and 100 mM of Na_2CO_3/NaOH buffer (pH 11.0–12.0). To determine the temperature range for growth, each species was cultivated on DBCM2 agar and incubated at 4, 10, 15, 20, 25, 30, 35, 37, 40, 45, and 55°C for 3–7 days. Catalase and oxidase tests were performed using 3% (v/v) H_2O_2 and 1% tetramethyl-p-phenylenediamine, respectively. Energy sources used in assimilation tests included D-glucose, D-mannose, D-galactose, D-maltose, D-sucrose, D-lactose, D-fructose, D-ribose, D-arabinose, L-rhamnose, D-xylose, trehalose, raffinose, glycerol, L-sorbose, pyruvate, D-mannitol, succinate, DL-lactate, L-malate, fumarate, citrate, acetate, L-glycine, L-histidine, L-alanine, L-glutamate, L-glutamic acid, L-arginine, L-lysine, and L-ornithine, each at 0.1% (w/v) based on DBCM2 broth without any carbon and nitrogen sources.

Genomic DNA was extracted using the LaboPass™ Tissue Genomic DNA Isolation kit (Cosmogenetech, Seoul, Korea). The partial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using archaeal-specific primers (Cui *et al*., 2009). The obtained PCR products were purified using the LaboPass™ PCR purification kit (Cosmogenetech, Seoul, Korea) and sent to Macrogen Co., Ltd. (Seoul, Korea) for sequence analysis. Sequencing was performed using archaeal universal primer including 18F (5′-ATTCCGGTTGATCCTGCC-3′), 344F (5′-CGGG YGCASCAGGCGCGAA-3′), 520R (5′-GWATTACCGC GGCKGCTG-3′), 787F (5′-ATTAGATACCCSBGTAG TCC-3′), and 1518R (5′-AGGAGGTGATCCAGCCGC-3′) (Abdallah *et al*., 2018). The sequences were assembled using the SeqMan™ II expert sequence analysis software (Thombre *et al*., 2016). Assembled sequences were identified based on the EzBioCloud 16S-based ID (https://

www.ezbiocloud.net/identify). Closely related taxa were obtained from the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon) (Yoon *et al*., 2017). Multiple sequence alignments were conducted using the Clustal W multiple sequence alignment program in BioEdit 7.2.6.1 software (Thompson *et al*., 1994; Hall, 1999). Phylogenetic trees were constructed using the MEGA X software (Kumar *et al*., 2018), based on the 16S rRNA gene sequence. Sequence relatedness was calculated using the Maximum likelihood (ML) algorithm (Felsenstein, 1981). Phylogenetic tree robustness was assessed using the bootstrap method with 1,000 replications. The Kimura two-parameter model was applied to calculate evolutionary distances (Kimura, 1980).

Results and Discussion

The 16S rRNA gene sequence analyses revealed that a total of 7 species belonged to previously unreported species in Korea. The strain information, identification, taxonomic assignment from species to classes, isolation source, and sequence accession numbers, including the National Institute of Biological Resources (NIBR) in Korea and GenBank, are listed in Table 1. The 7 species were distributed across the order *Halobacteriales* and belonged to the family *Haloferacaceae*. The family *Haloferacaceae* included the genera *Haloferax* (4 species) and *Haloplanus* (3 species). The isolated unrecorded haloarchaeal species exhibited various morphologies, including pleomorphic coccus and coccus shaped (Fig. 1). All these species were Gram-staining negative and lysed in distilled water. Some of the species required Mg^{2+} for growth, and most of them were found to grow optimally in to be mesophilic (20–40°C) and neutrophilic (pH 7.0) conditions. Most species were found to grow more than 5% NaCl (w/v). All species tested positive for catalase and negative for oxidase. Additionally, growth was observed in all species when using D-glucose, pyruvate, and fumarate as sole energy source. The identification of the 7 unrecorded species based on the 16S rRNA gene sequence similarities, was supported by the phylogenetic tree using the ML algorithm (Fig. 2). Detailed morphological, physiological, and basic biochemical characteristics of the 7 unrecorded haloarchaeal species are described in the following strain descriptions.

Description of *Haloferax sulfurifontis* MBLA0249

Cells are Gram-stain-negative and pleomorphic coccus shaped with 1.5–1.7 μm in diameter. Colonies are red, circular, and flat after 3 days of incubation on DBCM2 at 37°C. Catalase is positive, but oxidase is negative. Growth occurs in the 20–50°C (optimum, 37°C) at 5–25% NaCl (optimum, 15%) at pH 5.0–9.0 (optimum, pH 7.0) and

Table 1. Summary of all strains isolated in this study and their taxonomic affiliations.

Order	Family	Order	Strain ID	Accession/ NIBRARC number	Closest species	Similarity (%)	Media	Isolation source
Halobacteriales	*Haloferacaceae*	*Haloferax*	MBLA0249	OR646564 NIBRARC000510481	*Haloferax sulfurifontis*	99.93	DBCM2 at 37°C, 3 days	Seawater
			MBLA0250	OR646565 NIBRARC000510482	*Haloferax elongans*	99.78		
			MBLA0251	OR646566 NIBRARC000510483	*Haloferax larsenii*	99.85		
			MBLA0252	OR646567 NIBRARC000510484	*Haloferax namakaokahaiae*	99.68		
		Haloplanus	MBLA0253	OR646568 NIBRARC000510485	*Haloplanus litoreus*	99.78	DBCM2 at 37°C, 7 days	
			MBLA0254	OR646569 NIBRARC000510486	*Haloplanus ruber*	99.56		
			MBLA0255	OR646570 NIBRARC000510487	*Haloplanus aerogenes*	99.85		

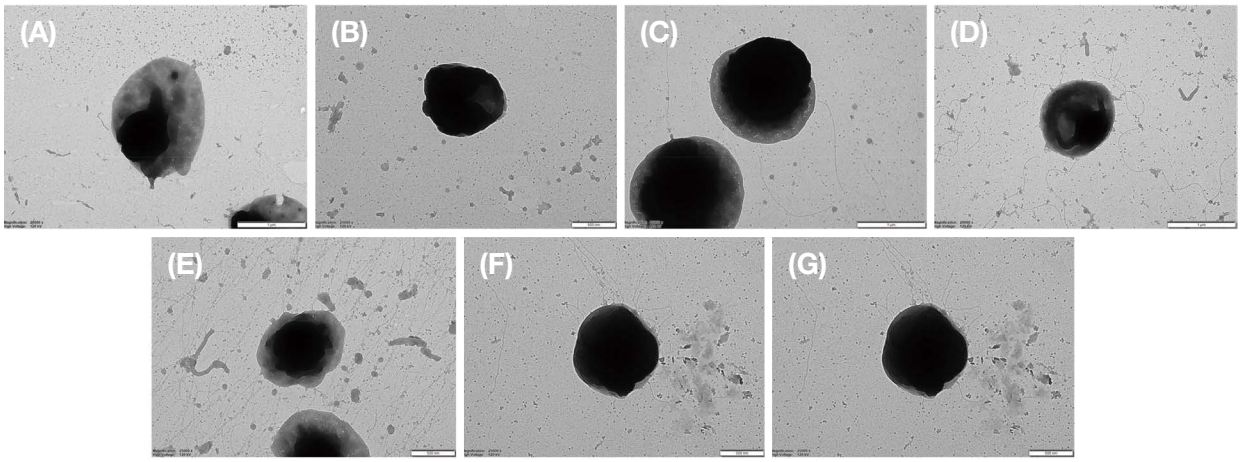


Fig. 1. Transmission electron micrographs of the 7 unrecorded species isolated in this study. Strains: A, MBLA0259; B, MBLA0250; C, MBLA0251; D, MBLA0252; E, MBLA0253; F, MBLA0254; G, MBLA0255.

lysed in distilled water. Mg^{2+} is required for growth. D-Glucose, D-galactose, D-fructose, D-sucrose, D-mannose, D-xylose, L-rhamnose, starch, DL-lactate, fumarate, pyruvate, citrate, acetate, glycine, L-asparagine, and L-glutamine are utilized as sole energy sources for growth, but D-maltose, D-lactose, D-trehalose, D-raffinose, D-mannitol, D-sorbitol, glycerol, DL-malate, succinate, L-alanine, L-arginine, L-histidine, L-ornithine, and L-glutamic acid are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OR646564. Strain MBLA0249 (=NIBRARC000510481) was isolated from seawater near Dokdo island, Korea (37°14′29.4″N 131°52′00.9″E).

Description of *Haloferax elongans* MBLA0250

Cells are Gram-stain-negative and pleomorphic-coccus shaped with 1.7−1.9 μm in diameter. Colonies are red, circular, and flat after 3 days of incubation on DBCM2 at 37°C. Catalase is positive, but oxidase is negative. Growth occurs in the 25−50°C (optimum, 37°C) at 10−30% NaCl (optimum, 15%) at pH 6.0−8.0 (optimum, pH 7.0) and lysed in distilled water. Mg^{2+} is required for growth. D-Glucose, D-fructose, D-sucrose, L-rhamnose, D-sorbitol, fumarate, pyruvate, glycine, L-histidine D-xylose, glycerol, DL-lactate, succinate, pyruvate, DL-malate, fumarate, citrate, acetate, and L-alanine are utilized as sole energy sources for growth, but D-galactose, D-maltose, lactose, D-mannose, D-xylose, D-trehalose, D-raffinose, D-mannitol, glycerol, starch, citrate DL-lactate, acetate, succinate, L-alanine, L-arginine, L-ornithine, L-asparagine, L-glutamine, and L-glutamic acid are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OR646565. Strain MBLA0250 (=NIBRARC000510482) was isolated from seawater near Dokdo island, Korea (37°14′29.4″N 131°52′00.9″E).

Description of *Haloferax larsenii* MBLA0251

Cells are Gram-stain-negative and coccus shaped with 1.5−1.7 μm in diameter. Colonies are red, circular, and flat after 3 days of incubation on DBCM2 at 37°C. Catalase is positive, but oxidase is negative. Growth occurs in the 30−55°C (optimum, 37°C) at 5−25% NaCl (optimum, 15%) at pH 6.0−8.0 (optimum, pH 7.0) and lysed in distilled water. Mg^{2+} is required for growth. D-Glucose, D-fructose, D-sucrose, L-rhamnose, starch, DL-lactate, fumarate, pyruvate, citrate, acetate, glycine, L-alanine, L-histidine, L-ornithine, L-asparagine, L-glutamine, and L-glutamic acid are utilized as sole energy sources for growth, but D-galactose, D-maltose, lactose, D-mannose, D-xylose, D-trehalose, D-raffinose, D-mannitol, D-sorbitol, glycerol, DL-malate, and succinate are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OR64 6566. Strain MBLA0251 (=NIBRARC000510483) was isolated from seawater near Dokdo island, Korea (37°14′ 29.4″N 131°52′00.9″E).

Description of *Haloferax namakaokahaiae* MBLA0252

Cells are Gram-stain-negative and coccus-shaped with 1.1−1.2 μm in diameter. Colonies are red, circular, and flat after 3 days of incubation on DBCM2 at 37°C. Catalase is positive, but oxidase is negative. Growth occurs in the 20−40°C (optimum, 37°C) at 5−25% NaCl (optimum, 15%) at pH 6.0−8.0 (optimum, pH 7.0) and lysed in distilled water. Mg^{2+} is required for growth. D-Glucose, D-galactose, D-fructose, D-sucrose, lactose, D-xylose, L-rhamnose, D-raffinose, D-sorbitol, starch, fumarate, pyruvate, DL-malate, acetate, glycine, L-ornithine, L-asparagine, L-glutamine, and L-glutamic acid are utilized as sole energy sources for growth, but D-maltose, D-mannose,

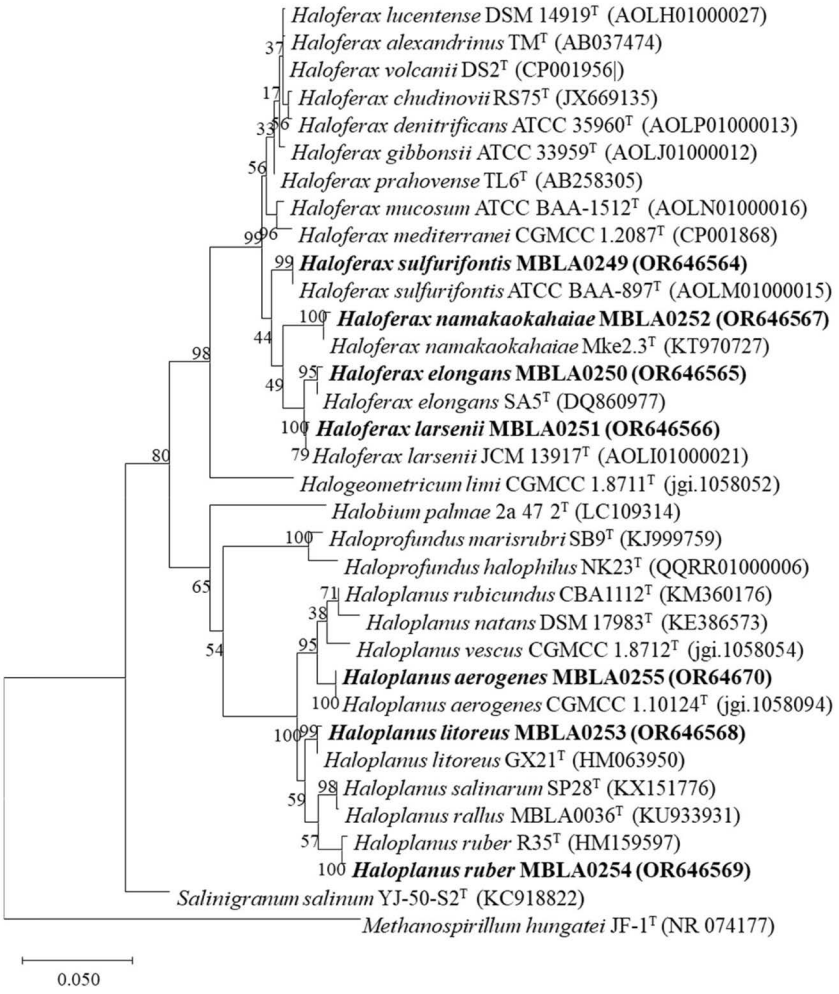


Fig. 2. Maximum likelihood (ML) phylogenetic tree based on the 16S rRNA gene sequences between the species isolated in this study. A phylogenetic tree was constructed with their relatives of the genera *Haloferax*, *Haloplanus*, *Halogeometricum*, *Halobium*, *Haloprofundus*, and *Salinigranum*. The numbers on the nodes indicate the bootstrap values (>70%). Bar, 0.05 accumulated changes per nucleotide, respectively.

D-trehalose, D-mannitol, glycerol, DL-lactate, citrate, succinate, L-alanine, and L-histidine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OR646567. Strain MBLA0252 (=NIBRARC000510484) was isolated from seawater near Dokdo island, Korea (37°14′29.4″N 131°52′00.9″E).

Description of *Haloplanus litoreus* MBLA0253

Cells are Gram-stain-negative and pleomorphic coccus shaped with 1.0−1.1 μm in diameter. Colonies are red, irregular, and flat after 7 days of incubation on DBCM2 at 37°C. Catalase is positive, but oxidase is negative. Growth occurs in the 20−50°C (optimum, 37°C) at 15−25% NaCl (optimum, 15%) at pH 6.0−9.0 (optimum, pH 7.0) and lysed in distilled water. Mg^{2+} is required for growth. D-Glucose, starch, DL-lactate, fumarate, pyruvate, citrate, DL-malate, acetate, L-asparagine, L-glutamine, and L-glutamic acid are utilized as sole energy sources for growth, but D-galactose, D-fructose, D-sucrose, D-maltose, lactose, D-mannose, D-xylose, L-rhamnose, D-trehalose, D-raffinose, D-mannitol, D-sorbitol, glycerol, succinate, glycine, L-alanine, L-arginine, L-histidine, and L-ornithine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OR646568. Strain MBLA0253 (=NIBRARC000510485) was isolated from seawater near Dokdo island, Korea (37°14′29.4″N 131°52′00.9″E).

Description of *Haloplanus ruber* MBLA0254

Cells are Gram-stain-negative and coccus shaped with 1.0−1.1 μm in diameter. Colonies are red, irregular, and flat after 7 days of incubation on DBCM2 at 37°C. Catalase is positive, but oxidase is negative. Growth occurs in the

20−45°C (optimum, 37°C) at 15−25% NaCl (optimum, 15%) at pH 6.0−9.0 (optimum, pH 7.0), lysed in distilled water, and growth occur without Mg^{2+}. D-Glucose, D-galactose, D-fructose, D-mannose, D-xylose, starch, fumarate, pyruvate, citrate, DL-malate, acetate, glycine, L-alanine, L-asparagine, and L-glutamic acid are utilized as sole energy sources for growth, but D-sucrose, D-maltose, lactose, L-rhamnose, D-trehalose, D-raffinose, D-mannitol, D-sorbitol, glycerol, starch, succinate, L-alanine, L-arginine, L-histidine, and L-glutamine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OR646569. Strain MBLA0254 (=NIBRARC 000510486) was isolated from seawater near Dokdo island, Korea (37°14′29.4″N 131°52′00.9″E).

Description of *Haloplanus aerogenes* MBLA0255

Cells are Gram-stain-negative and coccus shpaed with 0.8−0.9 μm in diameter. Colonies are red, irregular, and flat after 7 days of incubation on DBCM2 at 37°C. Catalase is positive, but oxidase is negative. Growth occurs in the 25−50°C (optimum, 37°C) at 10−25% NaCl (optimum, 15%) at pH 6.0−9.0 (optimum, pH 7.0) and lysed in distilled water, and growth occur without Mg^{2+}. D-Glucose, D-galactose, D-sucrose, lactose, D-mannose, D-trehalose, D-raffinose, D-mannitol, glycerol, starch fumarate, pyruvate, citrate, DL-malate, acetate, succinate, L-alanine, L-arginine, L-histidine, L-ornithine, L-asparagine, and L-glutamic acid are utilized as sole energy sources for growth, but D-fructose, D-maltose, D-xylose, L-rhamnose, D-sorbitol, DL-lactate, and glycine are not utilized. The NCBI accession number for the 16S rRNA gene sequence is OR646570. Strain MBLA0255 (=NIBRARC000510 487) was isolated from seawater near Dokdo island, Korea (37°14′29.4″N 131°52′00.9″E).

Conflicts of Interest

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

Acknowledgements

This work was supported by a grant from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202304104).

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Submitted: July 19, 2024
Revised: October 25, 2024
Accepted: October 25, 2024

Journal of Species Research 13(4):398-403, 2024

Open Access

Isolation and characterization of two unreported yeast species from wildflowers and mountain soil in Korea in the families *Filobasidiaceae* and *Piskurozymaceae*

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The purpose of this study was to isolate and identify wild yeasts from wildflower samples in Okcheon-gun and soil samples in Nowon-gu, Republic of Korea. Out of the fourteen strains identified, twelve had been previously reported, whereas two strains were unreported in the Republic of Korea. Identification of wild yeast strains was performed through pairwise sequence comparisons of the D1/D2 region of the 26S rRNA gene using the Basic Local Alignment Search Tool (BLAST) (Fell *et al*., 2000). Cell morphology was examined using a phase contrast microscope, and assimilation tests were conducted with the API 20C AUX kit. All strains were classified under the phylum *Basidiomycota*. The two unreported yeast strains, MFL-1 and NYB3-16, are classified under the genus *Naganishia* (family *Filobasidiaceae*, order *Filobasidiales*, class *Tremellomycetes*) and the genus *Solicoccozyma* (family *Piskurozymaceae*, order *Filobasidiales*, class *Tremellomycetes*), respectively. These two strains exhibited morphologies consistent with their type species. MFL-1 cells were circular, while NYB3-16 cells were oval. Both strains demonstrated polar budding. This study highlighted the morphological and biochemical characteristics of the two unreported yeast species that had not been previously documented in Korea.

Keywords: 26S rRNA, *Naganishia*, *Solicoccozyma*, unreported yeasts

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DOI:10.12651/JSR.2024.13.4.398

Introduction

Yeasts, belonging to the *Ascomycota*, *Basidiomycota*, and *Deuteromycota* phyla, have been used for centuries in the fermentation of traditional foods and beverages (Lee *et al*., 1997). Among these, basidiomycetous yeasts hold particular economic, agricultural, and medical significance. However, despite their potential, only 1 to 5% of these species have been identified, leaving much of their diversity unexplored (Boekhout *et al*., 2022). These yeasts are valuable in industrial applications, such as the production of astaxanthin in aquaculture and enzymes like xylanases and cellulases (Fell *et al*., 2001). This highlights the need for systematic classification and research to fully understand their ecological roles and harness their potential.

Within the *Basidiomycota*, families like *Filobasidiaceae* and *Piskurozymaceae* are particularly noteworthy due to their adaptations to extreme environments. The *Naganishia* genus, part of *Filobasidiaceae*, includes yeasts that thrive in cold, UV-intense, and nutrient-poor habitats, supported by genetic traits for trehalose synthesis and lipid metabolism. They also produce bioactive compounds like carotenoids and mycosporines, which offer UV protection (Nizovoy *et al*., 2021). Similarly, *Solicoccozyma* species from the *Piskurozymaceae* family are often found in soils and are known for their lipid accumulation, crucial for survival in resource-limited environments and promising for industrial applications like biodiesel production (Passoth, 2017). Their metabolic versatility further enhances their ecological and biotechnological value (Fonseca *et al*., 2011). Understanding these yeast families deepens our knowledge of microbial diversity and provides pathways for sustainable biotechnologies.

Until now, yeasts have mainly been isolated from fermented foods and raw materials, with some isolates applied in the food fermentation industry (Kim *et al*., 1999). However, research on the classification of yeasts from natural habitats and their industrial applications remains limited.

We have previously isolated yeasts from various soil

environments, including cold winter soils (Park *et al*., 2011), urban soils (Park *et al*., 2021; Maeng *et al*., 2022), and mountainous soils (Seok *et al*., 2024), and identified them using molecular biological tools. In this study, wild yeasts were isolated from wildflower samples collected in Okcheon-gun and soil samples from Nowon-gu, Republic of Korea. Two unreported yeast strains were discovered, and their phenotypic characteristics were investigated.

Materials and Methods

Wildflower samples were collected from Okcheon-gun, and soil samples were collected from Nowon-gu, Republic of Korea. The samples were serially diluted in distilled water, and the dilutions were spread on Yeast Extract Peptone Dextrose (YPD) agar (Difco, USA) plates and incubated at 25°C for 3 days. The strains have been preserved in a metabolically inactive state at the Korean Agricultural Culture Collection, Korea.

Cell morphologies of the strains were examined using a LEICA DM500 microscope after 3 days of incubation on YPD agar. Phase contrast microscope images and colony morphologies of strains MFL-1 and NYB3-16 are presented in Fig. 1. For biochemical characterization, the API 20C AUX system (bioMérieux) was used according to the manufacturer's instructions.

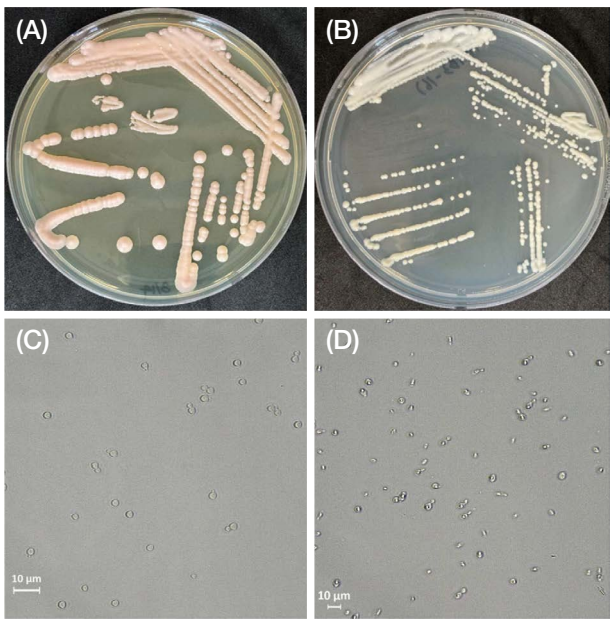


Fig. 1. Morphology of cells from the unreported strains MFL-1 and NYB3-16 incubated at 25°C. All strains were grown on YPD agar for 3 days. The colonies of *Naganishia liquefaciens* MFL-1 (A) and *Solicoccozyma gelidoterrea* NYB3-16 (B). The budding cells of *Naganishia liquefaciens* MFL-1 (C) and *Solicoccozyma gelidoterrea* NYB3-16 (D). Bars, 10 µm.

Genomic DNA was extracted after 3 days of incubation on YPD agar. The D1/D2 region of the 26S rRNA gene was amplified by PCR using the primers NL1 (5′-GCATATCA ATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGT GTTTCAAGACGG-3′) (Kurtzman and Robnett, 1998).

Pairwise sequence comparisons were conducted using the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., 1997) to align with sequences of related species retrieved from GenBank. The MYCOBANK database (https://www.mycobank.org/) was used to identify strain types for each species, and the gene sequences of closely related strains for the 26S rDNA region were obtained from NCBI (https://www.ncbi.nlm.nih.gov/).

Phylogenetic trees based on the D1/D2 domain of the LSU rRNA gene were reconstructed using the neighbor-joining method (Saitou and Nei, 1987) with the MEGA 11 program (Tamura *et al*., 2021). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983) for the neighbor-joining analysis, and the confidence levels of the clades were assessed through bootstrap analysis with 1,000 replicates (Felsenstein, 1985). Outgroup species for the phylogenetic tree were selected based on established yeast taxonomy practices, ensuring the inclusion of distantly related species to appropriately root the tree (Guarro *et al*., 1999; Rainey and Oren, 2011).

Results

Fourteen wild yeast strains were collected from wildflower samples in Okcheon-gun and soil samples in Nowon-gu, Korea. The yeast strains were identified by sequence analysis of the D1/D2 domain of the 26S rRNA gene using NCBI BLAST for similarity calculation. Based on this analysis, two yeast strains were identified as previously unreported species in Korea. All strains were classified into the families *Mrakiaceae* (1 strain), *Filobasidiaceae* (1 strain), *Piskurozymaceae* (6 strains), *Holtermanniella* (1 strain), and *Trimorphomycetaceae* (5 strains) within the phylum *Basidiomycota*.

Among the yeast strains collected from wildflower samples in Okcheon-gun, one strain, *Naganishia liquefaciens* MFL-1, was identified as an unreported species in Korea. From the soil samples in Nowon-gu, the isolated species included *Guehomyces pullulans* YB6-2-1, *Solicoccozyma gelidoterrea* NYB3-16, *Solicoccozyma terrea* YB1UV-4, YB1UV-6, YB1UV-3, *Solicoccozyma terricola* NYB3-14, NYB3-15, *Holtermanniella wattica* YB6-1, and *Saitozyma podzolica* YB2UV-1, YB3UV-1, YB3UV-4, NYB3-12, and NYB2-1. Additionally, *Solicoccozyma gelidoterrea* NYB3-16 was identified as another previously unreported species. The taxonomic composition and identification results are detailed in Table 1.

The unreported yeast strains MFL-1 and NYB3-16 both

Table 1. Yeasts isolated strains from soil in Republic of Korea. All strains were cultured under 25°C and 10°C for 3 days.

Phylum	Class	Order	Family	Strain ID	Most closely related species	26S rRNA similarity	Record in Korea
Basidiomycota	*Tremellomycetes*	*Cystofilobasidiales*	*Mrakiaceae*	YB6-2-1	*Guehomyces pullulans*	525/529 (99.24%)	Reported
		Filobasidiales	*Filobasidiaceae*	MFL-1	*Naganishia liquefaciens*	603/603 (100.00%)	Unreported
			Piskurozymaceae	NYB3-16	*Solicoccozyma gelidoterrea*	527/527 (100.00%)	Unreported
				YB1UV-4	*Solicoccozyma terrea*	620/620 (100.00%)	Reported
				YB1UV-6	*Solicoccozyma terrea*	620/620 (100.00%)	Reported
				YB1UV-3	*Solicoccozyma terrea*	620/620 (100.00%)	Reported
				NYB3-14	*Solicoccozyma terricola*	527/527 (100.00%)	Reported
				NYB3-15	*Solicoccozyma terricola*	527/527 (100.00%)	Reported
		Holtermanniales	*Holtermanniella*	YB6-1	*Holtermanniella wattica*	524/526 (99.62%)	Reported
		Tremellales	*Trimorphomycetaceae*	YB2UV-1	*Saitozyma podzolica*	523/524 (99.81%)	Reported
				YB3UV-1	*Saitozyma podzolica*	523/524 (99.81%)	Reported
				YB3UV-4	*Saitozyma podzolica*	523/524 (99.81%)	Reported
				NYB3-12	*Saitozyma podzolica*	523/524 (99.81%)	Reported
				NYB2-1	*Saitozyma podzolica*	523/524 (99.81%)	Reported

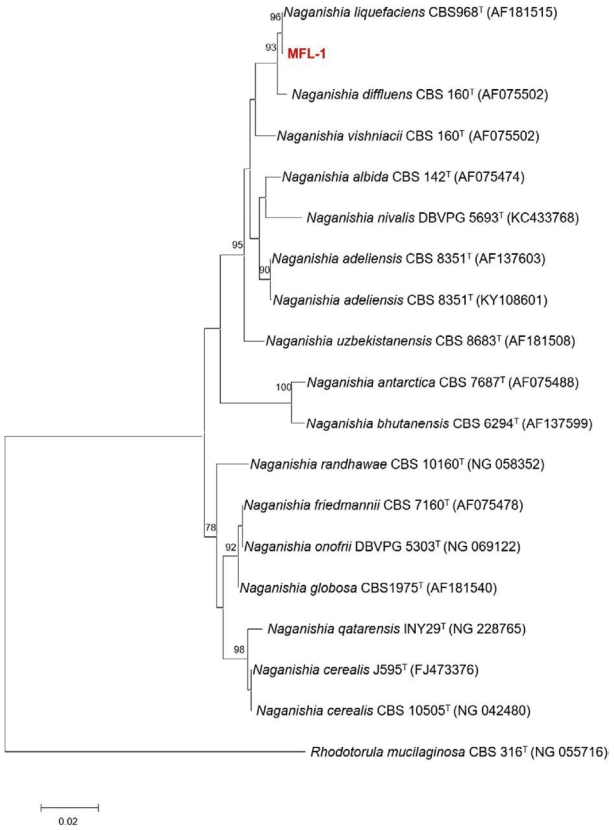


Fig. 2. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strains MFL-1 with closely related species. *Rhodotorula mucilaginosa* CBS 316^T was used as the outgroup. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position.

belong to *Basidiomycota*, with MFL-1 classified in the genus *Naganishia* and NYB3-16 in the genus *Solicoccozyma*. Phylogenetic analysis indicates that MFL-1 is closely related to species of *Naganishia*, while NYB3-16 is closely related to species of *Solicoccozyma*, based on the highest 26S rRNA gene sequence similarity (Figs. 2 and 3), supporting their close phylogenetic relationships.

Discussion

Species of *Solicoccozyma* have been isolated from diverse environments such as soil and crater lakes (Li *et al*., 2019), while *Naganishia* species have been found in environments like blue cheese, air, and dry moss (Luo *et al*., 2019; Farias *et al*., 2022). The genus *Solicoccozyma* includes eleven species, with *Solicoccozyma aeria* as the type species, while the genus *Naganishia* consists of twenty-three species, with *Naganishia globosa* as the type species (https://www.mycobank.org).

Despite belonging to different families, *Solicoccozyma*

and *Naganishia* share several characteristics typical of their respective taxonomic groups. For example, the *Piskurozymaceae* family, to which *Solicoccozyma* belongs, is characterized by the possible presence or absence of pseudohyphae and true hyphae, along with budding cells and occasional clamp connections. Notably, members of this family do not form basidiocarps and often utilize nitrate, with CoQ-10 as the primary CoQ system.

Similarly, the *Filobasidiaceae* family, which includes *Naganishia*, shares features such as the absence of basidiocarps and the presence of budding cells. However, *Filobasidiaceae* members are distinguished by their tubular holobasidia with terminal, sessile basidiospores. They also have a varied CoQ system, typically CoQ-9 or CoQ-10, and frequently utilize a range of organic acids, including L-malic and p-hydroxybenzoic acids (Liu *et al*., 2015).

These shared and distinct characteristics underline the taxonomic diversity within *Basidiomycota* and suggest potential ecological adaptations worthy of further investigation. These discoveries increase our understanding of the taxonomic diversity of *Basidiomycota* and demonstrate the ecological adaptability of *Solicoccozyma* and *Naganishia* in varied environments. Their potential roles in nutrient cycling and microbial ecosystems remain underexplored but hold promise for biotechnological applications, such as in food production, waste management, and bioremediation.

By documenting these unreported species, this study contributes to a better understanding of Korean yeast biodiversity and emphasizes the need for further research into their ecological functions and environmental sustainability contributions.

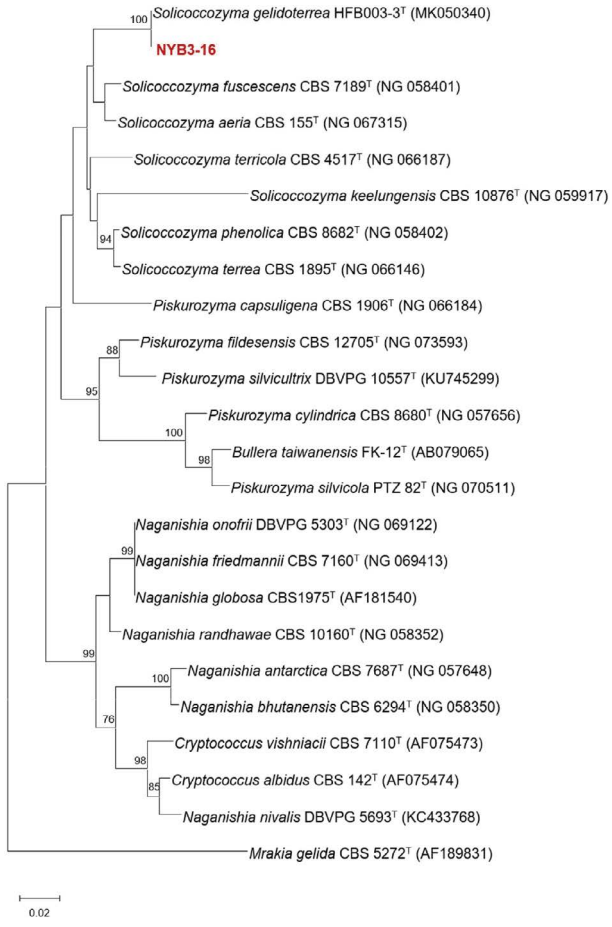


Fig. 3. A Neighbor-joining phylogenetic tree reconstructed from a comparative analysis of 26S rRNA gene sequences showing the relationships of strain NYB3-16 with closely related species. *Mrakia gelida* CBS 5272^{T} was used as the outgroup. Bootstrap values (>70%) based on neighbor-joining methods are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position.

Description of *Naganishia liquefaciens* MFL-1

Cells are circular and exhibit polar budding (Fig. 1). After 3 days of incubation on YPD agar at 25°C, colonies appear convex, glistening, and light pink. In the API 20C AUX test, strain MFL-1 tests positive for glucose, calcium-2-keto-D-gluconate, L-arabinose, D-xylose, D-sorbitol, methyl-α-D-glucopyranoside, D-cellobiose, D-maltose,

Table 2. Phenotypic characteristics that differentiate strain MFL-1 and related species.

	1	2	3	4
Growth on				
Temp (°C)	15–30	19–30	4–30	4–21
Assimilation of				
Glucose	+	+	+	+
Glycerol	−	w	−	−
Calcium 2-Keto-Gluconate	+	+	+	+
L-Arabinose	+	+	+	+
D-Xylose	+	+	+	v
Adonitol	w	−	+	−
Xylitol	−	w	+	−
D-Galactose	w	−	−	−
Inositol	−	−	+	−
D-Sorbitol	+	−	v	−
Methyl-α-D-Glucopyranoside	+	−	v	v
N-Acetyl-Glucosamine	−	−	ND	ND
D-Cellobiose	+	+	+	v
D-Lactose	w	+	−	−
D-Maltose	+	+	+	+
D-Saccharose	+	+	v	v
D-Trehalose	+	+	+	+
D-Melezitose	+	+	+	+
D-Raffinose	+	+	+	v

Taxa: 1, *Naganishia liquefaciens* MFL-1; 2, *Naganishia liquefaciens* CBS 968^{T}; 3, *Naganishia diffluens* CBS 160^{T}; 4, *Naganishia vishniacii* CBS 7110^{T}. +, positive; w, weak positive; −, negative; v, variable; ND, no data.

Table 3. Phenotypic characteristics that differentiate strain NYB3-16 and related species.

	1	2	3	4
Growth on				
Temp (°C)	15−30	19−30	4−30	19−25
Assimilation of				
Glucose	+	+	+	+
Glycerol	−	v	−	−
Calcium 2-Keto-Gluconate	+	ND	+	+
L-Arabinose	+	+	v	+
D-Xylose	+	+	+	+
Adonitol	w	+	+	−
Xylitol	−	ND	v	−
D-Galactose	+	+	+	−
Inositol	w	+	v	+
D-Sorbitol	w	+	v	−
Methyl-α-D-Glucopyranoside	+	+	+	−
N-Acetyl-Glucosamine	w	+	ND	ND
D-Cellobiose	+	+	v	+
D-Lactose	−	+	v	−
D-Maltose	+	+	+	−
D-Saccharose	w	+	v	−
D-Trehalose	−	v	+	−
D-Melezitose	+	+	+	−
D-Raffinose	−	+	+	−

Taxa: 1, *Solicoccozyma gelidoterrea* NYB3-16; 2, *Solicoccozyma gelidoterrea* CGMCC 2.5814^T; 3, *Solicoccozyma aeria* CBS 155^T; 4, *Solicoccozyma fuscescens* CBS 7189^T.
+, positive; w, weak positive; −, negative; v, variable; ND, no data.

D-saccharose (sucrose), D-trehalose, D-melezitose, and D-raffinose; weakly positive for adonitol, D-galactose, and D-lactose (bovine origin); but negative for glycerol, xylitol, inositol, and *N*-acetyl-D-glucosamine.

Strain MFL-1 (KCTC 37299) was isolated from wildflowers collected in Okcheon-gun, North Chungcheong Province, Republic of Korea.

Description of *Solicoccozyma gelidoterrea* NYB3-16

Cells are oval and show polar budding (Fig. 1). After 3 days of incubation on YPD agar at 25°C, colonies are convex, glistening, and light-yellow. In the API 20C AUX test, strain NYB3-16 tests positive for glucose, calcium-2-keto-D-gluconate, L-arabinose, D-xylose, D-galactose, methyl-α-D-glucopyranoside, D-cellobiose, D-maltose, and D-melezitose; weakly positive for adonitol, inositol, D-sorbitol, *N*-acetyl-D-glucosamine, and D-saccharose (sucrose); but negative for glycerol, xylitol, D-lactose (bovine origin), D-trehalose, and D-raffinose.

Strain NYB3-16 (KCTC 37289) was isolated from soil collected in Nowon-gu, Seoul, Republic of Korea.

Conflicts of Interest

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

Acknowledgements

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBRE202403 and NIBR202402104) and was also supported by Seoul Women's University (2024).

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Submitted: August 20, 2024
Revised: September 10, 2024
Accepted: September 11, 2024

Journal of Species Research 13(4):404-416, 2024

Open Access

List of insect collection by Du-Myung Seok preserved at Kyushu University in Japan

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This report lists and illustrates 35 insect species from a collection of 129 specimens gathered by D. M. Seok, the first Korean entomologist. Most of Seok's collections were preserved at Kyushu University in Japan for about 90 years before being relocated to NIBR (National Institute of Biological Resources) in Korea in September 2024.

Keywords: D. M. Seok, insect collection, Korean peninsula, Kyushu University, list, NIBR

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DOI:10.12651/JSR.2024.13.4.404

Introduction

Du-Myung Seok (석주명, 石宙明, 1908−1950) was the first Korean entomologist who made significant contributions to the taxonomy of butterflies of Korea. During his lifetime, he classified Korean butterflies into 248 species (Seok, 1973) and collected 750,000 butterfly specimens.

However, his collections were completely destroyed when the Korean War broke out (Lee, 1985; Sibatani, 1985; 1987; Yoon, 2018). To be precise, upon his retirement from teaching at Songdo High School (Gaeseong, North Korea) in April 1942, he destroyed 600,000 specimens due to lack of storage, and an additional 150,000 were thought to have been lost during the Korean War (Lee, 1985; Yoon, 2018).

To date, only 32 butterfly specimens of his collections are known to remain in Korea. However, during our investigation of dried specimens in the Entomological Laboratory, Kyushu University in Japan from 2010 to 2024, we discovered additional specimens collected by D. M. Seok in the Korean Peninsula in the 1930s and 1940s had been stored there.

In this paper, we identified a total of 129 Korean specimens representing 35 insect species collected by D. M. Seok and provided a list of specimens with illustrations. The specimens comprise five species of Order Orthoptera, seven of Order Hemiptera, five of Order Neuroptera, one of Order Megaloptera, one of Order Mecoptera, four of Order Trichoptera, ten of Order Lepidoptera, and two of Order Hymenoptera.

We compiled the list by comparing the collection site notation on the labels with current local names. All photographs of the labels for each specimen are shown in Figs. 1−106. All specimens were relocated from Kyushu University to the insect collections storage at the National Institute of Biological Resources (NIBR) in Korea in September 2024.

Comparison of collection site notation on labels and current local names (Chang *et al*., 2015)

Chouhei: Jangpyeong, Hamgyeongnamdo, N. Korea
Eiko: Yeongheung, Hamgyeongnamdo, N. Korea
Hannasan Saisyum: Hanrasan, Saeseom, Jejudo, S. Korea
Kanhoku Keigen: Gyeongweon, Hamgyeongbukdo, N. Korea
Kanhoku Shinbusei: Shinmuseong, Yanggangdo, N. Korea
Keikido Kaijo: Gaeseong, N. Korea
Kinsen: Geumcheon, Hwanghaebukdo, N. Korea

Kokaido Kinshu: Geumju, Hwanghaedo, N. Korea
Kongosan: Geumgangsan, Gangweondo, N. Korea
Koryo: Gwangneung, Gyeonggido, S. Korea
Kyujo: Gujang, Pyeonganbukdo, N. Korea
Nansen (Namcheon): Pyeongsan, Hwanghaebukdo, N. Korea
Nansetsurei: Namseolryeong, Yanggangdo, N. Korea
Shariin: Sariweon, Hwanghaebukdo, N. Korea

The List of Insect Collection by Du-myung Seok

Order Orthoptera 메뚜기목

Family Tettigoniidae 여치과

Conocephalus maculatus (Le Guillou, 1841) 점박이쌕쌔기
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0059 (fig. 1)

Gampsocleis sedakovii obscura (Walker, 1869) 여치
1 ex. (COREA) Keikido Kaijo, 22. Jun. 1935, D. M. Seok, NIBR2024-0062 (fig. 2)

Ruspolia interrupta (Walker, 1869) 왕매부리
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0060 (fig. 3)

Family Acrididae 메뚜기과

Acrida cinerea (Thunberg, 1815) 방아깨비
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0061 (fig. 4)

Arcyptera coreana Shiraki, 1930 참어리삽사리
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0055 (fig. 5)
2 exs. (COREA) Keikido Kaijo, 23. Jun. 1935, D. M. Seok, NIBR2024-0056−57 (figs. 6, 7)
1 ex. (COREA) Keikido Kaijo, 23. Jul. 1935, D. M. Seok, NIBR2024-0058 (fig. 8)

Order Hemiptera 노린재목

Family Cicadellidae 매미충과

Bothrogonia ferruginea (Fabricius, 1787) 끝검은말매미충
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0049 (fig. 9)
1 ex. (COREA) Keikido Kaijo, 16. Aug. 1935, D. M. Seok, NIBR2024-0050 (fig. 10)

Family Dictyopharidae 상투벌레과

Raivuna patruelis Stål, 1859 상투벌레
1 ex. (COREA) Keikido Kaijo, 19. Jul. 1935, D. M. Seok, NIBR2024-0051 (fig. 11)

Family Belostomatidae 물장군과

Appasus japonicus (Vuillefroy, 1864) 물자라
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0003 (fig. 12)

Family Nepidae 장구애비과

Laccotrephes japonensis Scott, 1874 장구애비
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0001 (fig. 13)

Ranatra chinensis Mayr, 1865 게아재비
1 ex. (COREA) Shariin, 17. Aug. 1935, D. M. Seok, NIBR 2024-0002 (fig. 14)

Family Pentatomidae 노린재과

Pentatoma japonica (Distant, 1882) 분홍다리노린재
10 exs. (COREA) Keikido Kaijo, 9. Oct. 1937, D. M. Seok, NIBR2024-0004−13 (figs. 15−24)

Pentatomidae sp.
35 exs. (COREA) Keikido Kaijo, 9. Oct. 1937, D. M. Seok, NIBR2024-0014−48 (figs. 25−36)

Order Neuroptera 풀잠자리목

Family Ascalaphidae 뿔잠자리과

Libelloides sibiricus sibiricus (Eversmann, 1850) 노랑뿔잠자리
2 exs. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0073−74 (figs. 37, 38)
1 ex. (COREA) Keikido Kaijo, 12, Apr. 1935, D. M. Seok, NIBR2024-0077 (fig. 39)
1 ex. (COREA) Keikido Kaijo, 20, Apr. 1935, D. M. Seok, NIBR2024-0078 (fig. 40)
1 ex. (COREA) Keikido Kaijo, 21, Apr. 1935, D. M. Seok, NIBR2024-0075 (fig. 41)
1 ex. (COREA) Keikido Kaijo, 28, Apr. 1935, D. M. Seok, NIBR2024-0076 (fig. 42)


[Corea]
Kaijo
(Keikido)
. . 1935
D. M. Seok
1


[Corea]
Kaijo
(Keikido)
24.vi.* 1935
D. M. Seok
2


[Corea]
Kaijo
(Keikido)
. . 1935
D. M. Seok
3


[Corea]
Kaijo
(Keikido)
. . 1935
D. M. Seok
4


[Corea]
Kaijo
(Keikido)
. . 1935
D. M. Seok
5


[Corea]
Kaijo
(Keikido)
23.vi.* 1935
D. M. Seok
6


[Corea]
Kaijo
(Keikido)
23.vi.* 1935
D. M. Seok
7

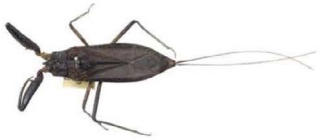

[Corea]
Kaijo
(Keikido)
23.vii.* 1935
D. M. Seok
8


[Corea]
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(Keikido)
. . 1935
D. M. Seok
9


[Corea]
Kaijo
(Keikido)
16.viii 1935
D. M. Seok
10


[Corea]
Kaijo
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. . 1935
D. M. Seok
11


[Corea]
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(Keikido)
. . 1935
D. M. Seok
12


[Corea]
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(Keikido)
. . 1935
D. M. Seok
13


[Corea]
Shariin
17. viii. 1935
14


[Corea]
Kaijo
(Keikido)
9. x. 1937
D. M. Seok
15


[Corea]
Kaijo
(Keikido)
9. x. 1937
D. M. Seok
16

Figs. 1–16. Photographs of Seok's collection and its label. Information on each specimen are given in the text.



Figs. 17–32. Photographs of Seok's collection and its label. Information on each specimen are given in the text.

Family Chrysopidae 풀잠자리과

Chrysopa formosa Brauer, 1851 갑오풀잠자리
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR2024-0064 (fig. 43)

Chrysopa sp.
1 ex. (COREA) Keikido Kaijo, 19. Jun. 1935, D. M. Seok, NIBR2024-0063 (fig. 44)
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0065 (fig. 45)

Family Myrmeleontidae 명주잠자리과

Baliga micans (MacLachlan, 1875) 명주잠자리
1 ex. (COREA) Keikido Kaijo, 22, Jun. 1935, D. M. Seok, NIBR2024-0071 (fig. 46)
2 exs. (COREA) Keikido Kaijo, 23, Jun. 1935, D. M. Seok, NIBR2024-0068–69 (figs. 47, 48)
1 ex. (COREA) Keikido Kaijo, 26, Jun. 1935, D. M. Seok, NIBR2024-0070 (fig. 49)

Distoleon nigricans (Okamoto, 1910) 알락명주잠자리
1 ex. (COREA) Keikido Kaijo, 30, Jul. 1935, D. M. Seok, NIBR2024-0072 (fig. 50)

Order Megaloptera 뱀잠자리목

Family Corydalidae 뱀잠자리과

Neochauliodes formosanus (Okamoto, 1910) 얼룩뱀잠자리
1 ex. (COREA) Keikido Kaijo, 11. Jun. 1935, D. M. Seok, NIBR2024-0067 (fig. 51)
1 ex. (COREA) Keikido Kaijo, 25. Jun. 1935, D. M. Seok, NIBR2024-0066 (fig. 52)

Order Mecoptera 밑들이목

Family Panorpidae 밑들이과

Panorpa coreana Okamoto, 1925 참밑들이
1 ex. (COREA) Keikido Kaijo, 7. Jun. 1935, D. M. Seok, NIBR2024-0053 (fig. 53)
1 ex. (COREA) Keikido Kaijo, 25. Jun. 1935, D. M. Seok, NIBR2024-0054 (fig. 54)

Order Trichoptera 날도래목

Family Goeridae 가시날도래과

Goera sp.
1 ex. (COREA) Keikido Kaijo, 25. Jun. 1935, D. M. Seok, NIBR2024-0080 (fig. 55)

Family Molannidae 날개날도래과

Molanna moesta Banks, 1906 날개날도래
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0079 (fig. 56)

Family Phryganeidae 날도래과

Semblis phalaenoides (Linnaeus, 1758) 굴뚝날도래
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0089 (fig. 57)
1 ex. (COREA) Keikido Kaijo, 28. Apr. 1935, D. M. Seok, NIBR2024-0087 (fig. 58)
1 ex. (COREA) Keikido Kaijo, 20. May. 1935, D. M. Seok, NIBR2024-0086 (fig. 59)
1 ex. (COREA) Keikido Kaijo, 22. May. 1935, D. M. Seok, NIBR2024-0085 (fig. 60)
1 ex. (COREA) Keikido Kaijo, 25. May. 1935, D. M. Seok, NIBR2024-0084 (fig. 61)
1 ex. (COREA) Keikido Kaijo, 7. Jun. 1935, D. M. Seok, NIBR2024-0083 (fig. 62)
1 ex. (COREA) Keikido Kaijo, 10. Jun. 1935, D. M. Seok, NIBR2024-0082 (fig. 63)
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR 2024-0090 (fig. 64)
1 ex. (COREA) Keikido Kaijo, 13. Jun. 1935, D. M. Seok, NIBR2024-0088 (fig. 65)

Family Stenopsychidae 각날도래과

Stenopsyche sp.
1 ex. (COREA) Keikido Kaijo, 29. Jun. 1935, D. M. Seok, NIBR2024-0081 (fig. 66)

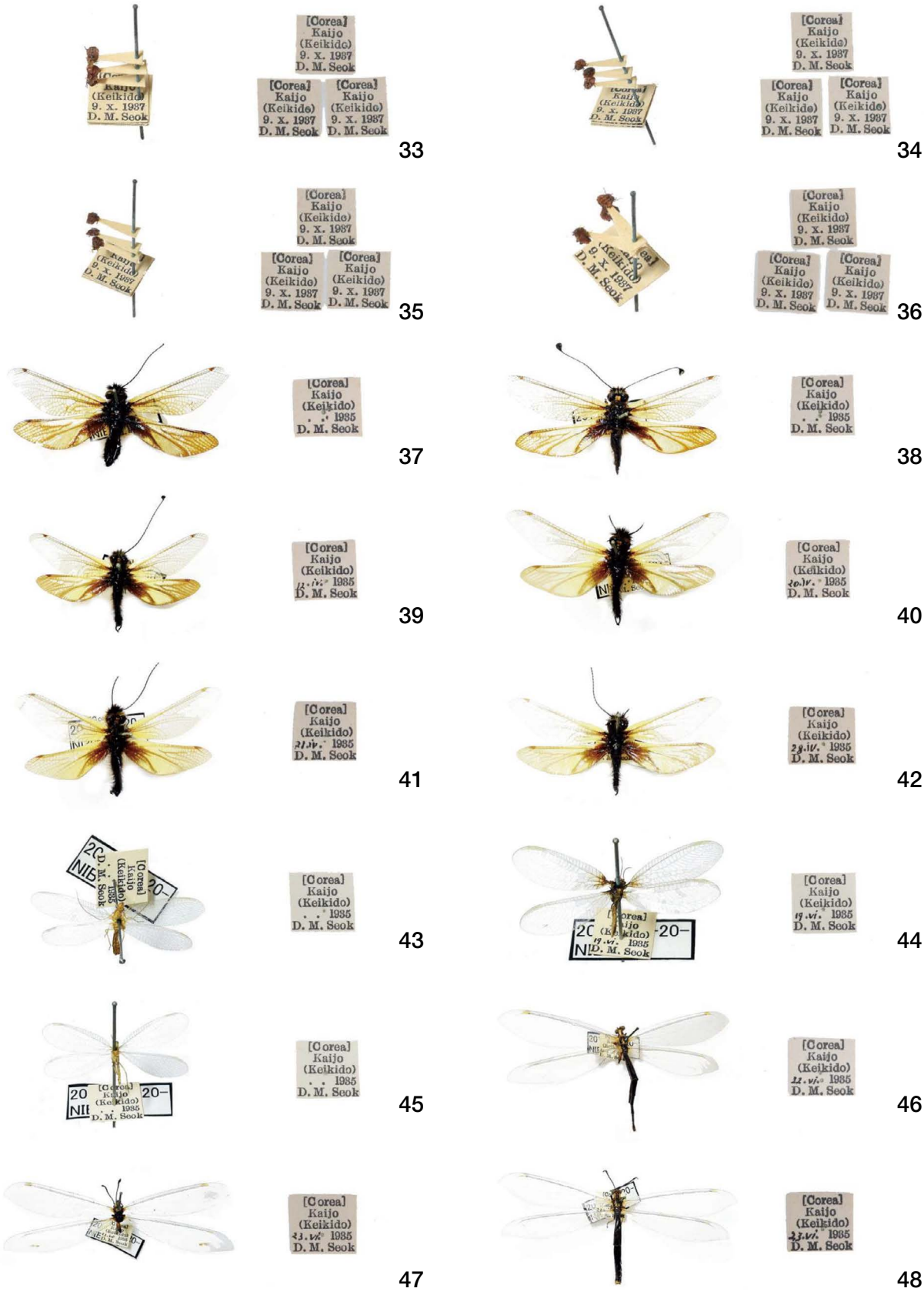
Order Lepidoptera 나비목

Family Lasiocampidae 솔나방과

Malacosoma neustria testacea (Motschulsky [1861]) 천막벌레나방
1 ex. (COREA) Keikaido Kaijo, 1935, D. M. Seok, NIBR 2024-0125 (fig. 67)
1 ex. (COREA) Keikaido Kaijo, Jun. 1935, D. M. Seok, NIBR2024-0127 (fig. 68)

Family Lycaenidae 부전나비과

Celastrina argiolus (Linnaeus, 1758) 푸른부전나비
1 ex. (KOREA) Kinsen, 2. Aug. 1934, D. M. Seok, NIBR



Figs. 33–48. Photographs of Seok's collection and its label. Information on each specimen are given in the text.



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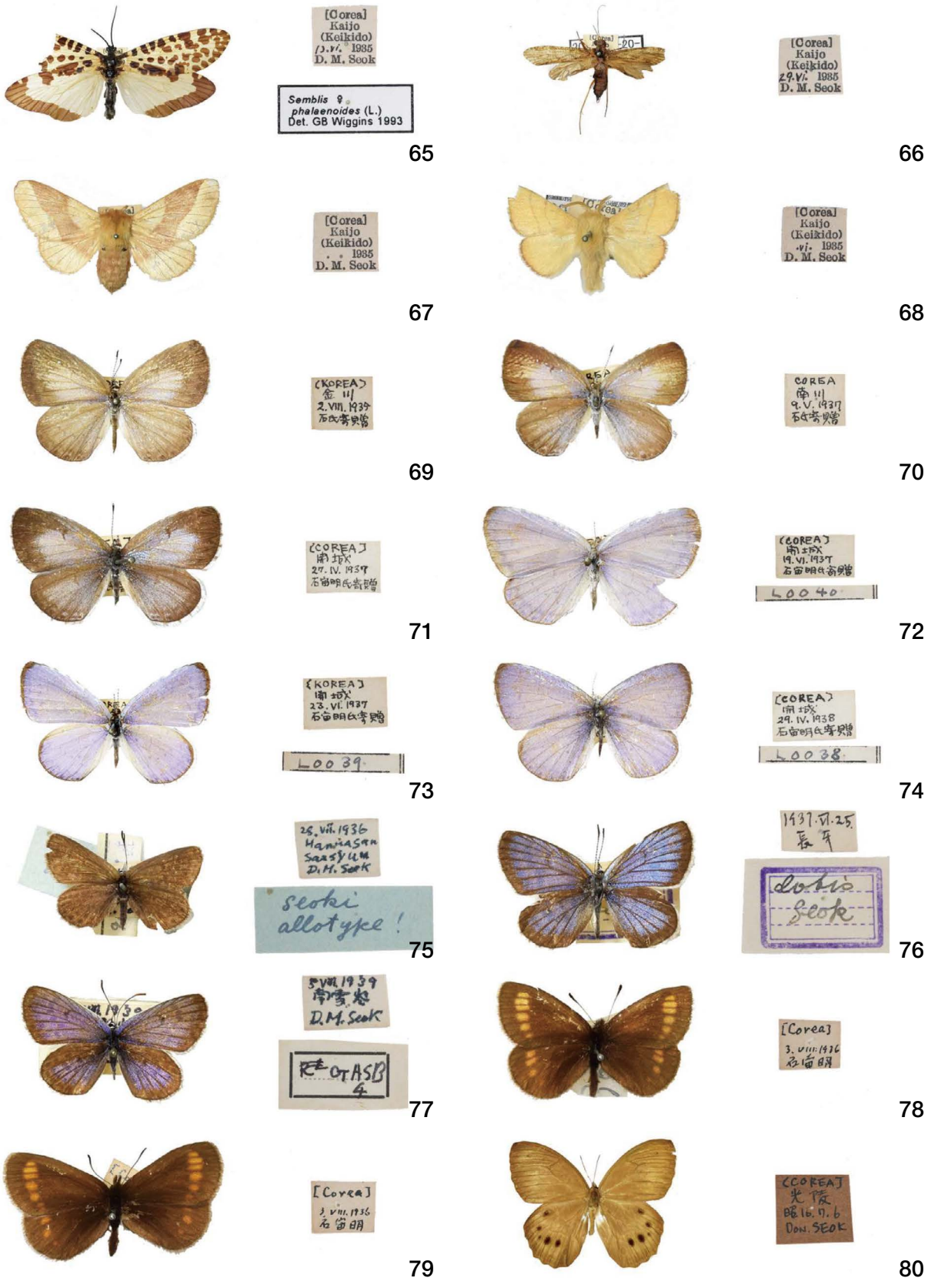


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64

Figs. 49–64. Photographs of Seok's collection and its label. Information on each specimen are given in the text.



Figs. 65–80. Photographs of Seok's collection and its label. Information on each specimen are given in the text.

2024-0097 (fig. 69)
1 ex. (KOREA) Nansen, 9. May. 1937, D. M. Seok, NIBR 2024-0096 (fig. 70)
1 ex. (COREA) Kaijo, 27. Apr. 1937, D. M. Seok, NIBR 2024-0100 (fig. 71)
1 ex. (COREA) Kaijo, 19. Jun. 1937, D. M. Seok, NIBR 2024-0094 (fig. 72)
1 ex. (COREA) Kaijo, 23. Jun. 1937, D. M. Seok, NIBR 2024-0095 (fig. 73)
1 ex. (COREA) Kaijo, 29. Apr. 1938, D. M. Seok, NIBR 2024-0093 (fig. 74)

Plebejus argus (Linnaeus, 1758) 산꼬마부전나비
1 ex. Hannasan Saisyum, 28. Jul. 1936. D. M. Seok, NIBR2024-0098 (fig. 75)
1 ex. Chouhei, 25. Jun. 1937. Seok, NIBR2024-0101 (fig. 76)
1 ex. Nanseturei, 5. Aug. 1939. D. M. Seok, NIBR2024-0099 (fig. 77)

Family Nymphalidae 네발나비과

Erebia pawlowskii Ménétriès, 1859 차일봉지옥나비
2♂. (COREA), 3. Aug. 1936, D. M. Seok, NIBR2024-0102–103 (figs. 78, 79)

Ninguta schrenckii (Ménétriès, 1859) 왕그늘나비
1 ex. (COREA) Koryo, 6. Jul. 1941, D. M. Seok, NIBR 2024-0104 (fig. 80)

Oeneis urda (Eversmann, 1847) 함경산뱀눈나비
2 exs. (KOREA) Eiko, 4. May. 1942, D. M. Seok, NIBR 2024-0106, 108 (figs. 81, 82)
2 exs. (KOREA) Eiko, 9. May. 1942, D. M. Seok, NIBR 2024-0105, 107 (figs. 83, 84)

Polygonia c-album (Linnaeus, 1758) 산네발나비
1 ex. (COREA) Kaijo, Unknown, D. M. Seok, NIBR 2024-0111 (fig. 85)
1 ex. (COREA), Unknown, D. M. Seok, NIBR2024-0120 (fig. 86)
1♂. Kanhoku Shinbusei, 31. Jul. 1931, D. M. Seok, NIBR2024-109 [Allotype, Holotype] (Fig. 87)
1 ex. Kanhoku Shinbusei, 31. Jul. 1931, D. M. Seok, NIBR 2024-123 (fig. 88)
1 ex. (COREA) Kaijo, 9. Jun. 1933, D. M. Seok, NIBR 2024-0112 (fig. 89)
1 ex. (COREA) Kaijo, 11. Jun. 1933, D. M. Seok, NIBR 2024-0113 (fig. 90)
2 exs. (COREA) Kyujo, 14. Jun. 1933, D. M. Seok, NIBR 2024-0115–0116 (figs. 91, 92)
1 ex. (COREA) Kaijo, 14. Jun. 1933, D. M. Seok, NIBR 2024-0117 (fig. 93)
1 ex. (COREA) Kaijo, 18. Jun. 1933, D. M. Seok, NIBR 2024-0114 (fig. 94)
1 ex. (COREA) Kaijo, 13. May. 1934, D. M. Seok, NIBR 2024-0119 (fig. 95)
1 ex. (COREA) Kaijo, 15. Jun. 1934, D. M. Seok, NIBR 2024-0121 (fig. 96)
1♀. (COREA) Kaijo, 22. Jul. 1934, D. M. Seok, NIBR 2024-0110 [Allotype, Holotype] (fig. 97)
1 ex. (COREA) Kokaido Kinshu, 28. Jul. 1934, D. M. Seok, NIBR2024-0122 (fig. 98)
1 ex. (COREA) Kanhoku Keigen, 4. Aug. 1934, D. M. Seok, NIBR2024-0124 (fig. 99)
1 ex. (COREA) Kaijo, 14. Aug. 1934, D. M. Seok, NIBR 2024-0118 (fig. 100)

Family Pieridae 흰나비과

Pontia daplidice (Linnaeus, 1758) 풀흰나비
2 pupae, (COREA) Keikido Kaijo, 23. Jun. 1935, D. M. Seok, NIBR2024-0091–92 (figs. 101, 102)

Family Crambidae 풀명나방과

Chilo sp.
1 ex. (COREA) Keikaido Kaijo, Jun. 1935, D. M. Seok, NIBR2024-0126 (fig. 103)

Family Thyrididae 창나방과

Striglina cancellata Christoph, 1914 창나방
1 ex. (COREA) Keikaido Kaijo, 1935, D. M. Seok, NIBR 2024-0128 (fig. 104)

Order Hymenoptera 벌목

Family Vespidae 말벌과

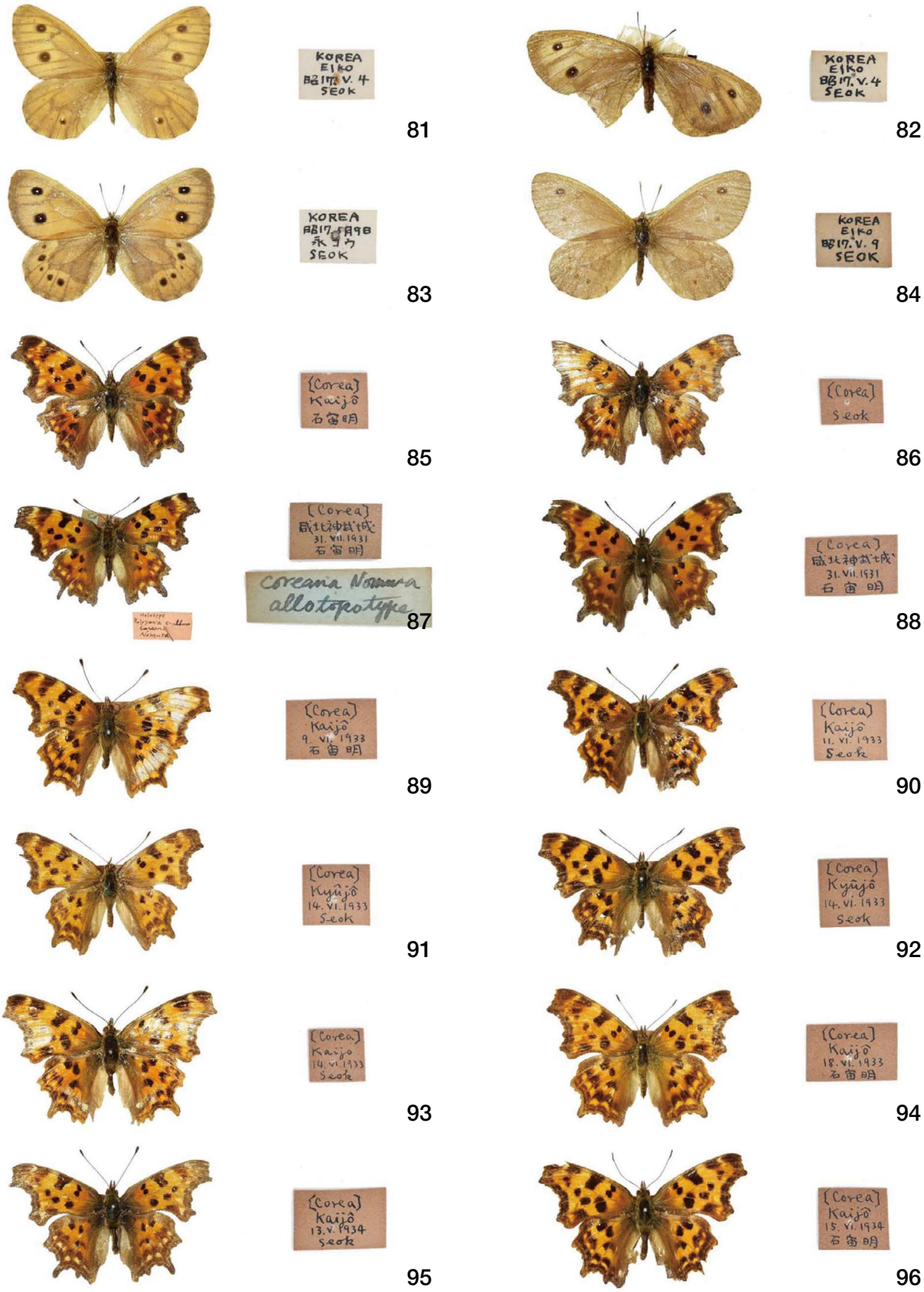
Vespa crabro flavofasciata Cameron, 1903 말벌
1 ex. (COREA) Keikido Kaijo, 1935, D. M. Seok, NIBR2024-0052 (fig. 105)

Family Braconidae 고치벌과

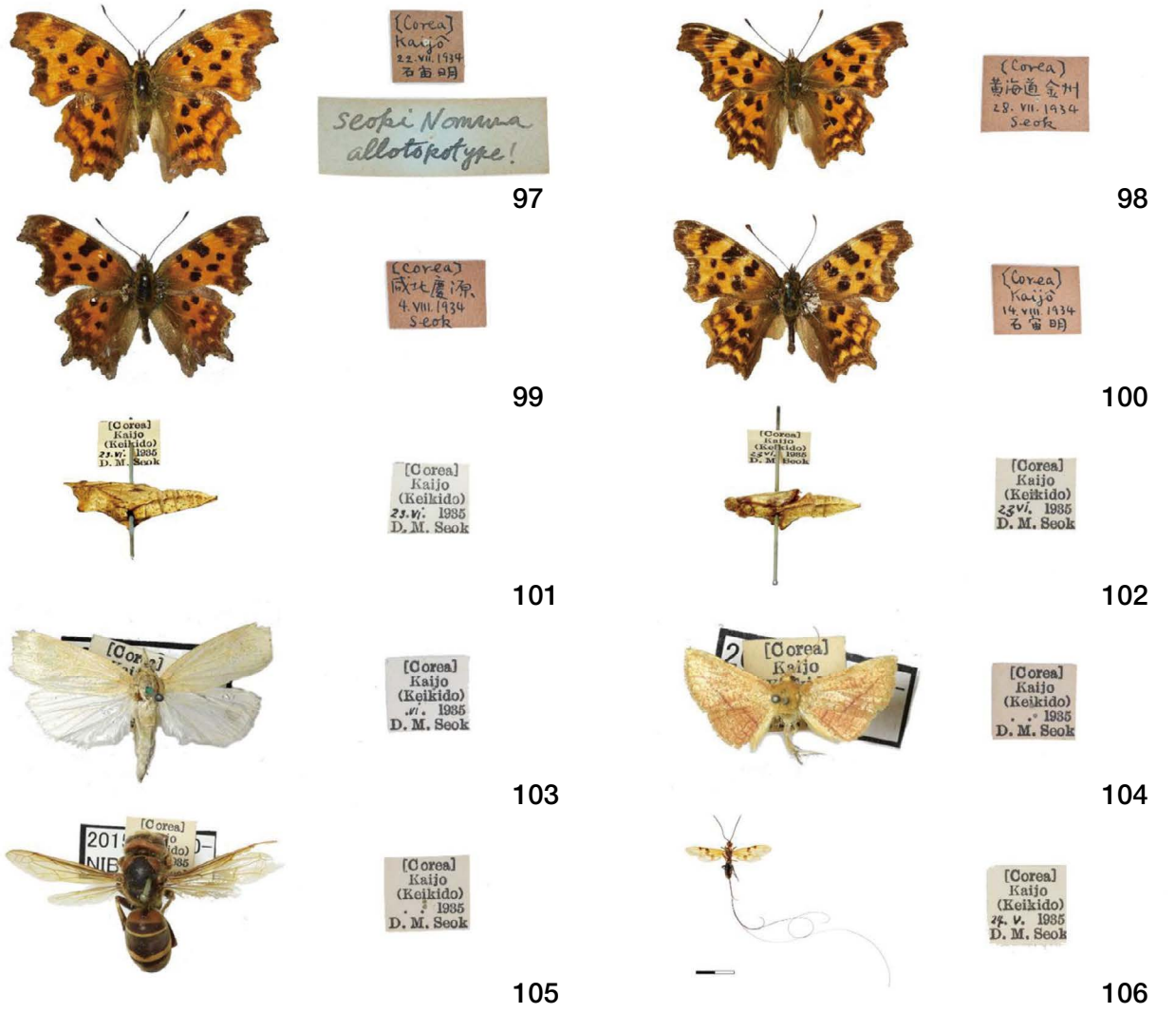
Euurobracon yokahamae (Dalla Torre, 1898) 말총벌
1 ex. (COREA) Kaijo, 24. May. 1935, D. M. Seok, NIBR 2024-0129 (fig. 106)

Discussion

According to Sibatani (1985; 1987), Seok entered the Kagoshima Agricultural and Forestry College in Japan in 1926 to study agriculture. Later, he became interested in natural history and studied entomology under Ginji



Figs. 81–96. Photographs of Seok's collection and its label. Information on each specimen are given in the text.



Figs. 97–106. Photographs of Seok's collection and its label. Information on each specimen are given in the text. Scale bar = 20 mm (Fig. 106).

Okajima, a teacher at the same college. Seok returned to Korea in 1929 and got a teaching job at Songdo High School in his hometown of Gaeseong.

Seok's teacher, Ginji Okajima, established the three laboratories of Zoology, Entomology, and Silkworm Science at Kyushu University between 1921 and 1922 and built the foundation by organizing the literature related to entomology before the currently recognized first professor of the Entomological laboratory, Teiso Esaki, took up the position. Ginji Okajima's name is also associated with the species names "*Neptis okajimai*" and "*Zephyrus ginzii*," which were named after him by Seok (Seok, 1936). Currently, the former is treated as a synonym of *Neptis philyra* and the latter as *Protantigius superance ginzi* (Paek *et al*., 2010; Joo *et al*., 2021).

Atsuhiro Sibatani, who studied Seok's work, had direct contact with him. Sibatani received materials from Seok from the Korean Peninsula while publishing a book on Lepidopteran genitalia. Seok also had been in contact with Iwahiko Sugitani, a famous entomologist and mathematics teacher at Kyoto Sanko High School at the time, who visited the Korean Peninsula many times and built up a valuable collection.

In fact, Seok dedicated the species named *Clossiana selene sugitanii* to Sugitani (Seok, 1939; 1947). This species was called 'Sugitani-eunjeomseon-pyobeomnabi' in Korea until very recently (Shin, 1996; Paek *et al*., 2010).

Sugitani's collection of 36,000 butterflies and moths is currently preserved at Kyushu University and was also utilized in the taxonomic revision of the genus *Maculinea* conducted by Sibatani *et al*. (1993), contributing to the understanding of lepidopteran fauna of the Korean

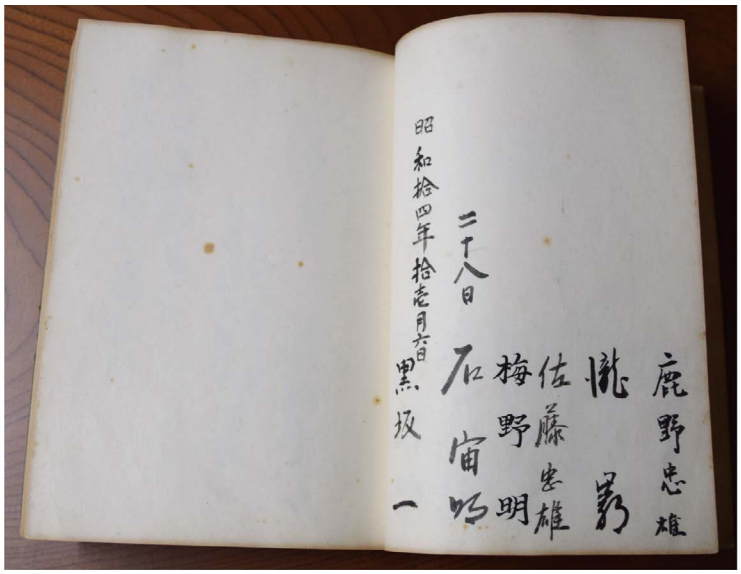


Fig. 107. Seok's signature when he visited the Entomological laboratory of Kyushu University on October 28, 1936.

Peninsula.

We identified a total of 129 specimens of 35 species at Kyushu University collected by Seok in 1931–1942 in the Korean Peninsula. Most specimens were collected in Gaeseong between 1933 and 1937 when Seok was working at Songdo High School in Gaeseong. Other specimens include those from what is now North Korea, such as Eiko (Yeongheung) Korea, and some from other parts of what is now South Korea, such as Koryo (Gwangneung), Seoul, and Jeju Island. The details of how Seok's specimens were donated to Kyushu University are unclear, but Seok's teacher, Ginji Okajima, was enrolled at Kyushu University, and there appears to have been some interaction with Seok and their Entomological laboratory during the time of Teiso Esaki.

In fact, in 1939, the Royal Asiatic Society of Great Britain and Ireland published 'A Synonymic List of Butterflies of Korea,' which included a preface by Okajima and an afterword by Esaki. Furthermore, during his visit to the Entomological Laboratory of Kyushu University on October 28, 1936, Seok signed the guest book designated for outside visitors (Fig. 107). Since some specimens collected between 1935 and 1937 bear the label "Donated by D.M.Seok," these specimens were considered to have been donated to Kyushu University personally by Seok at that time.

Below, we introduce some of the noteworthy species identified in the current lot.

Plebejus argus is a rare species found only on Jeju Island in South Korea, with other populations found in the northern part of North Korea (Joo *et al*., 2021). This species inhabits high altitudes in Japan, but populations are declining in various places. This lycaenid deposited at Kyushu University was collected by Seok from Hanrasan Mountain on Jeju Island. The label of the female has the place name "Hannasan, Saisyum" written by hand, which is thought to be the Japanese reading of "Hanrasan, Jeju." The specimen also has a note "Allotype!" (Taf. 4, Fig. 2), which represents the subspecies that Shirôzu and Sibatani (1943) dedicated to Seok as *Plebejus argus seoki*. Shirôzu and Sibatani (1943) stated that all the type series were collected by Seok on the summit of Hanrasan Mountain on July 27–28, 1936. However, the label of the male holotype indicates that Seok collected it on August 5, 1939, at Namseturei (Namseolryeong, Yanggangdo, North Korea). Thus, the authenticity of some of these labels needs further verification.

Polygonia c-album is distributed in the Korean peninsula from Gangwon-do northward. Nomura (1937) designated the subspecific name *coreana* to *Polygonia c-album* using a specimen collected by Seok at Kanboku Shinbusei (Shinmuseong Yanggangdo, North Korea) as the type specimen and further described *f. seoki* within this subspecies. However, *P. c-album coreana* is currently treated as a synonym of *P. c-album hamigera* (Butler, 1877) (Joo *et al*., 2021), and *f. seoki* is regarded as an invalid name.

Eurobracon yokahamae is a large parasitic wasp characterized by its extremely long ovipositor. This species parasitizes the larvae of large long-horned beetles and is distributed throughout Asia, including the Korean peninsula and the Japanese archipelago, but its population has been declining in recent years. The specimen deposited

at Kyushu University was collected in Seoul on May 24, 1935.

Libelloides sibiricus (Eversmann, 1850) is distributed throughout the Korean Peninsula. This species lives in moist, low shrub forests and grassland environments, but as these environments decline, its population is thought to be decreasing and will require thorough monitoring in the future. Currently, this species is listed as LC (Least Concern) in the Red Data Book of the Republic of Korea (NIBR, 2022). Several individuals of *L. sibiricus* collected by Seok in April 1935 at Gaeseong were confirmed in the current lot.

A closely related species, *Ascalaohus ramburi*, is distributed in Japan, but its population is also decreasing nationwide.

Except for some types of specimens, the insect collection by D. M. Seok was donated from Kyushu University to NIBR (National Institute of Biological Resources) in Korea in September 2024 and was transferred to the insect collections storage in NIBR. All specimens were well-preserved thanks to the efforts of former staff at Kyushu University, who carefully managed these insect specimens for over 90 years. Their dedication is a good example of how important specimen storage and management are in taxonomy.

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

Conflicts of Interest

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

Acknowledgements

We wish to our cordial thanks to Dr. Mun-Ki Paek (Institute of Korean Peninsula Insects Conservation, Korea), Dr. Sangwook Park (Research Institute of Forest Insect Diversity, Korea), Dr. Seulki Kim (Department of Agricultural Biology, Jeonbuk National University, Korea), Dr. Sun-Jin Park (Kyonggi University, Korea) and Dr. Taewoo Kim (National Institute of Biological Resources) for their assistance in the identification of species.

We also wish to express thanks to Dr. Chang-Woo Hyun (National Institute of Biological Resources) for providing information about the collecting locality in Jeju lsland.

This work was supported by grants from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202402108; A study on the species diversity of insects from Korea).

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Submitted: September 9, 2024
Revised: October 30, 2024
Accepted: October 30, 2024

Journal of Species Research 13(4):417-422, 2024

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New record of marine scuticociliates and euplotids (Protozoa, Ciliophora) from coastal waters of South Korea

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During a field survey of marine ciliate diversity in South Korea, four ciliate species were newly recorded as follows: *Falcicyclidium citriforme*, *Homalogastra setosa*, *Euplotes orientalis*, and *Euplotes shini*. The identification of these species was based on morphological examination through observations of both living cells and silver-impregnated specimens. A brief description, remarks, and photomicrographs are provided for each species.

Keywords: infraciliature, morphology, redescription, silver impregnation, taxonomy

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DOI:10.12651/JSR.2024.13.4.417

Introduction

Scuticociliates are a species-rich group of ciliates commonly found in coastal habitats (Carey, 1992; Lynn, 2008; Jung, 2021). According to the National Institute of Biological Resources (2023), thirty species of scuticociliates have been reported in Korea. Some species are known to cause severe diseases, such as scuticociliatosis in wild and mariculture fish populations (Noga, 2010). Although scuticociliates are facultative parasites capable of living independently, they may become parasitic under certain environmental conditions (Thompson Jr. and Moewus, 1964).

The genus *Euplotes* Ehrenberg in Hemprich and Ehrenberg, 1831, is one of the most species-rich genera in the phylum Ciliophora, with approximately 160 species. Euplotids are benthic ciliates found in terrestrial, freshwater, and marine environments worldwide (Curds, 1975; Song *et al*., 2009; Foissner, 2016; Živaljić *et al*., 2020; Abraham *et al*., 2021; Do *et al*., 2024). Despite their cosmopolitan distribution, only 23 species have been reported in Korea, according to the National Institute of Biological Resources. Despite the prevalence and diversity of the scuticociliates and euplotids, our understanding of their diversity in South Korea remains limited.

In this study, we provide brief descriptions, remarks, photomicrographs, and locality information for four newly recorded ciliates, two scuticociliate and two euplotid species from South Korea.

Materials and Methods

Sample collection and identification

The four species were collected from coastal waters, with detailed locality information provided in the "Material examined" section for each species. Water samples were transferred to the laboratory and incubated in Petri dishes, with mealworms or sterile wheat grains added as food sources. All four species were examined using a stereomicroscope (Olympus SZ11, Tokyo, Japan) and light microscopes (Olympus BX53), under bright-field and differential interference contrast (DIC) at magnifications ranging from 50× to 1000×.

The protargol impregnation 'Procedure A' of Foissner (2014) was performed using synthesized protargol and an acetone developer to reveal infraciliature and nuclear apparatus. Protargol powder was synthesized according to the methods described by Pan *et al*. (2013) and Kim and Jung (2017). Additionally, the Chatton-Lwoff silver nitrate impregnation technique was used to reveal the infraciliature and silverline system (Corliss, 1953). Terminology and classification follow Curds (1975) and Lynn (2008).

Results and Discussion

Class Oligohymenophorea de Puytorac *et al*., 1974
Subclass Scuticociliatia Small, 1967
Order Philasterida Small, 1967
Family Cyclidiidae Ehrenberg, 1838

Genus *Falcicyclidium* Fan *et al*., 2011

1. *Falcicyclidium citriforme* Fan *et al*., 2017

Material examined. Marine water (salinity 33.7‰, temperature 27.9°C) collected from Anin Beach, Gangdong-myeon, Gangneung-si, Gangwon-do, Korea (37°44′2″N, 128°59′26″E) on August 03, 2021.

Diagnosis. Body size 30–35 × 15–20 μm in vivo, on average 30 × 20 μm after protargol impregnation (n = 5) (Fig. 1A, B). Body shape ordinary to broadly oval (Fig. 1A–C). One globular macronucleus, 6 μm across on average after protargol impregnation, located slightly above the body center; nucleoli globular to ellipsoidal and evenly distributed; micronucleus lacking or indistinguishable (Fig. 1B). Contractile vacuole terminal, 4–5 μm across in vivo at end of diastole (Fig. 1A). Cytoplasm with food vacuoles, crystals, and lipid (Fig. 1A). Somatic and caudal cilia 6–10 μm and 14–18 μm long in vivo, respectively; invariably ten bipolar somatic kineties; kineties 1 and 10 with 24–27 and 22–26 mono/dikinetids, respectively (Fig. 1A–C). Oral apparatus occupies about 66% of body length after silver nitrate impregnation, composed of three adoral membranelles and paroral membrane; paroral membrane about 16 μm long (Fig. 1A–C). Silverline consists of primary meridians, which connect the somatic kinetids of each kinety, and transverse connectives without secondary meridians (Fig. 1C).

Distribution. China (Fan *et al*., 2017) and Korea (present study).

Remarks. The Korean population of *Falcicyclidium citriforme* is similar to the Chinese type population described by Fan *et al*. (2017). However, the Korean population has a slightly smaller body length compared to the type population (30–35 μm vs. 40–60 μm in vivo; Fan *et al*., 2017). In comparison to other species in the genus, *F. atractodes* Fan *et al*., 2011 resembles *F. citriforme* but can be easily distinguished by its body shape, with *F. atractodes* having spiny anterior and posterior ends, while *F. citriforme* lacks these spines (Fan *et al*., 2017). Additionally, *Falcicyclidium fangi* Fan *et al*., 2011 differs from *F. citriforme* in the ratio of the buccal field to body length (0.75 vs. 0.54; Fan *et al*., 2011; 2017).

Compared to other similar genera, *Cyclidium plouneouri* Dragesco, 1963 resembles *F. citriforme* but can be easily distinguished by a lower number of somatic kineties (14–16 vs. 10) and according to Wilbert, 1986 also by a lower number of somatic kineties (11 or 12 vs. 10). Additionally, *Cyclidium borrori* Borror, 1965 differs from *F. citriforme* by a lower number of somatic kineties (12 or 13 vs. 10).

Voucher slides. Two slide with protargol-impregnated specimens (MABIK PR00045077, MABIK PR00045078) and one slide with silver nitrate-impregnated specimens (MABIK PR00045079) were deposited at the National Marine Biodiversity Institute of Korea.

Family Uronematidae Thompson, 1964
Genus *Homalogastra* Kahl, 1926

2. *Homalogastra setosa* Kahl, 1926

Material examined. Marine water (salinity 35‰) collect-

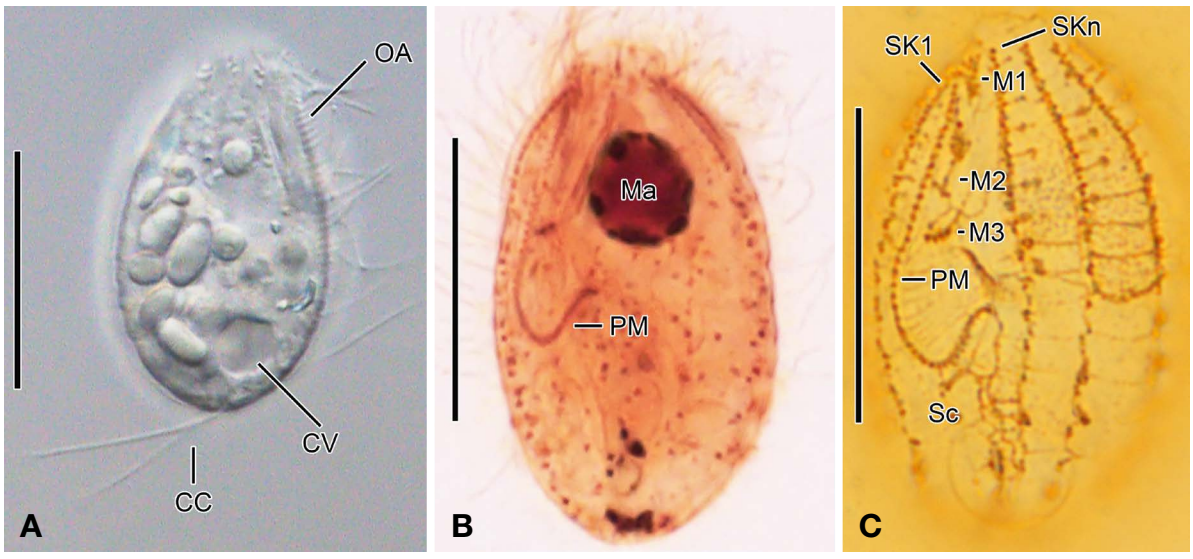


Fig. 1. *Falcicyclidium citriforme* from life (A), after protargol (B) and Chatton-Lwoff silver nitrate (C) impregnation. (A) Ventral view of a representative specimen showing the body shape, oral apparatus, contractile vacuole, somatic and caudal cilia. (B, C) Ventral views showing the infraciliatures and nuclear apparatus. CC, caudal cilia; CV, contractile vacuole; M1–3, membranelles 1–3; Ma, macronucleus; OA, oral apparatus; PM, paroral membrane; SK1 and n, somatic kineties 1 and n; Sc, scutica. Scale bars: 20 μm.

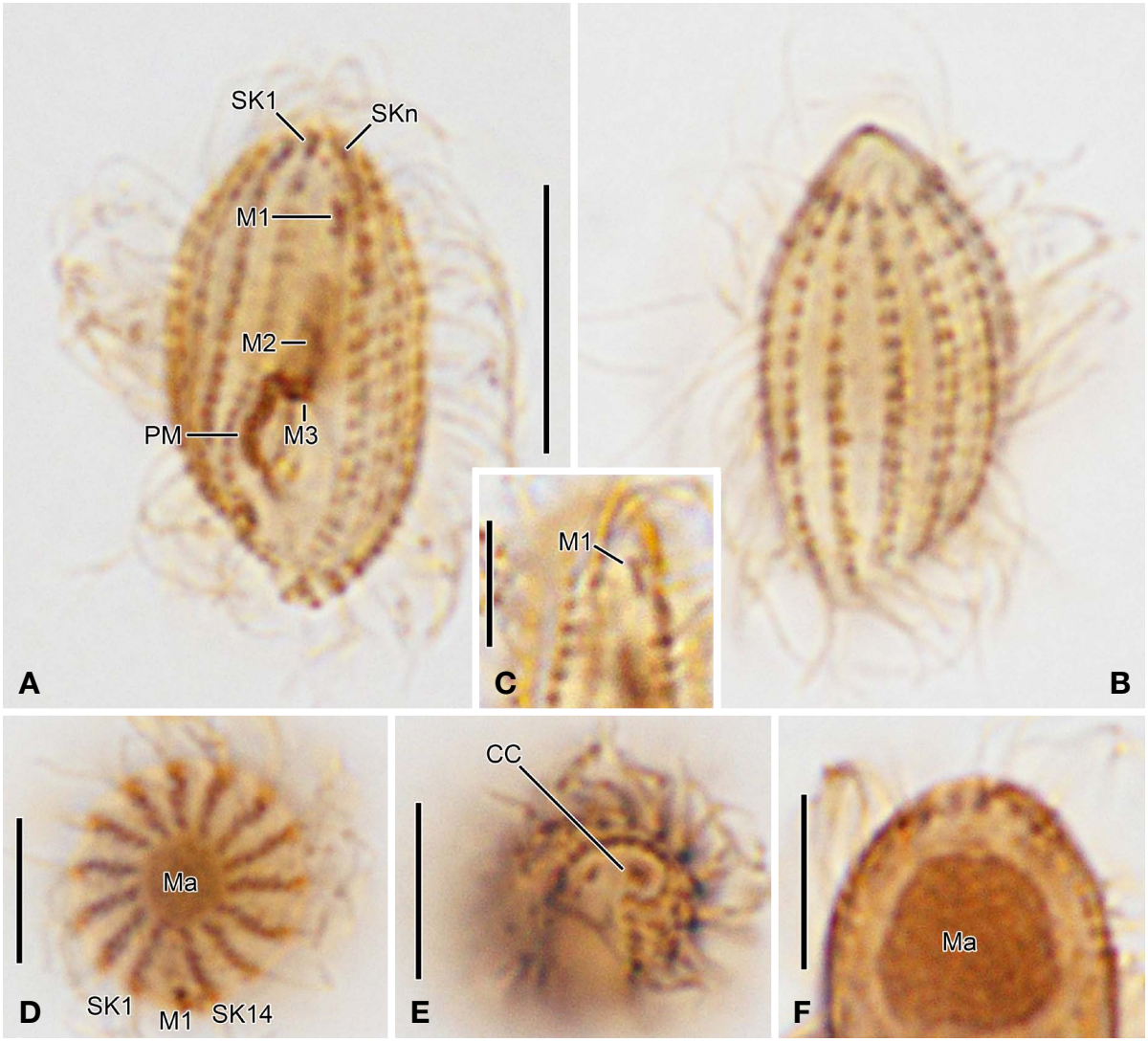


Fig. 2. *Homalogastra setosa* after protargol impregnation. (A, B) Ventral (A) and dorsal (B) view of the same specimen. The ventral view shows the body shape, cilia, and oral apparatus, while the dorsal view displays the somatic kineties and the barren apical plate. (C) Ventral view showing the single rowed membranelle 1. (D, E) Apical views of the anterior (D) and posterior (E) end of the cell, showing the somatic kineties, membranelle 1, macronucleus and caudal basal bodies. (F) Macronucleus in anterior portion of cell. CC, caudal cilium; M1−3, membranelles 1−3; Ma, macronucleus; PM, paroral membrane; SK1, 14 and n, somatic kineties 1, 14 and n. Scale bars: 10 μm (A), 3 μm (C), and 5 μm (D−F).

ed from Udo-myeon, Jeju-si, Jeju-do, Korea (33°30′51″N, 126°56′50″E) on May 29, 2024.

Diagnosis. Body size 12.6−15.3 × 7.4−8.3 μm after protargol impregnation (n = 11) (Fig. 2A, B). Body spindle-shaped, both ends pointed, posterior end slightly asymmetrical (Fig. 2A, B). One globular macronucleus size 4.0−6.1 × 3.7−5.1 μm after protargol impregnation, located slightly above the body center (Fig. 2F). 13−15 bipolar somatic kineties (Fig. 2A, B); anterior apical plate conical and distinctly baren (Fig. 2B, D); one caudal cilium (Fig. 2E). Oral apparatus occupies about 74% of body length after protargol impregnation, composed of three adoral membranelles and paroral membrane; membranelle 1 with one basal bodies row (Fig. 2C); paroral membrane on average 5 μm long after protargol impregnation (Fig. 2A).

Distribution. Africa (Buitkamp, 1977), Australia (Pomp and Wilbert, 1988), Austria (Foissner *et al*., 1982), Azerbaijan (Alekperov, 2005), China (Liu *et al*., 2020), Germany (Kahl, 1926), and Korea (present study).

Remarks. The Korean population resembles the type population described by Foissner *et al*. (1982), but differs slightly in its smaller body length after protargol impregnation (12.6−15.3 μm vs. 20−25 μm), a higher number of somatic kineties (13−15 vs. 12), and a pointed anterior end (vs. truncated).

Homalogastra setosa and *H. parasetosa* Liu *et al*., 2020

closely resemble each other but can be distinguished by the number of ciliary rows in membranelle 1 (1 vs. 2). When Liu *et al*. (2020) established *H. parasetosa*, they considered populations of *H. setosa* with a double-row membranelle 1 as junior synonyms of *H. parasetosa*.

Voucher slides. One slide with protargol-impregnated specimens (MABIK PR00045076) were deposited at the National Marine Biodiversity Institute of Korea.

Class Spirotrichea Bütschli, 1889
Subclass Euplotia Jankowski, 1979
Order Euplotida Small and Lynn, 1985
Family Euplotidae Ehrenberg, 1838
Genus *Euplotes* Ehrenberg in Hemprich and Ehrenberg, 1831

3. *Euplotes orientalis* Jiang *et al*., 2010

Material examined. Marine water (salinity 34‰) collected from Udo-myeon, Jeju-si, Jeju-do, Korea (33°29′47″N, 126°57′59″E) on May 29, 2024.

Diagnosis. Body size 32.1–34.3 × 15.5–17.9 μm after silver nitrate impregnation (n = 5), shape rotund; 21–23 adoral membranelles; 10 frontoventral (including 2 non-ciliated reduced cirri), 5 transverse, 2 caudal and 2 marginal cirri (Fig. 3A). 7 dorsal kineties with 6 or 7 dikinetids in the middle one; dorsal argyrome pattern of double-*patella*-I type (Fig. 3B).

Distribution. China (Jiang *et al*., 2010) and Korea (present study).

Remarks. The Korean population corresponds well with the Chinese type population described by Jiang *et al*. (2010) in several characteristics: body size after silver impregnation (32.1–34.3 μm vs. 32–42 μm); number of adoral membranelles (21–23 vs. 18–25), frontoventral cirri (8 vs. 8), caudal cirri (2 vs. 2), marginal cirri (2 vs. 2), dorsal kineties (7 vs. 6 or 7), dikinetids in the mid-dorsal row (6 or 7 vs. 6–8); dorsal argyrome pattern (double-*patella*-I); and habitat (marine).

Among the approximately 160 congeners, the presence of a reduced cirrus is a unique feature, with only eight species exhibiting this trait (Do *et al*., 2024). Of these species, *Euplotes raikovi* Agamaliev, 1966 is similar to *E. orientalis* but can be distinguished by the number of frontoventral cirri (7 vs. 8) and reduced cirri (1 vs. 2). *Euplotes pseudoraikovi* Alekperov, 2005 also resembles *E. orientalis*, but differs in the number of frontoventral cirri (8 vs. 6).

Voucher slides. Four slides with silver nitrate-impregnated specimens (MABIK PR00045072–PR00045075) were deposited at the National Marine Biodiversity Institute of Korea.

4. *Euplotes shini* Lian *et al*., 2020

Material examined. Marine water (salinity 29‰) collected from Buk-myeon, Uljin-gun, Gyeongsangbuk-do, Korea

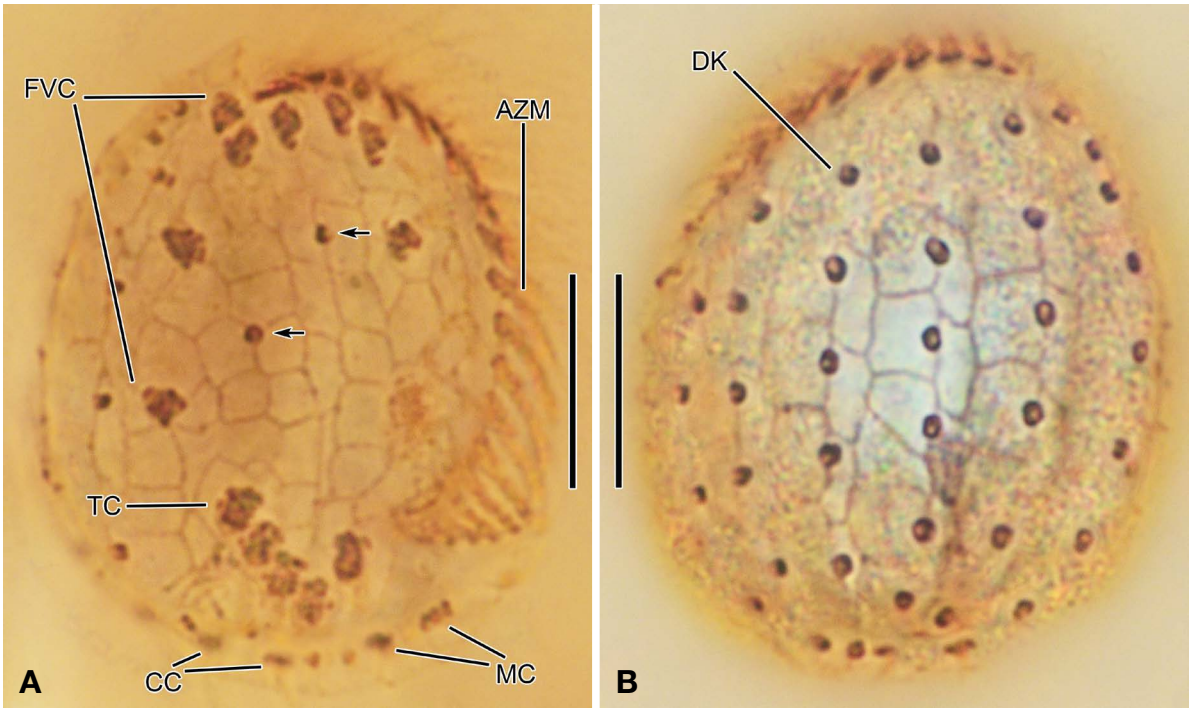


Fig. 3. *Euplotes orientalis* from Chatton-Lwoff silver nitrate impregnation. (A) Ventral view showing the body shape, ventral cirri, and oral apparatus. Arrows denote the reduced cirri. (B) Dorsal view showing the dorsal kineties and argyrome pattern. AZM, adoral zone of membranelles; CC, caudal cirri; DK, dorsal kineties; FVC, frontoventral cirri; MC, marginal cirri; TC, transverse cirri. Scale bars: 10 μm.

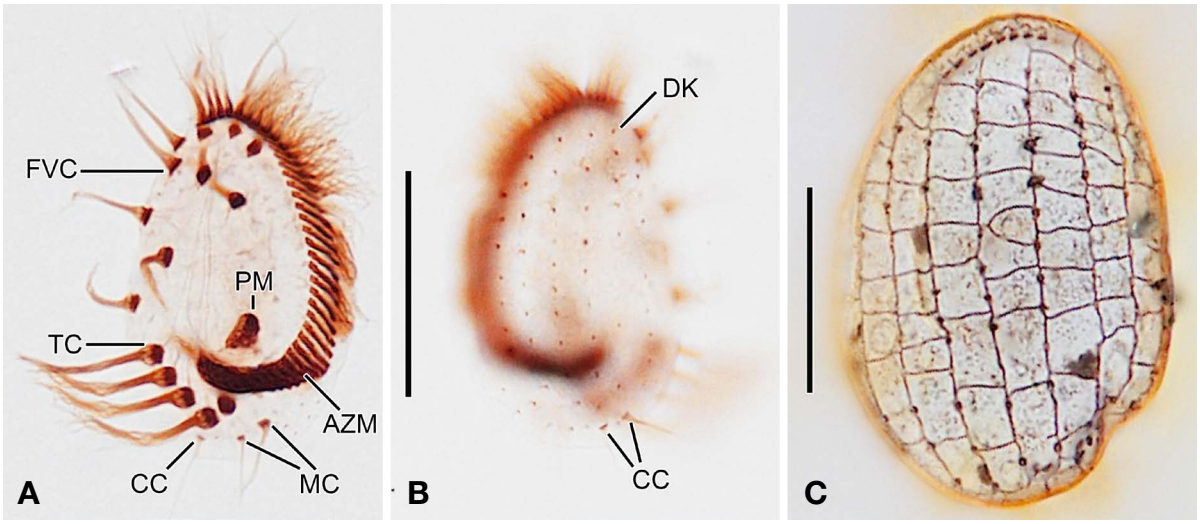


Fig. 4. *Euplotes shini* after protargol (A, B) and Chatton-Lwoff silver nitrate impregnation (C). (A, B) Ventral (A) and dorsal (B) view of the same specimen, showing body shape, ventral cirri, oral apparatus, and dorsal kineties. (C) Dorsal view showing the dorsal kineties and argyrome pattern. AZM, adoral zone of membranelles; CC, caudal cirri; DK, dorsal kineties; FVC, frontoventral cirri; MC, marginal cirri, PM, paroral membrane; TC, transverse cirri. Scale bars: 20 μm.

(37°32′5″N, 130°51′21″E) on May 23, 2023.

Diagnosis. Body size 43.8–55.0 × 20.7–29.1 μm after protargol impregnation (n = 11). Body shape broadly oval; macronucleus C-shaped, with a single small spherical micronucleus attached to it; 45–48 adoral membranelles; 10 frontoventral, 5 transverse, 2 caudal and 1 or 2 marginal cirri (Fig. 4A). 8 or 9 dorsal kineties with 11 or 12 dikinetids in the middle one; dorsal argyrome pattern of single-*vannus* type (Fig. 4B, C).

Distribution. China (Lian, 2020) and Korea (present study).

Remarks. The Korean population corresponds well with the Chinese type population described by Lian *et al.* (2020) in several aspects: buccal field length (72% vs. 75% on average); number of adoral membranelles (45–48 vs. 37–46), frontoventral cirri (10 vs. 10), caudal cirri (2 vs. 2 or 3), marginal cirri (1 or 2 vs. 2), dorsal kineties (8 or 9 vs. 9), dikinetids in the mid-dorsal row (11 or 12 vs. 10–14); dorsal argyrome pattern (single-*vannus*), and habitat (marine).

Compared to other species in the genus, *Euplotes minuta* Yocum, 1930 resembles *E. shini*, but the two species can be differentiated by the size of their marginal cirri, i.e., *E. minuta* has marginal cirri of approximately the same size (Park *et al.*, 2010), whereas *E. shini* has marginal cirri of different sizes. As noted by Lian *et al.* (2020), *E. minuta* is presumed to represent a species complex.

Voucher slides. Two slides with protargol-impregnated specimens (MABIK PR00045070, MABIK PR00045071) and one slide with silver nitrate-impregnated specimens (MABIK PR00045069) were deposited at the National Marine Biodiversity Institute of Korea.

Conflicts of Interest

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

Acknowledgements

We are grateful to Dr. Atef Omar for improving the manuscript. This work was supported by the management of Marine Fishery Bio-resources Center (2024) funded by the National Marine Biodiversity Institute of Korea (MABIK), and the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBRE202406).

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Submitted: September 21, 2024
Revised: October 31, 2024
Accepted: October 31, 2024

Journal of Species Research 13(4):423-433, 2024

Open Access

Taxonomic review of Korean *Ephemera* Linnaeus with lectotype designation of *E. sachalinensis* Matsumura (Insecta: Ephemeroptera: Ephemeridae)

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The common mayfly genus *Ephemera* Linnaeus from South Korea was taxonomically reviewed, with emphasis on the sympatric *E. orientalis* McLachlan and *E. sachalinensis* Matsumura. A taxonomic key to South Korean *Ephemera* species is provided for larval and adult stages. The thick and continued pair of dark stripes on mesonotum and presence of pigmentation on abdominal tergum I−II are the diagnostic characters to distinguish *E. orientalis* from *E. sachalinensis* in both male and female adults and larvae. In the larvae, the shape and length of foreleg claws, and the shape of posterior margin of tergum X are useful diagnostic characters to distinguish between these two species. In addition, a female specimen deposited in the Hokkaido University Museum, Sapporo, Japan, that was used in the original description of *E. sachalinensis* by Matsumura in 1911 is designated as the lectotype of the species. A DNA barcoding analysis on the East Asian *Ephemera* species with paired multi-stripes on the abdominal terga well substantiated their species status.

Keywords: DNA barcoding, *Ephemera sachalinensis*, Ephemeridae, Korean *Ephemera*, lectotype, taxonomic review

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DOI:10.12651/JSR.2024.13.4.423

Introduction

The burrowing mayfly family Ephemeridae has a worldwide distribution with exception in the poles and Oceania, and the family consists of four extant genera. The genus *Ephemera* Linnaeus is represented by approximately 60 nominal species; majority of them occur in China (30 species) (Yoon and Bae, 1985; Hwang and Bae, 2008; Hwang *et al*., 2008). Despite the *Ephemera* species are relatively large in body size among mayflies and easily sampled from diverse freshwater habitats, morphological identifications of some closely related species still are challenging (Hwang *et al*., 2009; Lee *et al*., 2011). Since the early classical studies by Eaton in the late nineteenth century (Eaton, 1871; 1883-1888; 1892), East Asian *Ephemera* were studied by Tshernova (1973), Gose (1981), and Hwang and Bae (2008).

Historically, the *Ephemera* species have been identified by taxonomical keys by original descriptions and type information (Tshernova, 1973; Hwang and Bae, 2008). Paired multi-stripes on the abdominal terga as one of important taxonomical keys to differentiate them used in closely related *Ephemera* species (Tshernova, 1973; Hwang and Bae, 2008). Some of *Ephemera* species often exhibit a wide range of intraspecific variations in all the life stages that make accurate species identification difficult (Hwang *et al*., 2003; Hwang and Bae, 2008). For example, accurate species identification of widely distributed and sympatric *E. orientalis* McLachlan, 1875 and *E. sachalinensis* Matsumura, 1911 have frequently been occurred in ecological or biomonitoring studies in South Korea (Bae *et al*., 1994; Hwang *et al*., 2003). Particularly, the repeated mass emergence of *E. orientalis* with *E. sachalinensis* in the lower reaches of streams and large rivers in South Korea requires detailed taxonomic identification of these two species. Thus, the purpose of this study is to taxonomically review Korean *Ephemera* species with emphasis on distinct species delimitation of the two sympatric species, *E. orientalis* and *E. sachalinensis*. In addition, we designate a female *Ephemera* specimen

preserved in the Matsumura's collection in the Hokkaido University Museum as a lectotype of *E. sachalinensis*. We also provided DNA barcoding information using mitochondrial *COI* gene sequences to support identification of the East Asian *Ephemera* species.

Materials and Methods

All specimens used in this study are deposited in the Korea University Entomological Museum, Seoul, Republic of Korea (KUEM). Approximately 150 specimens (larvae, subimagoes, and adults) from different localities in South Korea were examined and identified using the original description and taxonomic keys (McLachlan, 1875; Eaton, 1892; Matsumura, 1911; Demoulin, 1965; Tshernova, 1973; Bae, 1995; Hwang *et al*., 2003).

For *COI* gene sequencing of the *Ephemera* species, genomic DNA was extracted from the leg or coxa muscle following the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following instructions. Mitochondrial *COI* genes were amplified and sequenced using the PCR conditions and primers used in previous studies (Hwang *et al*., 2013; Suh *et al*., 2019). Phylogenetic analyses were conducted using the T92 + G + I model determined as the best-fit evolutionary substitution model by AICc from jModeltest 2.1.7 (Darriba *et al*., 2012) and subsequent phylogenetic analyses using the maximum likelihood (ML) with 1,000 bootstrap repeats, implemented in MEGA X (Kumar *et al*., 2016).

Taxonomic Accounts

***Ephemera orientalis* McLachlan, 1875** (Figs. 1A, 1B, 1C, 2A, 2C, 2E, 3A, 3B, 4A, 4E, 4I, 5A)

Ephemera orientalis McLachlan, 1875: 167; Demoulin, 1965: 215; Tshernova, 1973: 225; Gose, 1981: 13; Bae *et al*., 1994: 70; Bae and Yoon, 1997: 148; Hwang *et al*., 2003: 430.

Material examined. South Korea: Seoul: 6 males & 23 female subimagoes, Gwangnaru, Hangang (R.), 2018-V-14, Park SH, Seok SW. **Gyeonggi-do:** 10 male subimagoes & 19 female subimagoes, Gapyeong, 2011-V-2; 4 males & 13 female subimagoes, Gapyeong, 2011-VI-2; 17 males & 23 females, Gapyeong, Yeopgwang-gyo (Br.), 2017-V-8; 16 males & 19 females, Namyangju-si, Deokso, 2018-V-13; 12 males & 20 females, Pocheon-si, Hwanhyunmeon, 1991-VII-18; 3 males & 14 females, Yangpyeong, 2002-IV-29. **Gangwon-do:** 25 males & 37 females, Jeongsun-gun, Hwaam-myeon, 2018-V-7, Park SH, Kim JS; 5 larvae, Jeongseon, 2018-VI-2, Park SH, Kim JS; 4 larvae, Jeongseon-gun, Bungyang-ri (E 128°40′02.11″ N 37°22′41.04″), 2018-IX-14, Park SH, Kim JS, Mayorga A; 6 larvae, Wonju-si, Cheondungsan (Mt.) (E 127°56′52.88″ N 37°04′31.31″), 2016-IX-11, Park SH, Seok SW. **Gyeongsangnam-do:** 9 larvae, Sancheong-gun, Jirisan National Park, 2017-V-31, Bae YJ, Park SH.

Diagnosis. Body length 16.4 ± 0.3 mm in female adult and 14.1 ± 0.1 mm in male adult (Fig. 1A, 1B). Pronotum with lateral dark stripes and yellowish pale median band (Fig. 2A, 2D, 2E). Abdomen with paired multi-striped markings; segments I–II of abdominal tergum with dark pigment covering all or almost all the segments; pigmentation can be as dots, bars or irregular-shaped (Figs. 3A, 3B, 4A).

Adult. Forewing transparent with dark brown marks on crossveins; costal area dark brown; MP1–MP2 with a dark spot; CuA and MP2 fused (Fig. 1C). Forelegs anterior half of fore femur pale brown and posterior half dark brown; fore tibia and tarsus dark brown (Fig. 1A). Male genital lobes square-shaped, sclerotized and without prominent apical inner concavity; penis with distinct titillators triangular-shaped (Fig. 5A).

Mature larva. Claw pointed and elongated (Fig. 4I). Posterior margin of tergum X trapezoid-shaped and pronounced (Fig. 4E).

Distribution. China, Japan [type locality], Korea, Mongolia, Russia.

Remarks. Among the *Ephemera* species with paired multi-striped markings on abdominal segments, this species is one of the most widely distributed species in Far East Asia, and is well studied especially in Japan and South Korea in terms of life history (Gose, 1970; Lee *et al*., 1995; 1999; 2008; Hwang *et al*., 2009; Lee *et al*., 2011; Seok *et al*., 2019), the level of genetic diversity and its ecology (Kuroda *et al*., 1984; Lee *et al*., 1995; Kang *et al*., 2005; Hwang *et al*., 2009; Takenaka *et al*., 2023).

This species was redescribed based on the holotype (Demoulin, 1965), although the redescription lacked details of larva and male genitalia. Because of its wide distribution in a variety of habitats and geographical regions, morphological variations between local populations or cohorts are still poorly understood. Based on a close examination of adult and larval materials, we found that the shape of the pigmentation on the abdominal segment I–II (Fig. 3A, 3B; also illustrated by Gose, 1981) could be a useful character to distinguish *E. orientalis* from its sympatric *E. sachalinensis*.

In addition, despite its wide distribution across various habitats and countries, studies on the morphological variability among populations and cohorts remain incomplete. For instance, this study observed some morphological variations. Specimens from different sites in South Korea exhibited certain variations in the pigmentation patterns on tergal segments I–II (Fig. 3A, 3B), such as complete dark pigmentation, or incomplete dark pigmentation (Fig.

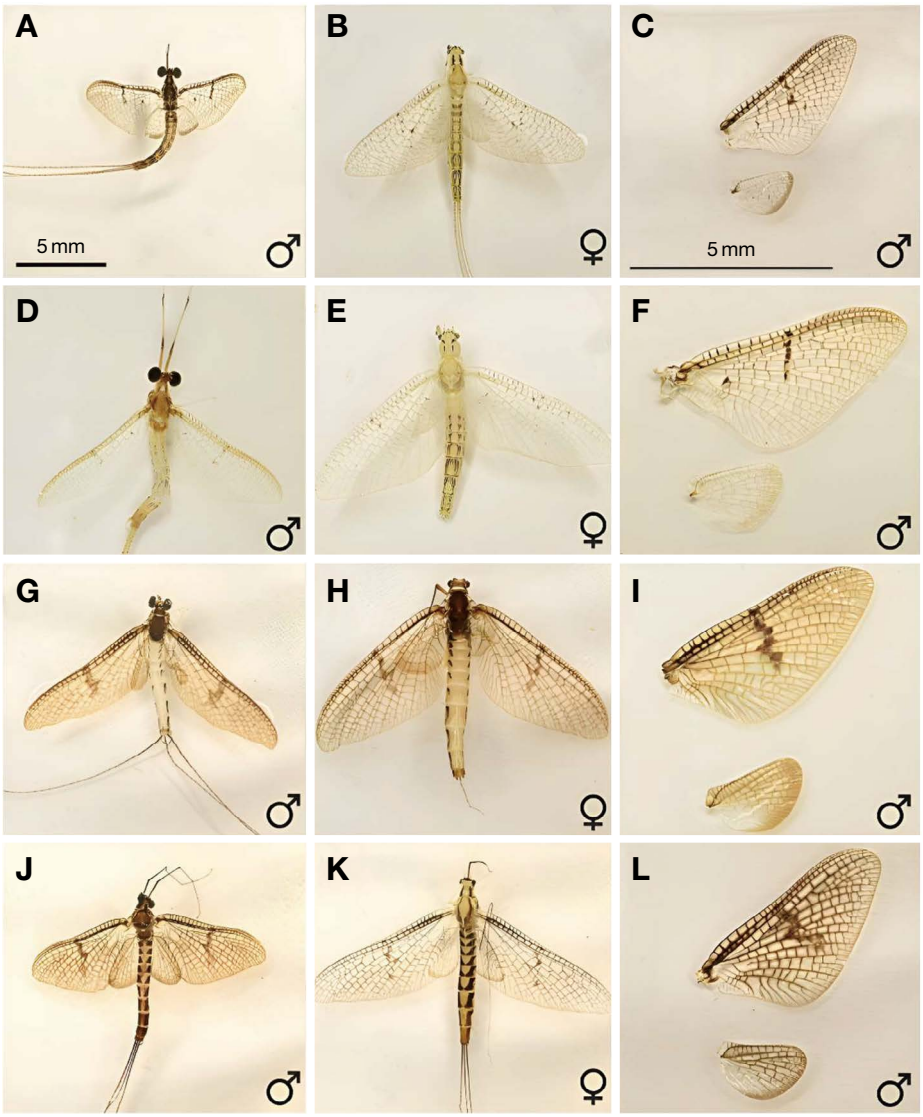


Fig. 1. Adults of South Korean *Ephemera* Linnaeus: A. male *E. orientalis* McLachlan; B. female *E. orientalis*; C. male wings of *E. orientalis*; D. male *E. sachalinensis* Matsumura; E. female *E. sachalinensis*; F. male wings *E. sachalinensis*; G. male *E. separigata* Bae; H. female *E. separigata* Eaton; I. male wings *E. separigata*; J. male *E. strigata* Bae; K. female *E. strigata*; L. male wings *E. strigata*.

3A). However, other distinctive morphological features proposed in this study remained consistent across sex, both larval and adult stages.

***Ephemera sachalinensis* Matsumura, 1911** (Figs. 1D, 1E, 1F, 2B, 2D, 2F, 3C, 4B, 4F, 4J, 5B, 6)

Ephemera sachalinensis Matsumura, 1911: 7; Tshernova, 1973: 329; Hwang *et al*., 2003: 430.

Lectotype (New designation). Female subimago (pinned; Fig. 6A), カラフト (Karafuto＝Sakhalin), 三宅 (Miyake: Collector's name), Matsum (＝Matsumura); ス川ヤ山 - 7/1907, deposited at the Hokkaido University Museum, Sapporo, Japan.

Other material examined. South Korea: Seoul: 34 female subimagoes, Amsa-dong, Hangang (R.), 2017-VI-1, Kang JH, Seok SW, Lim CS; 1 male imago & 14 female subimagoes, Seoul, Gwangnaru, Hangang (R.), 2018-V-14, Park SH, Seok SW. **Gyeonggi-do:** 1 male, Namyangju-si, Gu-am-dong-san, 1994-VII-20, Bae YJ; 3 male imagoes, Gapyeong, 2005-VIII-21; 12 female imagoes, Namyangju-si, Deokso, 2017-VI-29, Kang JH, Park SH, Lim CS; 4 larvae, Gapyeong, 1997-V-30, Bae YJ; 5 male subimagoes & 12 female subimagoes, Gapyeong, 2017-V-8; 8 male subimagoes, Gapyeong, 2017-V-20, Park SH, Uy CJ. **Gangwon-do:** 3 larvae, Jeongseon-gun, Jeongseon-eup (E 128°42′50.5″ N 37°23′15.3″), 2018-VI-2, Park SH, Kim JS; 37 larvae, Jeongseon-

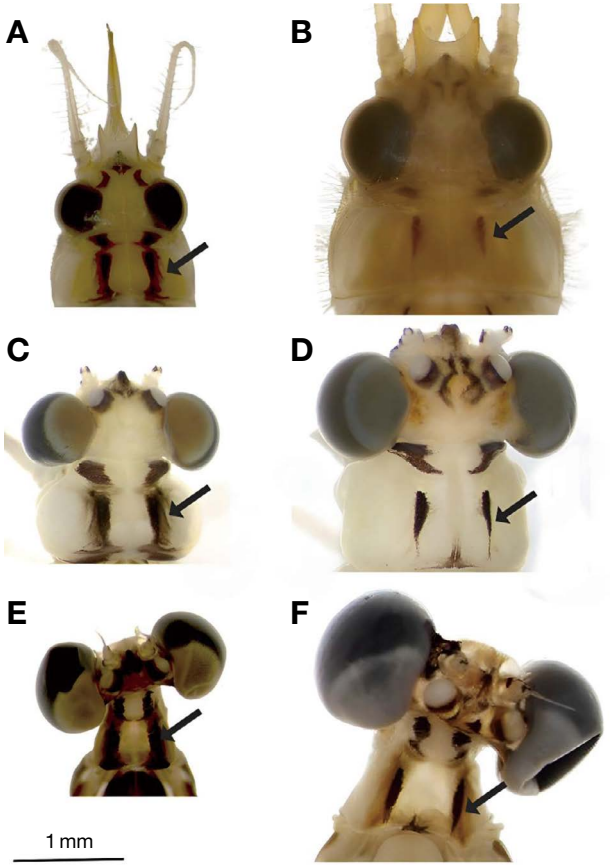


Fig. 2. Head of *Ephemera orientalis* McLachlan and *Ephemera sachalinensis* Matsumura: A. larval head of *E. orientalis*; B. larval head of *E. sachalinensis*; C. subimago head of *E. orientalis*; D. subimago head of *E. sachalinensis*; E. imago head of *E. orientalis*; F. imago head of *E. sachalinensis*.

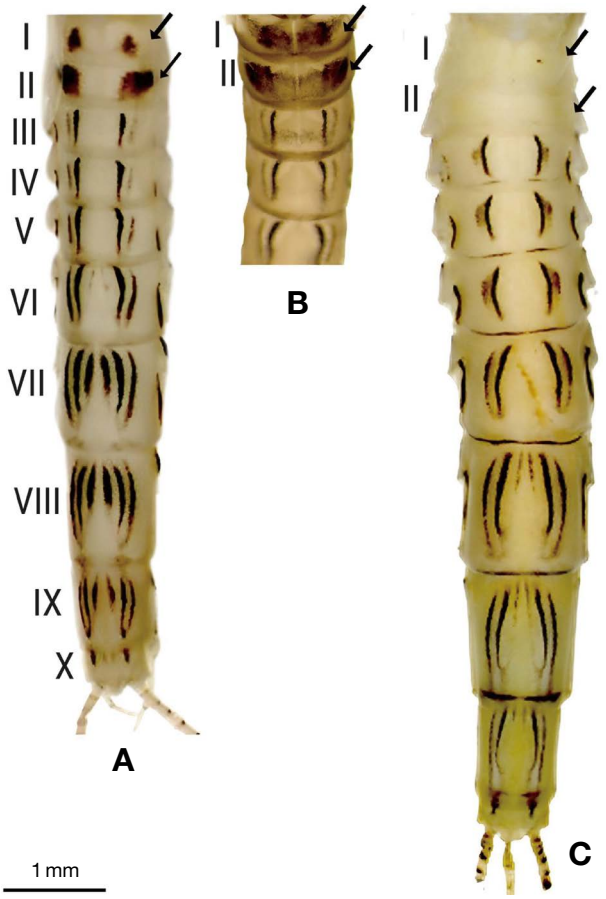


Fig. 3. Abdominal terga of *Ephemera orientalis* McLachlan and *Ephemera sachalinensis* Matsumura: A. variation I of abdominal terga of *E. orientalis*; B. variation II of abdominal terga of *E. orientalis*; C. abdominal terga of *E. sachalinensis*. Arrows indicate the key diagnostic characters between *E. orientalis* and *E. sachalinensis* at abdominal terga I and II.

gun, Hwaam-myeon, 2018-VI-2, 2018-V-7, Park SH, Kim J. **Jeollanam-do:** 2 male subimagoes & 3 female subimagoes, Yeongam-gun, Hoemun-ri (N 34°46′46.1″ E 126°40′47.6″), 2018-VII-18, Mayorga A, Uy CJ, Jung SW. **North Korea:** 1 male imago, Hwanghae Province (=Hwanghae-do): Haeju, 1986-VI-8, Kwang S. **Mongolia:** 3 female imagoes, Bulgan Aimag, Teshing Soum (wetland), 4.3 km SSW of Teshing (N 49°90′72.5″ E 102°65′66.5″), 970 m a.s.l., 2005-VII-10-11, Samaa. **Russia:** 1 male imago, Primosky Krai, Nakhodka City, Westshore of Rivadia Lake (N 42°51′46.43″ E 103°38′53.44″), 2013-VIII-17, Bae YJ, Kang JM, Lee JM, Vshivkova T.

Redescription (adult female lectotype). Specimen is pinned. Overall body yellowish and brownish (Fig. 6B). Pronotum with two lateral dark stripes becoming narrower basally and a yellowish pale median band (Fig. 6D). Mesonotum with a thick dark median band and lateral borders with dark pigmentation (Fig. 6C). Abdomen with paired multi-striped markings; abdominal terga I–II pale without dark pigmentation; terga III–VI with apparently dark multiple stripes but becoming unclear at last segments (Fig. 6C, 6B). Forewings and hindwings completely damaged (Fig. 6B). Three yellowish to brownish caudal filaments present. Forelegs broken; middle legs and hind legs completely yellowish (Fig. 6B).

Diagnosis. Body length 21.80±0.50 mm in female adult and 18.50±0.10 in male adult (Fig. 1D, 1E). Pronotum with lateral dark stripes becoming narrower basally and a yellowish median band (Fig. 2B, 2D, 2F). Abdominal terga I–II pale without dark pigmentation (Fig. 3C).

Adult. Forewings with dark brown marks on crossveins; costal area dark brown; CuA and MP2 fused (Fig. 1C). Foreleg trochanter, tibia-femur, tibia-tarsus junctions with dark pigmentation (Fig. 1D). Male genital lobes non-sclerotized, rounded at apex and elongated with inner concavity; penis with distinct thin titillators (Fig. 5B).

Mature larva. Claw neither elongated nor sharp (Fig. 4J).

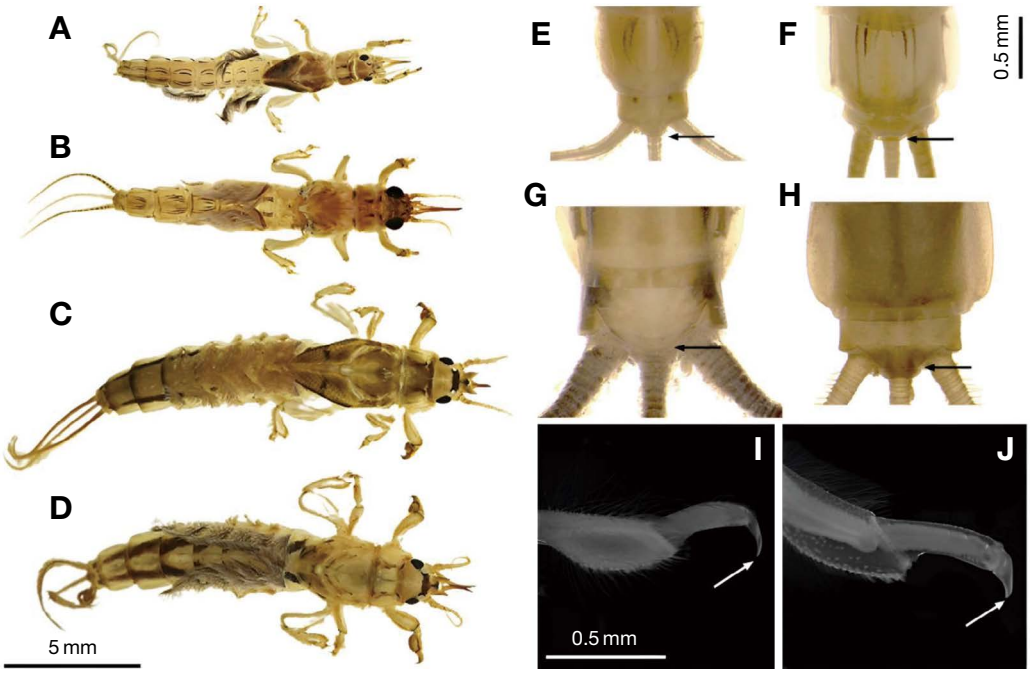


Fig. 4. South Korean *Ephemera* Linnaeus larvae: A. *E. orientalis* McLachlan; B. *E. sachalinensis* Matsumura; C. *E. separigata* Bae; D. *E. strigata* Eaton. Posterior margin of tergum X of South Korean *Ephemera* larvae: E. *E. orientalis*; F. *E. sachalinensis*; G. *E. separigata*; H. *E. strigata*. Claw of foreleg of the larvae: I. *E. orientalis*; J. *E. sachalinensis*.

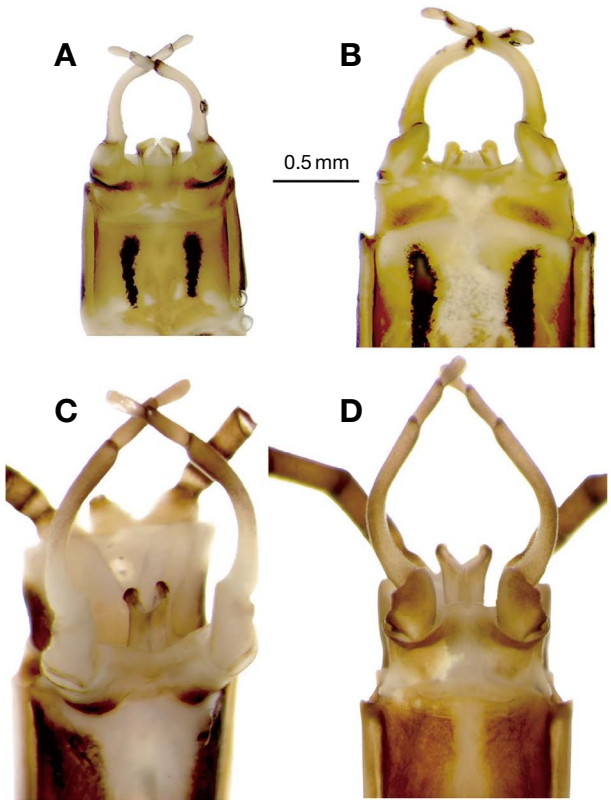


Fig. 5. Male genitalia of South Korean *Ephemera* Linnaeus males: A. *E. orientalis* McLachlan; B. *E. sachalinensis* Matsumura; C. *E. separigata* Bae; D. *E. strigata* Eaton.

Posterior margin of tergum X trapezoid-shaped and not pronounced, almost straight (Fig. 4F).

Distribution. China, Kazakhstan, Korea, Mongolia, Russia Far East [type locality].

Remarks. This species has not been well defined in terms of taxonomy and relations with other sympatric species in the same genus. Because the type information was lacked in the Matsumura's (1911) original description of this species, mayfly taxonomists like Tshernova (1973) were unable to locate any type specimens that were used in Matsumura's original description of this species. While the first author of this study had searched Matsumura's collection in the Hokkaido University Museum in Sapporo, a female subimago specimen (Fig. 6A) that was most probably labeled and used by Matsumura in the original description of *Ephemera sachalinensis* Matsumura, 1911 was found. The locality (Karafuto = Sakhalin) and collector (Miyake) also coincide with the information in the Matsumura's brief description of the species: "Fundorte: Chippsani und Suzuya, gesammelt in 2 females. Exemplaren von Herrn B. Miyake [= Locations: Chippsani and Suzuya, collected in 2 females. Specimens by Mr. B. Miyake]" (Fig. 6A). Although the condition of this Matsumura's specimen is not complete, some preserved characters such as the lateral dark stripes becoming narrower basally from pronotum and the lack of pigmentation at the abdominal terga I and II (Fig. 6B) well substantiate Matsumura's (1911) species concept of *E. sachalinensis*. The lectotype

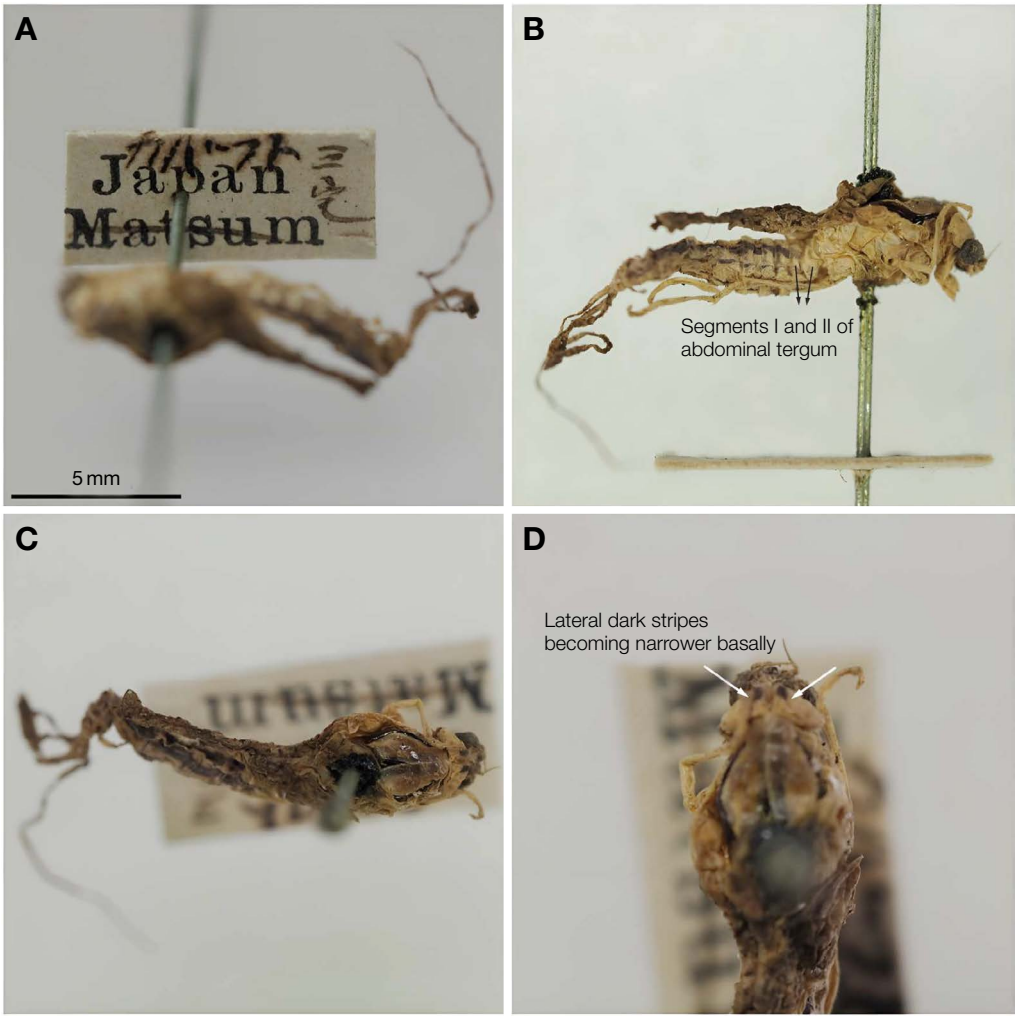


Fig. 6. Lectotype of *Ephemera sachalinensis* Matsumura (female) deposited in the Hokkaido University Museum: A. label; B. lateral view; C. dorsal view; D. head close-up.

designation must involve one of the original specimens used by the author for the description, which is the case in our study. Further revision of fresh specimens from the type locality (Sakhalin, Russia) will be beneficial to complement both Matsumura's original description (1911) and ours. The discovery of this historical specimen has significant scientific and historical relevance.

Ephemera sachalinensis is morphologically similar to *E. pieli* Navás based on the observation data of Chinese specimens provided by Li *et al.* (2023). Both *E. sachalinensis* and *E. pieli* lack dark pigmentation on the abdominal terga I–II, but *E. pieli* possesses two lateral dark spots on the abdominal tergum II. In addition, *E. sachalinensis* can be distinguished by different shape of male genitalia (Li *et al.*, 2023).

Ephemera sachalinensis is widely distributed in Northeast Asia and largely overlaps in distribution with its sympatric *E. orientalis* (Tshernova, 1973; Bae and Sóldan, 1997; Hwang *et al.*, 2003; 2008). Further investigations on life history, distribution and habitat, and other ecological features are needed to understand adaptation and evolution of these two closely related species. Compared to *E. orientalis*, in specimens from different sites around South Korea and other countries, it was not found important variability in the diagnostic characters for this species.

***Ephemera separigata* Bae, 1995** (Figs. 1G, 1H, 1I, 4C, 4G, 5C)

Ephemera separigata Bae, 1995: 160; Tiunova and Semenchenko, 2022: 94.

Specimens examined. South Korea: Gyeonggi-do: 37 larvae, Gapyeong-gun, Buk-myon (E 127°26′35.1″ N 38°01′31.3″), 2019-VI-4, Kang JH, Park SH, Lim C, Kim J.

Gangwon-do: 1 male subimago & 3 female subimagoes, Wonju-si, Chiaksan, 2002-VI-5, 2002-VI-15, Bae YJ. **Gyeongsangbuk-do:** 3 larvae & 1 male subimago, Gimcheon, Hwangaksan, 1996-IV-20, 2003-V-20; 11 larvae, Yeongju, Sobaeksan National Park, 2007-VI-5; 7 larvae, Yeongju-si, Dansan-myeon, 2007-IX-15. **Jeollanam-do:** 1 male imago, Jirisan, Dalgung, 1986-VI-25, Bae YJ, Hwang MJ.

Diagnosis. Abdominal tergum I pale with a pair of dark thick lateral bars; terga V–IX with a pair of dark narrow lateral bars; lateral bars marginally oriented, not reaching the median area (Fig. 1G, 1H).

Adult. Forewings whitish color with dark brown marks on crossveins; costal area dark brown; CuA–MP2 not fused (Fig. 1I). Male genital lobes narrow, Y-shaped with inner concavity; forks apex curved inertly and slightly rounded penis without distinct titillators (Fig. 5C).

Mature larva. Generally similar to adults in abdominal markings. Posterior margin of tergum X round-shaped (Fig. 4G).

Distribution. South Korea [type locality], Russian Far East.

Remarks. This species is not as common as the other *Ephemera* species in South Korea because of its exclusive distribution in mountain streams at relatively high altitude (Hwang *et al*., 2013). Monophyly of this species was well supported by previous molecular data (Hwang *et al*., 2013; Tiunova and Semenchenko, 2022).

***Ephemera strigata* Eaton, 1892** (Figs. 1J, 1K, 1L, 4D, 4H, 5D)

Ephemera strigata Eaton 1892: 302; Tshernova, 1973: 332; Gose, 1981: 16.

Specimens examined. South Korea: Gyeonggi-do: 4 male imagoes & 4 female imagoes, Gapyeong, 2003-V-18, 2013-VI-9. **Gangwon-do:** 36 larvae, 2 male subimagoes, 15 male imagoes & 26 female imagoes, Jeongseon, 2018-V-6-7, 2018-VI-2, Park SH, Kim JS, Mayorga A. **Chungcheongbuk-do:** 12 larvae, Danyang, 2007-VIII-25, Bae YJ.

Diagnosis. Abdominal tergum I pale with a pair of dark thick lateral bars. Abdominal terga V–IX with a pair of dark thick lateral bars reaching the median area basally (Fig. 1J, 1K). Adult: Forewings transparent with dark brown marks on crossveins; costal area dark brown; CuA–MP2 not fused (Fig. 1L). Male genital lobes wide Y-shaped with a slight inner concavity; forks apex curved inertly; penis without distinct titillators (Fig. 5D).

Mature larva. Generally similar to adults in abdominal markings. Posterior margin of tergum X quadrilateral-shaped (Fig. 4H).

Distribution. China, Japan [type locality], Korea, Mongolia, Russia.

Remarks. This species has been frequently treated as an ecological model species of life history studies, intermediate host of nematodes, and population analysis using machine-learning techniques (Gose, 1970; Kuroda *et al*., 1984; Takemon, 1990; Hirasawa and Urabe, 2003; Li *et al*., 2020).

In some immature larval specimens (7–12 mm in body length), abdominal terga VIII–IX are completely dark with a pair of pale median stripes, but this pigmentation changes to typical larval markings in the mature larvae.

Key to Korean *Ephemera* species

Mature larvae

1. Abdominal terga VII–IX with paired multi-striped markings (Fig. 4A, 4B)····· 2
– Abdominal terga VII–IX with paired single-striped markings (Fig. 4C, 4D)····· 3
2. Mesonotum yellowish pale with a continuous pair of dark stripes (Fig. 2A, 2C, 2E). Segments I–II of tergum with a dark pigment covering all or most of the segment (Fig. 3A). Fore claw pointed and elongated; posterior margin of tergum X trapezoid-shaped and pronounced (Fig. 3E) ····· *E. orientalis*
– Mesonotum pale with narrow discontinued pair of stripes becoming narrower at base (Fig. 3B). Segments I–II of tergum without dark pigment (Fig. 3B). Fore claws not elongated (Fig. 4J); posterior margin of tergum X almost square and not pronounced (Fig. 4F) ····· *E. sachalinensis*
3. Lateral dark bars of terga VII–IX narrow and only at borders, not reaching the median area of segments (Fig. 4C). Posterior margin of tergum X rounded (Fig. 4G) ····· *E. separigata*
– Lateral dark bars of terga VII–IX thick and reaching median area of segments terminally (Fig. 4D). Posterior margin of tergum X angled (Fig. 4H)····· *E. strigata*

Adults

1. Abdominal terga with paired multi-striped markings (Fig. 3A, 3C)····· 2
– Abdominal terga with paired single-striped markings (Fig. 1G, 1H, 1J, 1K)····· 3
2. Mesonotum yellowish pale with thick and continued pair of dark stripes (Fig. 2C, 2E). Segments Terga I–II with a dark pigment covering all or most of segment (Fig. 3A, 3B). Male genitalia sclerotized genital lobes without inner concavity (Fig. 5A). Foreleg dark brown, anterior half of femur pale····· *E. orientalis*
– Mesonotum pale with narrow and discontinued pair of narrow stripes (Fig. 2D, 2F). Terga I–II without dark pigment (Fig. 3C). Male genitalia with non-sclerotized and rounded apical genital lobes with inner concavity at apex (Fig. 5B)····· *E. sachalinensis*

3. Mesonotum with pale wide band on disc and dark lateral bands (Fig. 1G, 1H). Lateral dark bars of tergum narrow and only at borders not reaching median area of segment (Fig. 1G, 1H). Male genital lobes narrow Y-shaped with inner concavity; forks apex curved inertly and slightly rounded (Fig. 5C)·····*E. separigata*

– Mesonotum with pale wide band on disc and dark lateral bands (Fig. 1J, 1K). Lateral dark bars thick rhomboid-shaped and reaching median area of segment basally (Fig. 1J, 1K). Male genital lobes wide Y-shaped with a slight inner concavity; forks apex curved inertly (Fig. 5D)····· *E. strigata*

Discussion

Here, we reviewed the taxonomy of Korean *Ephemera* species, with an emphasis on clearly delimiting the two sympatric species, *E. orientalis* and *E. sachalinensis*. Detailed taxonomic examination is important since these two species are often confused in the field due to their similar morphology and shared habitat. Particularly, clear species identification keys will be very useful for the biomonitoring and the investigation of the recent mass emergence of *E. orientalis* in urban areas in Korea.

In addition, DNA barcodes using *COI* gene sequences clearly identified seven *Ephemera* species from Far East Asian region (Fig. 7). The intraspecific sequence divergence of the three *Ephemera* species (*E. orientalis*, *E. sachalinensis*, *E. separigata*) lies within the generally accepted species delimitation level of approximately 2% (Ratnasingham and Hebert, 2007; Suh *et al*., 2019) (Table 1). Although the genetic distance of *E. sachalinensis* from Korea and Russia shows a slightly higher species delimitation level of 3%, the morphological re-examination in this study confirmed that they belong to the same species, with no significant morphological differences. A high level of intraspecific divergence (5.80%) was observed in *E.*

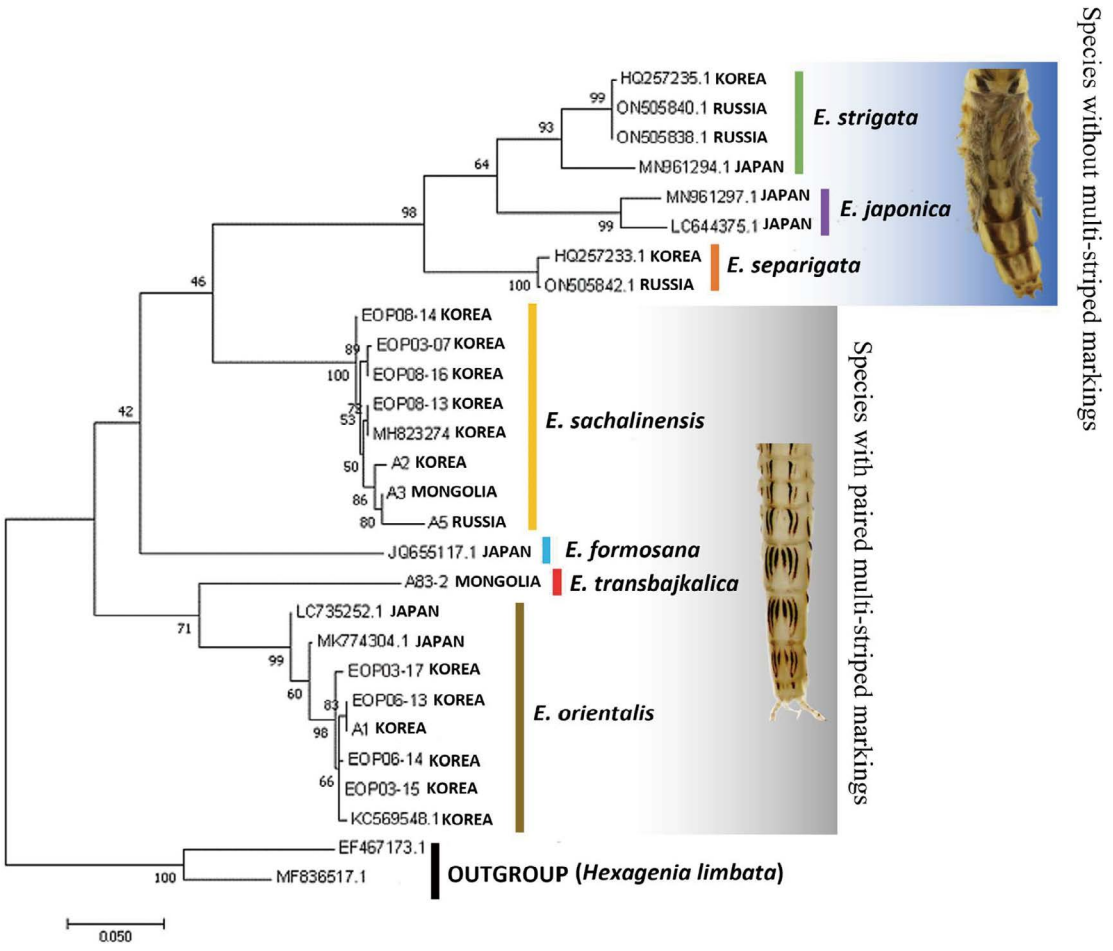


Fig. 7. Phylogenetic tree based on 26 mitochondrial *COI* gene sequences of *Ephemera* species from four countries from Far East Asia. The values on the branch indicate maximum likelihood (ML) bootstrap support values. Blue shade: non-multi-striped markings in the abdominal terga species. Gray shade: multi-striped markings in the abdominal terga species.

Table 1. Interspecific and intraspecific genetic diversity among *Ephemera* species based on the mean *p*-distance (%).

Interspecific diversity	Mean *p*-distance (%)	S.E. (%)
E. formosana vs. *E. japonica*	16.9	1.50
E. formosana vs. *E. orientalis*	14.5	1.02
E. formosana vs. *E. sachalinensis*	13.0	0.89
E. formosana vs. *E. separigata*	16.4	0.56
E. formosana vs. *E. strigata*	17.4	1.13
E. formosana vs. *E. transbajkalica*	14.2	1.10
E. japonica vs. *E. sachalinensis*	15.6	0.87
E. japonica vs. *E. separigata*	11.8	1.24
E. japonica vs. *E. strigata*	10.0	0.41
E. japonica vs. *E. transbajkalica*	16.7	0.65
E. orientalis vs. *E. sachalinensis*	12.7	1.37
E. orientalis vs. *E. separigata*	16.6	0.68
E. orientalis vs. *E. strigata*	15.0	0.74
E. orientalis vs. *E. transbajkalica*	10.8	1.49
E. sachalinensis vs. *E. separigata*	14.2	2.61
E. sachalinensis vs. *E. strigata*	15.0	1.06
E. sachalinensis vs. *E. transbajkalica*	15.3	0.93
E. separigata vs. *E. strigata*	11.1	0.92
E. separigata vs. *E. transbajkalica*	17.3	0.81
E. strigata vs. *E. transbajkalica*	16.6	0.75
E. formosana vs. *Hexagenia limbata* (Outgroup species)	16.9	2.80
E. japonica vs. *H. limbata*	17.0	0.88
E. orientalis vs. *H. limbata*	14.2	1.10
E. sachalinensis vs. *H. limbata*	15.8	2.52
E. separigata vs. *H. limbata*	17.3	1.80
E. strigata vs. *H. limbata*	16.9	0.70
E. transbajkalica vs. *H. limbata*	16.5	0.40
Intraspecific diversity	**Mean *p*-distance (%)**	**S.E. (%)**
E. japonica	3.9	0.76
E. orientalis	1.2	1.03
E. sachalinensis	1.4	0.30
E. separigata	0.7	0.50
E. strigata	2.9	1.10

S.E.: standard error

strigata individuals from Korea and Japan suggesting the possibility of cryptic species for this species in these countries, as reported by Suh *et al*. (2019) (see Table 2). This level of genetic divergence between the two regions suggests the need for a detailed morphological examination of specimens from Korea and Japan to clarify the taxonomic status of *E. strigata*.

More importantly, the species with multi-striped markings in the abdominal terga are well-distinguished and congruent with the taxonomic data presented herein; nonetheless, this species group is not monophyletic as expected, but it is clearly differentiated from the species with non-multi-striped markings (*E. separigata* - *E. japonica* - *E. strigata* clade) (Fig. 7). Multi-striped abdominal markings are neither synapomorphies nor phylogenetically informative characters in *Ephemera* species and, therefore, do not support the formation of a monophyletic group. Paired stripe patterns are likely homoplastic traits, indicating they may have evolved independently in different lineages rather than being inherited from a common ancestor.

In conclusion, we provided a detailed taxonomic review of Korean *Ephemera* species, along with the lectotype designation of *E. sachalinensis*. The DNA barcoding information of the East Asian *Ephemera* species provided in this

Table 2. Intraspecific genetic diversity of *Ephemera* species from Far East Asian countries based on the mean *p*-distance (%).

Comparisons between countries	Mean *p*-distance (%)	S.E. (%)
E. orientalis		
Japanese vs. Korean	2.10	0.70
E. sachalinensis		
Korean vs. Mongolian	1.20	0.45
Korean vs. Russian	3.00	1.12
Mongolian vs. Russian	1.90	0.81
E. separigata		
Korean vs. Russian	0.70	0.40
E. strigata		
Japanese vs. Korean	5.80	2.10
Japanese vs. Russian	5.60	1.50
Korean vs. Russian	0.75	0.64

S.E.: standard error

study will support clear species identification, contributing diverse ecological and evolutionary studies of this mayfly species group.

Conflicts of Interest

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

Acknowledgements

We deeply thank Masahiro Ohara and Tatsushi Takayanagi from the Hokkaido University Museum, Sapporo, Japan, who kindly guided us to the type material of *E. sachalinensis* Matsumura. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (grant number 2022R1A2C1009024, RS-2024-0035046).

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Submitted: September 23, 2024
Revised: October 30, 2024
Accepted: October 31, 2024

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Front Cover

This cover features a morphological characteristic (i.e., silverline) used for ciliate identification. A related article can be found on pages 417-422. Photo by Jeong Hyeon Yeo.